

Does the Side of Deviation of Nasal Septum have Concomitant Predictive Relation with Side of Sphenoid Septum Deviation?

G VENKATESH¹, TS GUGAPRIYA², N VINAY KUMAR³, SD NALINA KUMARI⁴



ABSTRACT

Introduction: The asymmetric hidden sphenoid sinuses get separated by a bony septum which rarely lies in the median plane. Deviated Nasal Septum (DNS) formed by bony and cartilaginous parts can be identified by clinical examination. Paucity of literature about the concomitant side deviation of both the septum, has been found.

Aim: To find the predictive relation between the side deviation of nasal and sphenoid septum.

Materials and Methods: A cross-sectional study was undertaken with coronal CT image sets of paranasal air sinuses at Chennai Medical College Hospital and Research Centre, Trichy, Tamil Nadu. Images of subjects above 18 years of age without chronic sinusitis blocking Infundibulo-osteal complex, space occupying lesion, previous sinonasal surgeries, and inflammatory polyps were included from December 2016 to January 2017. In 130 source image sets, side deviation of the nasal septum and sphenoid septum were observed and categorised. The data were

analysed using IBM SPSS Statistics for Windows, version 26 and a p-value of <0.05 was considered as statistically significant. The agreement of corresponding side deviation of nasal and sphenoid septum was tested using chi-square and Kappa test.

Results: The DNS was noted in 94.6% (123 of 130) of images. The right, left and median deviations were observed in 50.8% (66 of 130), 43.8% (57 of 130) and 5.4% (7 of 130), respectively. The sphenoid septum deviations of right, left and median was noted in 43.1% (56 of 130), 36.9% (48 of 130), and 19.2% (25 of 130), respectively. The right deviated sphenoid septum was seen in 39 out of 66 right DNS images and left deviated in 29 out of 57 left DNS images. Chi-square showed statistical significance (p-value=0.0031) and kappa [0.231 (SE of kappa=0.064, 95% confidence interval: from 0.104 to 0.357)] was assessed as a fair agreement between the observed values.

Conclusion: The fair agreement between sides of DNS with position of sphenoid septum concludes that DNS could be a probable predictor of position of sphenoid septum.

Keywords: Nasal septum deviation, Paranasal sinus, Sphenoid sinus, Tomography, Trans-sphenoid surgery

INTRODUCTION

The sphenoid sinuses are asymmetric air cavities located within the body of sphenoid bone and are separated by a bony septum [1]. The sphenoid sinuses present with varying degrees of pneumatization and are also related to vital structures near the sellar region [2]. The sphenoid septum lies rarely on the median plane but very often deviated laterally to one side or the other [3-5].

Minimal traumatization of nasal structures enabling quick recovery postoperatively with less complications in addition to providing panoramic view of operating field have made endoscopic endonasal trans-sphenoidal approach as the preferred surgical route to pituitary tumours now a days [6,7].

The sphenoid sinus is a hidden sinus and examination of it could be only possible by image analysis. There is a paucity of literature proposing any anatomical structure as predictor for sphenoid septal deviation by physical examination itself. An insignificant association between sides of nasal septal deviation and position of sphenoid septum in Mediterranean ethnicity was reported [8].

Nasal septum having bony and cartilaginous nasal components also occurs rarely in midline, causing DNS with a reported incidence of 62% that can be identified by clinical examination itself [8-13]. By mid-sixth week of development, the developing sphenoid bone cartilage contributes to the nasal septum formation [14]. This developmental sequence forms the basis for consideration that the side of deviation in sphenoid septum might result in concomitant side deviation of nasal septum [14].

Therefore, this study was done to test the hypothesis that the clinically observed side of deviation of nasal septum could act as a predictor for side of sphenoid septum deviation.

MATERIALS AND METHODS

A cross-sectional computerised tomographic study was undertaken with coronal CT image sets of paranasal air sinuses from archives of Department of Radiology, Chennai Medical College Hospital and Research Centre, Trichy, Tamil Nadu collected over a period of two months from December 2016 to January 2017. Without subject to any further sampling, universally we have included all the clinical records applicable during reference period. The study was initiated with approval from the Institutional Ethics Committee (IEC no. CMCH&RC/IEC NO- 34, dated 20/09/2016) and after obtaining informed consent from the study participants. The CT scan images were taken using GE Healthcare Lightspeed Ultra CT Scan Machine 8 slice with 3 mm thickness. The age of the subjects ranged from 18 to 60 years.

Images of subjects above 18 years of age were included in the study. The subjects with chronic sinusitis blocking Infundibulo-osteal complex, space occupying lesions, previous sinonasal surgeries, and inflammatory polyps were excluded from the study.

The 130 coronal CT source image sets obtained without subjecting to any further sampling, universally we have included all the image sets available during the reference period and after applying exclusion and inclusion criteria. They were analysed by RadiAnt DICOM Viewer (Medixant co., version-2020.1.1). The reference line for determining DNS was marked by two points. The first point was made at the superior attachment of nasal septum at the base of crista galli and the second point was made at the inferior attachment of septum at maxillary crest/anterior nasal spine. The bony part of the nasal septum was assessed for deviation in coronal images. The side of nasal septum convexity/deviation was determined posterior

to the reference line and was classified into right, left or median (no deviation) [15].

The deviation direction of sphenoid septum was observed from anterior to posterior plane. In images with more than one septum, a complete median or paramedian oriented septum extending from anterior to posterior was confirmed as the main septum. The deviation direction of sphenoid septum was determined as right, left and median with reference to posterior wall of the sphenoid sinus [8]. The number of images that showed corresponding side deviation of both nasal and sphenoid septum was also noted.

STATISTICAL ANALYSIS

The data collected were statistically analysed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). The p-value of <0.05 was considered statistically significant for all tests. Statistical agreement of two variables, corresponding side deviation of nasal and sphenoid septum was done using Chi-square and Kappa test.

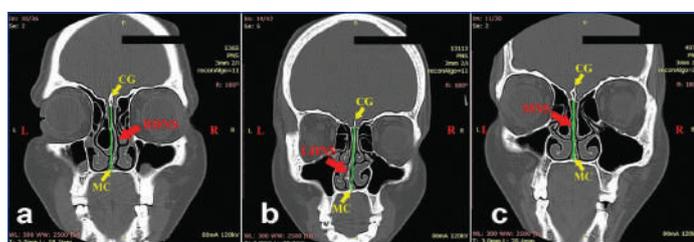
RESULTS

The computerised tomographic analysis of 130 coronal image sets of paranasal air sinuses with a mean±SD age of 40.3±1.63 years were analysed for deviation direction of nasal septum and sphenoid septum. In the study, male to female distribution was 64 and 66, respectively.

The DNS was noted in 94.6% of images (123 of 130) and the deviation direction of nasal septum was determined to be on right, left and median [Table/Fig-1,2].

| Deviation direction | Number of images | Percentage |
|---------------------|------------------|------------|
| Right | 66 | 50.8% |
| Left | 57 | 43.8% |
| Median | 7 | 5.4% |
| Total | 130 | 100% |

[Table/Fig-1]: Incidence of deviation direction of nasal septum.



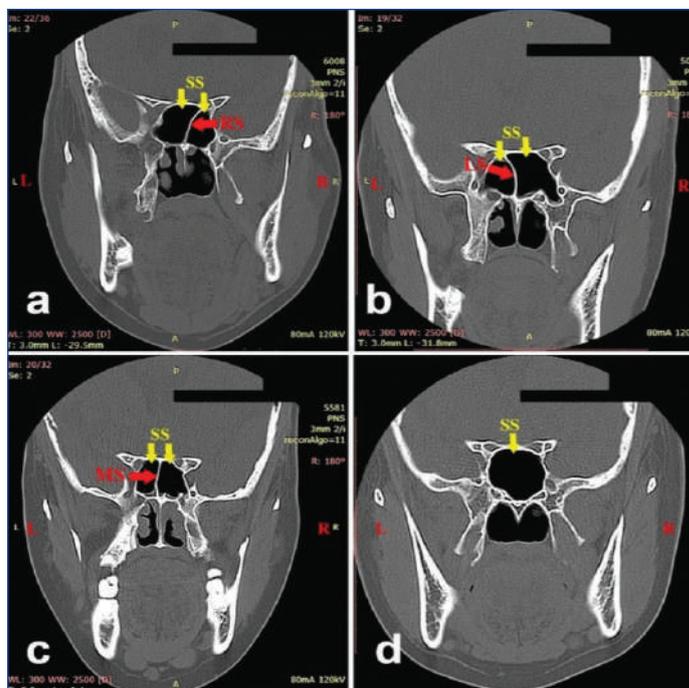
[Table/Fig-2]: Showing direction of deviation of nasal septum. a) Right Deviated Nasal Septum (RDNS)-red arrow; b) Left Deviated Nasal Septum (LDNS)-red arrow; c) Median Nasal Septum (MNS)-red arrow. CG: Crista galli; MC: Maxillary crest

The sphenoid septum was observed in 99.2% of images (129 of 130) and was absent in 0.8% of images (1 of 130). The incidence of deviation direction of sphenoid septum was observed to be on the right, left or median (no deviation) as tabulated [Table/Fig-3,4].

| Deviation direction | Number of images | Percentage |
|---------------------|------------------|------------|
| Right | 56 | 43.1% |
| Left | 48 | 36.9% |
| Median | 25 | 19.2% |
| Absent | 1 | 0.8% |
| Total | 130 | 100% |

[Table/Fig-3]: Incidence of deviation direction of sphenoid septum.

The sphenoid septum was observed in 99.2% of images (129 of 130) and was absent in 0.8% of images (1 of 130). The incidence of deviation direction of sphenoid septum was observed to be on the right, left or median (no deviation) as tabulated [Table/Fig-3,4].



[Table/Fig-4]: Showing direction of deviation of sphenoid septum: a) Right deviated sphenoid septum (RS)-red arrow; b) Left deviated sphenoid septum (LS)-red arrow; c) Median Sphenoid septum (MS)-red arrow; d) Absent sphenoid septum. SS: Sphenoid sinus

| Deviation direction of nasal septum | | Deviation direction of sphenoid septum | | | |
|-------------------------------------|---------------|--|-------------------|-------------------|-------|
| Side | No. of images | Right | Left | Median | Total |
| Right | 66 | 39 (28.65) [3.74] | 15 (24.56) [3.72] | 12 (12.79) [0.05] | 66 |
| Left | 57 | 16 (24.74) [3.09] | 29 (21.21) [2.86] | 12 (11.05) [0.08] | 57 |
| Median | 7 | 1 (2.60) [0.99] | 4 (2.23) [1.40] | 1 (1.16) [0.02] | 6* |
| Total | 130 | 56 | 48 | 25 | 129 |

[Table/Fig-5]: Statistical analysis of association between the side of DNS and sphenoid septal deviation. The chi-square statistic is 15.9516. *The p-value is 0.0031; The result is considered significant at p<0.05; Sphenoid septum was absent in one case†

The Kappa test was applied to know the intergroup agreements between the deviation direction of nasal and sphenoid septa. The value of kappa=0.231 (SE of kappa=0.064, 95% confidence interval: From 0.104 to 0.357) was assessed as a fair agreement between the observed values of direction deviations of nasal and sphenoid septa (kappa between 0.21 and 0.40: fair agreement).

The proposed null hypothesis was that there is no statistically significant association between the side of DNS and sphenoid septal deviation has been rejected as the association was shown to be statistically significant by applying chi-square test (p-value=0.0031).

DISCUSSION

A common phenomenon that occurs in the nasal septum is its deviation to either side. Previous literature shows references of such deviation of DNS with either right or left side predominance [Table/Fig-6] [8-19].

Right-sided dominance was observed in the present study and was reported by some previous studies as well [9,17, 18-19]. Such deviations were proposed to be as a result of trauma either during intrauterine life or postnatal life [20]. A developmental theory was also put forth as the probable cause for such deviation in the nasal septum with sphenoidal process playing the pivotal role [21,22]. The sphenoidal process of cartilaginous septum in cases of DNS was observed to be significantly longer and prominent with distinct histology [21]. A study on role of sphenoidal process in DNS showed that increasing angle of deviation noted in cases with long sphenoid process [22]. These observations could be

| Study | Right | Left | Median |
|--------------------------|-------|--------|--------|
| Battal B et al., [8] | 41% | 44.6% | 14.3% |
| Keles B et al., [9] | 51.1% | 42.2% | 6.7% |
| Tsai TL et al., [10] | 21.2% | 21.2% | 57.7% |
| Moorthy PNS et al., [11] | 36.5% | 54% | 9.5% |
| Orhan I et al., [16] | 49.5% | 50.5% | -- |
| Sistani SS et al., [17] | 65.3% | 34.7% | -- |
| Wojas O et al., [18] | 60.5% | 39.4% | -- |
| Ahmed SU et al., [19] | 47.8% | 41.3% | 10.9% |
| Present study | 50.8% | 43.8 % | 5.4 % |

[Table/Fig-6]: Comparing incidence of deviation direction of nasal septum.

explained as due to developmental delay in ossification of septum. The delayed septal ossification contributed to increased deviation of septum [21,22].

The present study's incidence of the positional deviation of sphenoid septum and absence of septum falls within the same range when compared to previous studies [23-25]. But the commonest side of deviation stands inconclusive [Table/Fig-7] [5,8,23-25].

| Study | Right | Left | Median | Absent |
|-------------------------------|--------|--------|--------|--------|
| Vidya CS and Raichurkar K [5] | 63.75% | 28.75% | -- | -- |
| Battal B et al., [8] | 43.3% | 38.9% | 17.7% | 1.3% |
| Kapur E et al., [23] | 64.4% | 35.6% | -- | 2% |
| Sirikci A et al., [24] | 20.6% | 21.7% | 38% | -- |
| Fasunla AJ et al., [25] | 26.4% | 43.6% | 18.2% | 2.7% |
| Present study | 43.1% | 36.9% | 19.2% | 0.8% |

[Table/Fig-7]: Comparing incidence of deviation direction of sphenoid septum [5,8,23-25].

The endoscopic endonasal trans-sphenoidal approach has wide application and recommended in treatment of pituitary tumours, craniopharyngiomas, germinomas of sellar and parasellar region in adult as well as paediatrics, due to its minimal invasiveness, less trauma and for maintaining anatomical integrity [26,27]. During this procedure, the excision of sphenoid septum is a necessity for adequate exposure of sella and the surgeon must have orientation to deviated direction of sphenoid septum pre operatively [6,7,26,27]. The anatomical variations of deviated sphenoid septum and its relationship to nearby vital structures like internal carotid artery, optic nerve, pituitary gland delineates the determination of its position for safety during surgery [1,2].

In concordance with present study's finding of same side deviation of DNS and sphenoid septum to either right side (59.09% i.e., 39 out of 66) or left side (50.87% i.e., 29 out of 57), another study also had reported concomitant deviation of both septa to right or left sides in 21.8% and 18.8% cases, respectively [8]. The possible predictive relation of side of deviation of DNS in determining the sphenoid septal position in this study stands in fair agreement statistically by kappa analysis of 0.231 and significant by Chi-square with $p=0.0031$.

Limitation(s)

In spite of this statistical agreement, the limited sample size and the cross-sectional study design limited the generalisability of the relation between sides of DNS to the side of sphenoid septum position.

CONCLUSION(S)

The present study findings shows a fair statistical agreement between sides of deviation of nasal septum with position of sphenoid septum, thus we conclude that the direction of DNS could be a probable

predictor of side deviation of sphenoid septum. These relational findings would aid the surgeon to preoperatively plan upon the side of main access during endoscopic endonasal trans-sphenoidal approaches. Further prospective studies are recommended with more sample size of endoscopic endonasal trans-sphenoidal approach regarding this relation between the septa are needed to confirm the relationship.

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Portal Annular Pancreas: An Unusual Pancreatic Pseudotumor with Clinical Significance

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Abstract

Portal annular pancreas (PAP) is a rare congenital anomaly due to abnormal fusion of ventral pancreatic bud left to the developing portal vein. Its incidence is 1.1%–3.4% in the literature. PAP is commonly involving the uncinate process; complete encircling is relatively rare. Proposed hypothesis for the congenital PAP is (1) hypertrophied ventral pancreatic bud fuses with the body of the pancreas left to the portal vein and (2) malformation of the portal vein. We found a bulky pancreas in a 51-year-old male during an autopsy. After careful dissection, we observed that the pancreas was completely encircling around the portal vein cranial to its formation. The encircled tissue was confirmed as normal pancreatic tissue by H and E and immunohistochemical staining. Pancreatic ductal pattern was observed anterior to the portal vein. This condition is usually asymptomatic; most of the time, the same was diagnosed incidentally during surgery. However, in cases of the malignant lesion in the head of the pancreas, the PAP is usually confused as the tumor's extension (pseudotumor). The management of posttransection pancreatic stump is challenging and usually results in complications such as pancreatic fistula. Knowledge about the PAP is necessary for the surgeon and radiologist for accurate diagnosis and prior planning to prevent the postoperative complications.

Keywords: Circumportal pancreas, congenital anomaly, pancreatic fistula, portal vein

INTRODUCTION

The portal annular pancreas (PAP) is a rare developmental anomaly. The incidence of the PAP is ranging from 1.1% to 3.4% in the literature.^[1,2] PAP commonly involves the uncinate process; complete encircling is relatively rare.^[3] It is usually asymptomatic; however, in patients with PAP, the surgical procedure near the pancreatic region is challenging, with a higher morbidity rate, usually results in postoperative complications such as pancreatic fistula.^[4,5] In the present study, the authors report a rare complete PAP with its histological features.

CASE REPORT

During the routine autopsy in a case of road traffic accident, the authors incidentally noticed the bulkier pancreas in a 51-year-old male. The pancreas was dissected from the posterior abdominal wall along with the duodenum and surrounding structures for further evaluation. The length and

weight of the pancreas were 25.5 cm and 196 g (doubled than the usual), respectively. After careful dissection, we observed that the pancreatic tissue was completely encircling around the portal vein cranial to its formation (suprasplenic type) [Figure 1a, b and c]. On hematoxylin and eosin staining, the wrapped aberrant tissue shows classical pancreatic acini and islets of Langerhans. Islets of the aberrant pancreatic tissue express specific immunoreactivity with the anti-synaptophysin antibody [Figure 2].^[6-8] We noticed that the pancreatic ductal system was lying in front of the portal vein (anteportal type) on piecemeal dissection.

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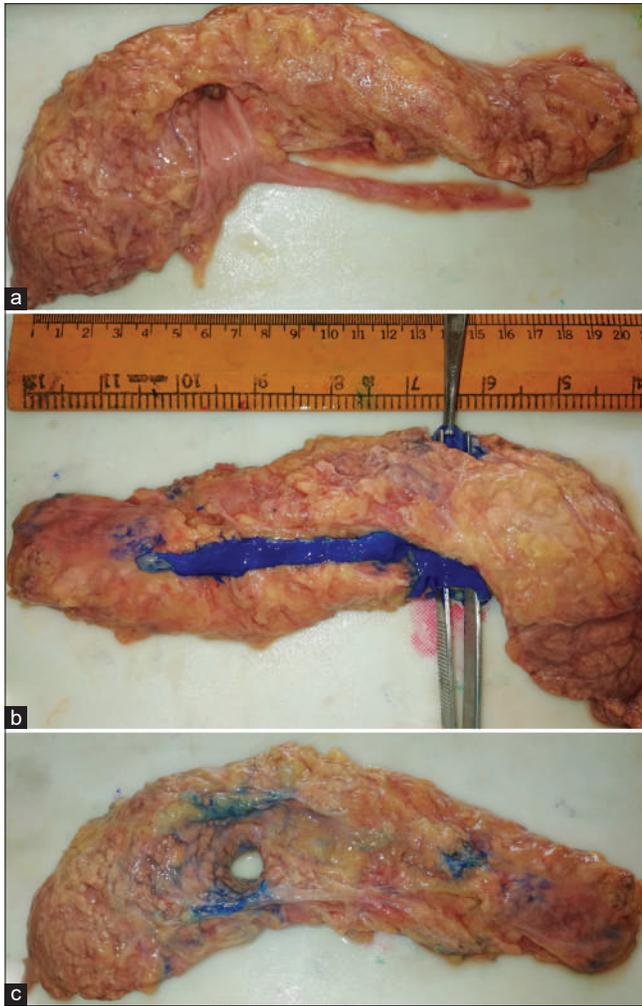


Figure 1: Dissected portal annular pancreas. (a) Antero-inferior view of pancreas showing the formation of portal vein and entry of the same into the substance of pancreas. (b) Posterior view of the pancreas with colored (blue) portal vein insitu. (c) Inferior view of pancreas showing the opening for portal vein within the pancreas

DISCUSSION

The annular pancreas is the most common congenital anomaly seen in the pancreas, in which the duodenal annular pancreas is the usual variant. The PAP is relatively rare when compared with other variants of the annular pancreas. The PAP may be due to abnormal fusion of ventral bud left to the developing portal vein resulting in the PAP [Figure 3].^[1,9,10] Like this, rarely pancreatic bud encircles the superior mesenteric vessels (SMV) results in entrapment of SMV into the substance of the pancreas.^[3] PAP is frequently seen in some animals such as swine.^[10,11] PAP commonly involves only the uncinata process; complete encircling is relatively rare.^[10,11] Variant hepatic artery was found to associated with the 25% of PAP which is similar to that of its incidence with nonportal annular pancreas.^[2]

The pancreas develops from the two endodermal evaginations from the caudal region of the foregut, namely dorsal and ventral pancreatic buds. During the pancreas development, the ventral

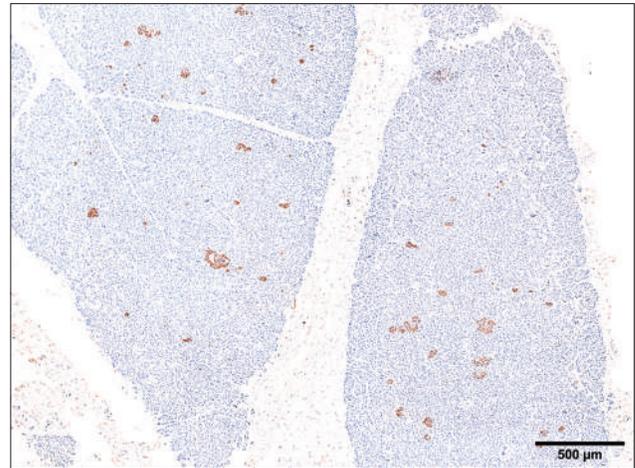


Figure 2: Immunohistochemical staining with anti-synaptophysin antibody showing the islets (brown – DAB positive areas) with background hematoxylin stain (exocrine area)

bud rotates to the left in the axis of the developing duodenum to fuse posteriorly with the dorsal pancreatic bud.^[12] Researchers proposed two hypotheses for congenital PAP. (1) Hypertrophied ventral pancreatic bud fuses with the body of the pancreas left to the portal vein and (2) malformation of the portal vein.^[1,13] In the present case, a bulky pancreas (doubled the weight of normal pancreas) strongly suggests the former hypothesis.

Based on its relation to the commencement of the portal vein, the PAP was classified into three types, namely suprasplenic (aberrant pancreatic parenchyma cranial to the formation of the portal vein), infrasplenic (caudal to its), and mixed type.^[14] Further, based on the relation of the pancreatic ductal pattern, it is classified into three more types, namely anteportal, retroportal, and retroportal with pancreatic divisum.^[9,15,16] In the present case, it belongs to the suprasplenic anteportal type of the PAP, the most common (92%) type of PAP.

This variation is usually asymptomatic. In cases of the malignant lesion in the head of the pancreas, the PAP was confused with the tumor's extension (pseudo tumour).^[1] In other way, if there is an extension of the tumor into the PAP, it may lead to incomplete removal of tumor cells. Contrast-enhanced computed tomography scan is the investigation of choice for diagnosing the PAP; magnetic resonance cholangiopancreatography may be required for further evaluation of ductal pattern before surgery.^[4,16] Precise planning of surgical procedures is necessary to avoid complications.^[4,17] Previous studies reported that the PAP is missed in more than half of the cases in preoperative magnetic resonance imaging (MRI) and computed tomography (CT) scans.^[9] A higher incidence of surgical complications is reported in this anomaly, especially the postoperative pancreatic fistula (45% of cases).^[4,11] Unlike the normal pancreas, the surgical procedure in this cases is different and challenging. There are no proper guidelines in the stump management of PAP. In classical pancreatoduodenectomy (Whipple resection), posttransection

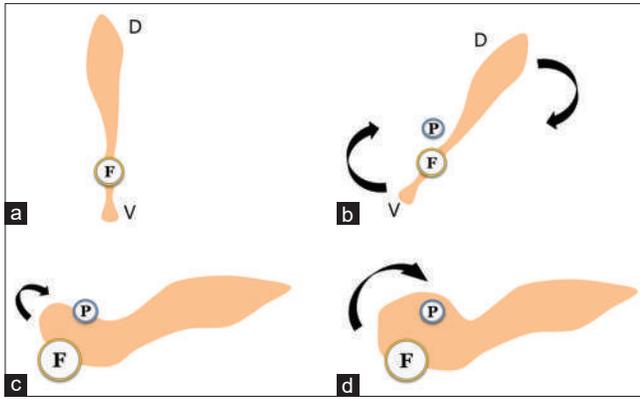


Figure 3: (a-c) Line diagrams show the mechanism of the normal development of the pancreas; D shows the hypertrophic ventral pancreatic bud (V) fusing to the dorsal bud (d) left to the portal vein; F – primitive foregut. The figure was adapted and modified from Yilmaz and Celik

of the pancreatic head, two pancreatic stumps (i.e., one stump anterior to the portal vein and another posterior to it) have to be managed in cases of the PAP. Dhanapal *et al.* reported a similar intra-operative case of anteportal type of PAP. They performed “duct to mucosa” pancreaticojejunostomy in the anterior stump and the posterior stump without ductal opening was managed with interrupted polypropylene sutures.^[4]

CONCLUSION

In conclusion, the PAP is predominantly asymptomatic in nature. However, in cases of a malignant lesion in the head of the pancreas, the PAP is usually confused as the extension of the tumor (pseudotumor). Postoperative pancreatic fistula is the most common complication in cases of PAP, thus the prior planning of pancreatic stumps management is necessary. Knowledge about the PAP is necessary for the surgeon and radiologist for the accurate diagnosis since the condition is missed in more than half of the cases in preoperative MRI and CT scans.

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Conflicts of interest

There are no conflicts of interest.

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Redefining the tail of pancreas based on the islets microarchitecture and inter-islet distance

An immunohistochemical study

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Abstract

Researchers divided the pancreas distal to the neck into 2 equal parts as the body and tail region by an arbitrary line. Surgeons considered the part of the pancreas, left to the aorta as the tail region. We performed this study to identify the transition zone of low-density to high-density islet cells for redefining the tail region.

We quantified islets area proportion, beta-cell area proportion, and inter-islet distance in 9 Indian-adult-human non-diabetic pancreases from autopsy by using anti-synaptophysin and anti-insulin antibodies. Data were categorized under 3 regions like the proximal body, distal body, and distal part of the pancreas.

Islet and beta-cell area proportion are progressively increased from head to tail region of the pancreas with a significant reduction in inter-islet distance and beta-cell percentage distal to the aorta. There is no significant difference in inter-islet distance and beta-cell percentage of the distal part of the body and tail region.

Crowding of islets with intermingled microarchitecture begins in the pancreas distal to the aorta, which may be the beginning of the actual tail region. This study will provide insight into the preservation of islets-rich part of the pancreas during pancreatectomy and future prediction of new-onset diabetes.

Abbreviations: AJCC = American Joint Committee on Cancer, B1 = part of body right to the aorta, B2 = part of body left to the aorta, DAB = 3,3'-diaminobenzidine, H = head, HRP = horseradish peroxidase, IHC = immunohistochemistry, IID = inter-islet distance, NODM = new-onset diabetes, T = tail, WSI = whole slide image.

Keywords: inter-islet distance, islets, pancreatic resection

1. Introduction

The pancreas has a unique scattered distribution of islets, which not only vary in their microarchitecture but also in its proportion to the exocrine part in various regions of the pancreas. Recent studies

documented the intra-pancreatic regional variability of islets area proportion.^[1] Islets of different parts of the pancreas are varying in their embryogenesis, architecture, and insulin secretory capacity.^[2-4] Islets in the tail region are derived from the dorsal pancreatic bud and these islets are relatively larger in size with the characteristic intermingling of relatively fewer beta cells with more alpha cells, which increase their insulin-secreting capacity via paracrine effect.^[5,6] Since the islet distribution/density is relatively higher (twice that of the head) in the tail region,^[7] obviously, the inter-islet distance (IID) will be less due to overcrowding of islets.

Even though many studies emphasizing on the importance of the pancreatic islets on the tail region, there is paucity on the studies which actually delineate the tail region.^[8] The classical anatomy textbook describes the tail region as part of the pancreas within the layers of the splenorenal ligament.^[9] Most of the pancreas researchers for the purpose of histomorphometric studies considered tail and body as 2 equal parts.^[10] As per the American Joint Committee on Cancer (AJCC) guidelines, surgeons considering the part of the pancreas left to the aorta as the tail region.^[11] Despite the importance of the islets of tail region in comparison with islets of other parts of the pancreas, there is a paucity of literature that exactly demarcates the tail region where the islets density is relatively higher than the rest of the pancreas. Detailed knowledge about the beginning of the tail region where the islet density starts rising is essential to preserve islet rich part during pancreatic surgeries, to predict the future risk of new-onset diabetes (NODM) following pancreatectomy and to improve the islet harvesting.

Furthermore, the quantification of exact islet area proportion or individual islet cell composition in different regions of the

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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human pancreas is challenging due to its complex distribution. The majority of the existing studies have quantified the endocrine part by taking a limited number of tissue sections, restricted to certain regions of the pancreas or by selecting the pancreatic tissue blocks randomly.^[12–14] The pancreatic islets are known for high regional variability, so this random or restricted selection of tissue will result in under or overestimation of islets area.^[15] Furthermore, few studies have selected random microscopic fields or islet-rich fields for measuring the proportion of endocrine part leading to selection bias.^[1]

So, the present study aimed to determine the location of transition zone in the pancreas, that is, the beginning of the actual tail region of the human pancreas where the islet density is start rising or differ significantly based on the inter-islet distance and endocrine area proportion (beta-cell percentage) by using large-scale computer-assisted analysis.

2. Materials and methods

2.1. Pancreas collection

A total of 9 adult human pancreases were collected from autopsy within 12 hours of death after obtaining the institutional ethical committee approval.^[16] In all the cases, the cause of death was the road traffic accident; none of the cases were suffering from any chronic disorders like diabetes, pancreatitis, etc. The pancreas was divided into anatomical head, body, and tail based on the previous studies^[10,17] (Fig. 1). Briefly, after removing the head (H) by dividing at the neck (using portal vein as a landmark), part of the pancreas distal to the portal vein is divided into 2 equal parts as the anatomical body and tail region (T) (Fig. 1). Secondly, based on AJCC surgical guidelines,^[11] the body is subdivided into 2 parts (part of body right to the aorta [B1] and part of body left to the aorta [B2]) by using the left margin of the aorta as the landmark. All 4 segments were labeled, as shown in Figure 1. Subsequently, complete coronal and transverse sections of all the 4 parts of the pancreas were grossed as described earlier.^[17] Approximately 40 sub-blocks from each pancreas were immersion fixed in 10% neutral buffered formalin, processed, and embedded in paraffin blocks. Pancreas showing

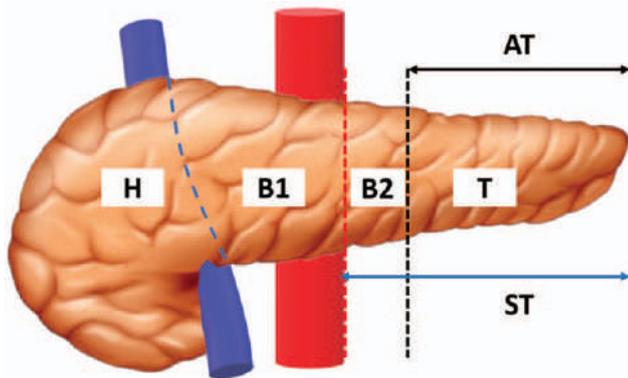


Figure 1. Pancreatic head (H) is divided by using portal vein as a landmark (blue dotted line); body and tail region (T) of the pancreas is divided into 2 equal halves (black dotted line); the body of the pancreas is subdivided as B1 (part of body right to the aorta) (red dotted line) and B2 (part of the body left to the aorta) based on AJCC guidelines. AJCC=American Joint Committee on Cancer; AT=anatomical tail (T); ST=surgical tail (B2+T).

autolytic changes and occult pancreatitis in Hematoxylin and Eosin staining were excluded from the study.

2.2. Immunohistochemistry

Immunohistochemistry (IHC) was done on 2 consecutive 4 μm thick paraffin sections from each block. Antigen retrieval was done by using the heat antigen retrieval method under high pressure with a citrate buffer solution. After blocking the endogenous peroxidase activity, primary antibodies such as rabbit monoclonal anti-synaptophysin antibody (1:300) (PathnSitu, Livermore, California) and rabbit monoclonal anti-insulin antibody (1:200) (PathnSitu, Livermore, California) were used. The primary antibody was detected by a secondary antibody labeled with horseradish peroxidase (HRP) and 3,3'-diaminobenzidine (DAB) chromogen (DAKO, Carpinteria, CA). Whole slide images of all IHC slides were captured in 10 \times magnification by using Carl Zeiss digital slide scanning system and Meta-Systems software. 10 \times magnification was sufficient for computer-assisted image analysis.^[18] Each whole slide image (WSI) sized several gigabytes were exported into the TIFF format and were transported to the workstation.

2.3. Quantification

All WSIs were analyzed by using Fiji/ImageJ free software from NIH (downloaded from <http://imagej.nih.gov/>). We quantified the islets and beta-cell area proportion as per our previous publication.^[17] In addition to that, following the segmentation of synaptophysin stained WSIs, the inter islet distance of each islet was measured by using the nearest neighbor distances plugin in ImageJ software^[19] (Fig. 2B and C). The IID was measured from the center of each islet to the center of nearby islets. The islet area less than 17 μm^2 was excluded as these are scattered islets cells rather than individual islets.

The formula used for various calculations are:

1. Islet area proportion = [total DAB positive area of the slide (anti-synaptophysin)]/[total tissue area of the slide] \times 100.
2. Beta-cell area proportion = [total DAB positive area of the slide (anti-insulin)]/[total tissue area of the slide] \times 100.
3. Beta-cell percentage = [total beta-cell area]/[total islet area] \times 100.

Here the authors calculated the beta-cell percentage against the total islet area in different parts of the pancreas.

2.4. Statistical analysis

Data are summarized and expressed as mean \pm SD. Paired “*t*” test was used to compare the data of means of the islet and beta-cell area proportion, beta-cell percentage, and IID in various regions of the pancreas. Pearson correlation test was used to compare the relation of islet areas proportion and inter-islet distance. *P* value $<$.05 taken as significant. The statistical test was performed by using SPSS software version 25 and graphs were plotted by Microsoft Excel 2016 software.

3. Results

Details of the 9 subjects, including age, gender, and BMI were given in Table 1. In all cases, BMI is within a reasonable limit.

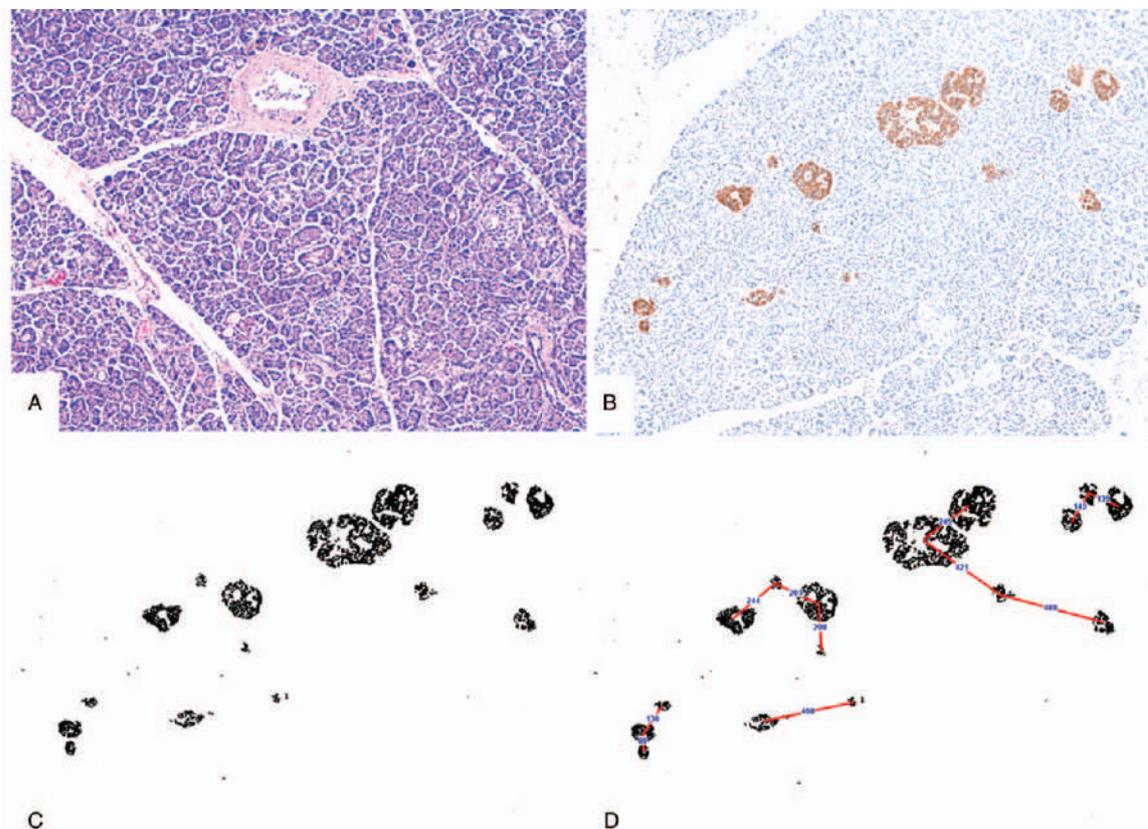


Figure 2. (A) Hematoxylin and Eosin-stained section showing pancreatic islets and acini without features of autolysis; (B) a single representative field of IHC image, stained by an anti-synaptophysin antibody from B1 segment of a pancreas; (C) processed image – segmentation of DAB positive area in ImageJ software; (D) schematic representation showing how the ImageJ software calculated the IID – centre of each islet to the centre of the nearby islet. Images B, C, and D are at same magnification for better comparison. IHC=immunohistochemistry, IID=inter-islet distance

3.1. Topographical variation of islets and beta-cell area proportion

Topographical variation of islet and beta-cell area proportion of all the 9 cases were plotted in the bar graph with its corresponding inter-islets distances in Figure 3. The mean islet area proportion of B1, B2, and T segment was $0.75 \pm 0.29\%$, $0.99 \pm 0.34\%$, and $1.37 \pm 0.65\%$ respectively. The mean beta-cell area proportion of B1, B2, and T segment was $0.58 \pm 0.26\%$, $0.7 \pm 0.31\%$, and $0.93 \pm 0.43\%$, respectively. The mean islets and beta-cell area proportions are progressively increasing from the

body to the tail region of the pancreas (Table 2). Islets and beta-cell area proportion of B2 segment (part of the body left to the aorta) is significantly higher than that of B1 segment with the P value of .007 and .021, respectively. Thus, there is a significant rise in endocrine proportion in the distal part of the body (B2 segment).

3.2. Topographical variation of islet dimension

The mean islet dimensions of B1, B2, and T segment of the pancreas are $42.34 \pm 5.19 \mu\text{m}$, $43.62 \pm 8.24 \mu\text{m}$, and $45.62 \pm 4.67 \mu\text{m}$, respectively. The mean islet dimension increased from the B1 to T segment; however, there is no significant difference (P value = .3).

3.3. Topographical variation of inter-islet distances

The mean inter-islet distances of B1, B2, and T segment of the pancreas are $268.1 \pm 62.3 \mu\text{m}$, $232.6 \pm 49.9 \mu\text{m}$, and $210.54 \pm 39.7 \mu\text{m}$, respectively. The mean inter-islet distance was progressively decreasing from body to tail region of the pancreas. The mean inter-islet distance of the B2 segment is significantly lower than that of the B1 segment; however, there is no significant difference between the IID of the B2 and T segment. Thus, crowding of islets starts in the distal part of the anatomical body. There is a strong negative correlation of IID and islet area

Table 1
Details of the subjects.

| Case No. | Age | Gender | BMI |
|----------|-----|--------|----------|
| 1 | 45 | F | 21.31659 |
| 2 | 34 | F | 18.91807 |
| 3 | 35 | F | 20.3428 |
| 4 | 49 | M | 18.02289 |
| 5 | 30 | F | 20.56881 |
| 6 | 34 | F | 20.88889 |
| 7 | 75 | M | 18.32963 |
| 8 | 27 | M | 19.77041 |
| 9 | 25 | M | 28.47898 |

BMI=body mass index.

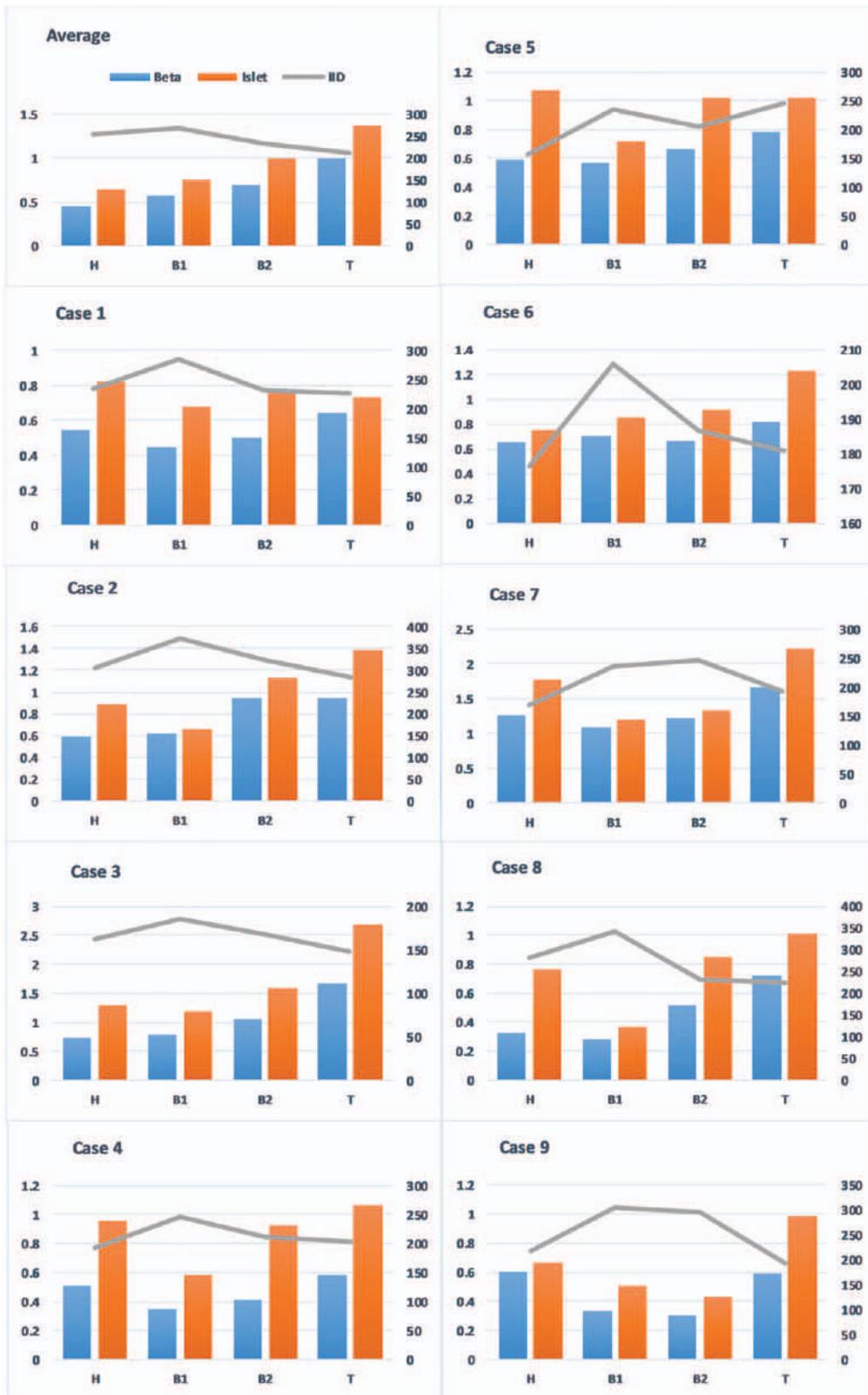


Figure 3. Bar chart representing the topographical variation of islets and beta-cell area proportion with its corresponding IID of all the 9 cases along with its mean values (average). IID was reduced in the B2 segment in most of the cases, which is almost similar to that of the T segment. Case nos. 5 and 7 show the difference in IID trend when compared with the general trend; out of that 2 cases, case no. 7 is the 70yr old male; thus, the cases might be because of age-related changes in the pancreas. IID=inter-islet distance.

Table 2
Regional variation of islet and beta-cell area proportion, inter-islet distance, and beta-cell percentage to total islet area.

| | Islet area proportion | | Beta-cell area proportion | |
|----|-----------------------|------------------|---------------------------|------------------|
| | Mean ± SEM | P | Mean ± SEM | P |
| B | 0.87 ± 0.30% | B vs T = .005* | 0.62 ± 0.28% | B vs T = .001* |
| B1 | 0.75 ± 0.29% | B1 vs B2 = .007* | 0.58 ± 0.26% | B1 vs B2 = .021* |
| B2 | 0.99 ± 0.34% | B2 vs T = .021* | 0.7 ± 0.31% | B2 vs T = .006* |
| T | 1.37 ± 0.65% | | 0.93 ± 0.43% | |

| | Inter-islet distance | | Beta-cell percentage to total islet area | |
|----|----------------------|------------------|--|------------------|
| | Mean ± SEM | P | Mean ± SEM | P |
| B | 250.1 ± 56.1 μm | B vs T = .013* | 70.1 ± 13.8% | B vs T = .844 |
| B1 | 268.1 ± 62.3 μm | B1 vs B2 = .016* | 75.7 ± 12.2% | B1 vs B2 = .036* |
| B2 | 232.6 ± 49.9 μm | B2 vs T = .130 | 69.1 ± 13.3% | B2 vs T = .986 |
| T | 210.54 ± 39.7 μm | | 69.2 ± 9.9% | |

B=anatomical body, B1=part of body right to the aorta, B2=part of the body left to the aorta, T=anatomical tail.

proportion, confirmed with Pearson correlation and had $r = -0.579$, $P < .002$; which is statistically significant (Fig. 4).

3.4. Topographical variation of beta-cell area percentage to total islet area

The mean beta-cell area percentage of B1, B2, and T segment was $75.7 \pm 12.2\%$, $69.1 \pm 13.3\%$, and $69.2 \pm 9.9\%$, respectively. The mean beta-cell area percentage of the B2 segment is significantly lower than that of the B1 segment; however, there is no significant difference between the mean beta-cell area percentage of the B2 and T segment.

4. Discussion

The pancreas is an exo-endocrine organ that regulates body metabolism. Endocrine cells are scattered within the exocrine

pancreas, which constitutes around 2% of the total pancreatic area. In standard textbooks, there is no clear demarcation of the tail region from the body. The proportion of islets and beta cells are higher in the distal part of the pancreas. This differential distribution may be due to the variable sources of its embryological origin.^[1,7,20] Even though the size of the pancreas varies widely from rodents to monkeys or humans, the size of the islet is almost remaining constant, with the maximum diameter being around 500 μm.^[6,7] Thus, several researchers hypothesized that the optimal size of the islet is essential for its adequate functional activity.^[2,4,21] To meet the insulin demand, the islet number (limiting islet size) and its cellular arrangement are increased during evolution.^[6] This intermingled arrangement of alpha and beta cells allows the contact of single beta cells with more number of alpha cells, which increase the insulin-secreting capacity of beta cells via the paracrine effect.^[6]

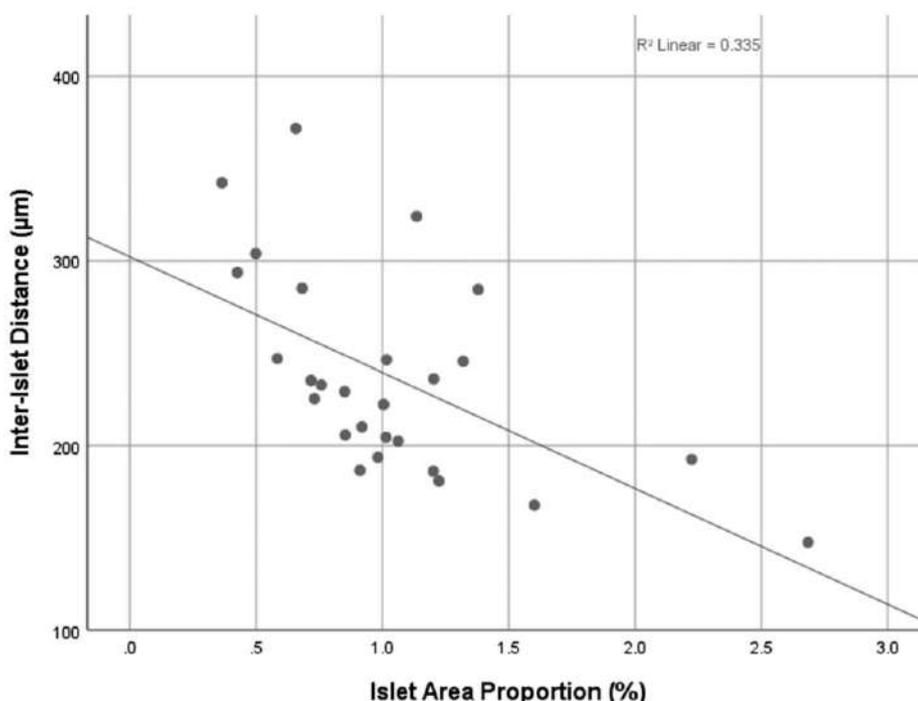


Figure 4. Relation of islet area proportion and inter-islet distance.

Topographical variation of islets morphology and functions is also linked with their development.^[2–4] Islets derived from the dorsal pancreatic bud (distal part of the pancreas) are relatively larger in size with the characteristic intermingled arrangement of alpha and beta cells, which has higher insulin-secreting capacity than that of islet-derived from the ventral bud (lower part of the head).^[4,7] Some animal studies demonstrated that the basal insulin-secreting capacity of islets derived from both ventral and dorsal buds is similar; however, the insulin-secreting capacity of islet-derived from the dorsal pancreatic bud is significantly higher even in the lower glucose concentration via the paracrine effect.^[2–4]

Topographical variation in islet distribution, like more than two-fold (100%) increase in islet area proportion in the distal part^[7] is probably due to an increase in islets number rather than size.^[6] The present study also documented no significant variation in islet dimension in the body and tail region of the pancreas. The increase in islet number leads to crowding of the islets, which results in the reduction of the IID. Though the larger islets with characteristic intermingling arrangement of alpha and beta cells are more efficient in insulin secretion, there is a reduction in beta-cell area percentage to total islets area.^[17] Thus, in the present study, we used the IID and beta-cell area percentages to determine the tail region.

In the present study, the authors observed a reduction in IID in the part of the pancreas left to the aorta (B2 and T segment) (Table 2). Even though both islet and beta-cell area proportion increase from the body to tail, it is increasing disproportionately. Islet area proportion increases 24% from B1 to B2 region, whereas beta-cell area proportion increases 12% only (Table 2). Thus, there is a reduction in beta-cell percentage to total islet area in the B2 region. Increasing in islet areas with a significant reduction in the beta-cell percentage in the B2 region shows the appearance of larger islets with characteristic intermingled alpha and beta cells, as seen in similar studies.^[6,17] In the region left to the aorta (B2 and T region), both islet and beta-cell area proportion increased ~40% symmetrically (Table 2) leading to no differences in the beta-cell percentage to total islet area, which shows there is no change in islet architecture (size and cellular arrangement) in both these areas. Even though there is a significant increase in islet and beta-cell area proportion between B2 and T segments, the B2 segment shows the characteristic islet crowding and intermingled beta and alpha cells arrangement as observed in the T region. Thus, the B2 and T segment has histomorphometric similarities in islets distribution and architecture. The entire part of the pancreas left to the aorta may be considered as the tail of the pancreas.

Distal or left pancreatectomy may range from resection of the smaller region of the tail to complete resection body and tail region, which is commonly done in the cancerous or premalignant lesions of the pancreas. Approximately 70% of islets are lost during total resection of the pancreas distal to the neck (extended left pancreatectomy). Thus, NODM (type 3c diabetes mellitus)^[22] is commonly encountered in 5% to 50% of the individuals based on the extent of the pancreatic resection and pre-existing disease status of the individual.^[23,24] The incidence of NODM is higher in the distal pancreatectomy individuals when compared with the other type of pancreatectomies (Table 3).^[23,25–34] Apart from endocrine insufficiency, exocrine insufficiency is also reported in 20.19% of distal pancreatectomy individuals.^[33] So optimal resection is essential to avoid metabolic derangement and subsequent morbidities.

Table 3

Review of literature shows the incidence of new-onset diabetes mellitus (NODM) in the distal pancreatectomy (DP).

| S. No. | Author (year) | NODM in DP (%) |
|--------|----------------------------|----------------|
| 1 | Schnelldorfer et al (2007) | 51 |
| 2 | King et al (2008) | 20–50 |
| 3 | Kim et al (2011) | 19.2 |
| 4 | Burkhardt et al (2015) | 31 |
| 5 | De Bruijin et al (2015) | 17–36 |
| 6 | Kwon et al (2015) | 30.5 |
| 7 | Kang et al (2016) | 25.3 |
| 8 | Nguyen et al (2017) | 44.6 |
| 9 | Woodcock et al (2019) | 4–5 |
| 10 | Jiro et al (2019) | 44.79 |
| 11 | Wu et al (2020) | 3–40 |

As the pancreas shows a high degree of intra-pancreatic regional variability in islets morphology and distribution, we quantified the islet and beta-cell areas proportion by examining the WSI of a complete representative coronal and sagittal section of the pancreas tissue (at least 20 cm² area microscopically in each pancreas) using large-scale computer-assisted analysis with Image J software. This method of analysis minimizes the sampling error and interobserver variation. Furthermore, researchers in various studies have determined the total islet area proportion by adding 3 major endocrine cells of islets like alpha-, beta-, and delta cells, which leads to an underestimation of total islets area due to ignoring the minor endocrine cells like epsilon and pancreatic polypeptide cells.^[1,15,20] The anti-synaptophysin antibody used in the present study is against the transmembrane vesicle protein of islets cell, which shows immunoreactivity toward all subtypes of islets cells resulting in less chance of underestimation of islet area.^[35]

The limitation of the present study is that even though we have collected the pancreas from individuals without a history of diabetes, there may be chances of subclinical diabetes. We have examined the reports of recent blood sugar values wherever available to exclude the diabetic pancreases. The other limitation of the present study is case no 7 (Table 1 and Fig. 3), which is the pancreas of a 75-years old male, who is a relatively older individuals in the study. The IID and beta-cell percentages show no significant change in B1 and B2 segment; this might be due to age-related changes in the pancreas.^[36,37]

In conclusion, for the first time, we provided information about the beginning of the tail of the pancreas based on the histomorphometric features by using large-scale computer-assisted analysis. Part of the pancreas distal to the aorta contains a higher density of relatively larger islet with more insulin-secreting capacity. Even though the amount of the pancreatic resection in distal pancreatectomy is decided based on the extent and nature of the disease, knowledge about the topographical variation of islet density in the pancreas will provide an insight into the better preservation of islets during pancreatic surgery and prediction of future risk of diabetes and other comorbid conditions.

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Topographical Variations of Wormian Bones in Eastern-Indian Dry Human Skulls: Current Perspective and Review of Literature

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Mini Review

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Abstract

Wormian bones occur due to the failure of fusion of the additional ossification centers with prominent skull bones. It may be misinterpreted as a fracture in cases of trauma. Several studies reported the racial differences in the incidence of Wormian bone. We aimed to determine the incidence of Wormian bone in the adult dry skull of the eastern Indian population with its location. A total of 30 adult dry (male – 14; female – 16) skulls of unknown age were included in the study. 53.33% (16) of the skull had the Wormian bones, out of which 13.33% (4) had a single Wormian bone; a maximum of ten Wormian bone was observed in a skull. The highest incidence of Wormian bone was observed in the lambdoid suture and pterion (62.5%) followed by asterion and coronal sutures with an incidence of 31.25% and 6.25%, respectively. Out of the four Inca bones, the largest Inca measured 63.8 x 59.6mm. Wormian bones are present in normal individuals with lower incidence. The number of Wormian bones increased in the pathological condition that shows its pathological and diagnostic implications. The knowledge about the Wormian bone is necessary to differentiate it from fracture or diagnosis of pathological condition underlying it. We hope the present study helps radiologists or surgeons in successfully differentiating a skull fracture from the Wormian bones by using modern-day imaging techniques.

Keywords: Sutural bone; Inca; Fracture; Skull; Calvaria

Introduction

Wormian bones or sutural bones are occurring due to the failure of fusion of the additional ossification centers with main skull bones [1]. It may vary in size, shape, location and number in different individuals. Few studies reported in the literature show racial or regional variation. The individual bone that occurs at the posterior fontanelle is named as Inca bone, preparital bone or Goethe's ossicle [2,3]. Wormian bones occurrence is related to both genetic and environmental factors. Though Wormian bones usually occur in few individuals, it is also reported in many autosomal dominant genetic disorders [4]. The most common site of occurrence is in the lambdoid suture, followed by other sutures and fontanelle. The least common site is bregma. Knowledge about the incidence and location of sutural bone is essential to interpret the radiological images of the skull to

distinguish the Wormian bone from fracture lines. In the case of misinterpretation, the treatment course is different and causes a delay in the timely treatment of emergencies. Thus, the present study was aimed to determine the incidence of Wormian bone in the adult dry skull of the eastern Indian population with its location.

Materials and Methods

A total of 30 adults dry (male – 14; female – 16) skulls of unknown age from the Department of Anatomy, All India Institute of Medical Sciences, Bhubaneswar were included in this study after institutional ethical committee approval. The skull with ante-mortem or post-mortem injuries or disarticulated skulls were excluded. Each skull was examined from anterior to posterior for the presence of sutural or wormian bones. The presence of wormian bone with its

location, side, number and dimension was recorded. The measurements are taken with the help of a digital Vernier caliper with 0.01mm precision.

Statistical analysis

The comparison of incidence in different locations and dimensions of right and left was performed using a student's t-test. The size of the wormian bones was expressed as mean \pm SEM. All statistical analyses were performed by using SPSS (Statistical Package for Social Sciences) version 25.0 (SPSS, Chicago, IL). A P value $<$ 0.05 was considered statistically significant.

Results

Out of 30 skulls examined, 16 (53.33%) skulls had single or multiple Wormian bones. The number of Wormian bones in a skull were ranging from one to ten. Single Wormian bone was found in four skulls (13.33%), two to five Wormian bones were found in eight skulls (26.67%). More than five Wormian bones were observed in four skulls (13.33%). The maximum number of Wormian bones were found in a single skull is ten (3.33%). In the present study, a total of 60 wormian bones were observed, out of which four (13.33%) are Inca bones. Incidence of wormian bones is maximum in lambdoid suture (62.5%) (right - 56.25%; left - 50%; bilateral - 43.75%) and pterion (62.5%) (right - 43.75%; left - 25%; bilateral - 6.25%), followed by asterion and coronal sutures composed of 31.25% and 6.25% of wormian bone. Wormian bones were not found in sagittal suture and bregma. Overall, 62.5% of skulls shown bilateral wormian bone. The incidence of wormian bones on the right side is slightly higher than the left side. Size of inca bone is relatively larger than Wormian bone. Out of the four Inca bone, the largest Inca was measuring 63.8 x 59.6mm was found in a female skull along with five wormian bones in the lambdoid suture (Figures 1-3).

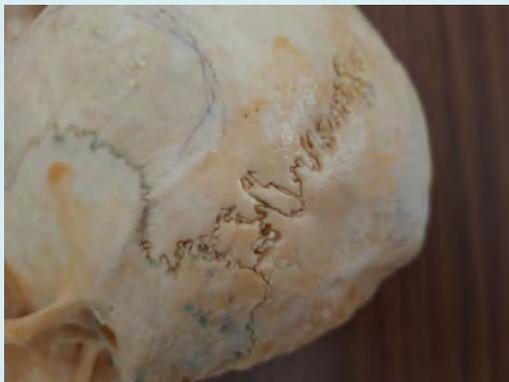


Figure 1: Photograph from posterolateral view of skull showing Wormian bone in left asterion.



Figure 2: Photograph of two large inca bone.



Figure 3: Wormian bone in the left lambdoid suture.

Discussion

Wormian bones was named after the Danish anatomist Olaus Worm, who mentioned it in a letter to Thomas Bartholin in 1643 [5,6]. The first description of wormian bone as "ossiculum antiepilepticum" in the literature was made by Paracelsus (1493-1541) [5]. They believed sutural bone has antiepileptic characters. Several authors mentioned it as ossa suturalis, accessory ossicles, Paracelsian ossicle, Ossa wormiana, ossicula Andernaci, ossa Goethiano, ossa triquetra, ossa triangularis, ossa quadratum, suturaux, fontanellaires, insules, intercalaria and raphogeminantia based on their discoverer, shape, location and function [7]. The morphogenesis of wormian bones is still controversial. In 1977, El-Najjar et.al., reported that wormian bones formation is not influenced by mechanical distortion [8]. However, recent studies suggesting that mechanical factor that spread sutures apart results in the formation of wormian bones [9]. Some authors suggesting the genetic factor for the formation of wormian bone as its incidence was higher

in few congenital disorder such as cleidocranial dysostosis, pycnodysostosis, rickets, kinky hair, osteogenesis imperfect, Menkes disease, hypothyroidism and hypophosphatasia, Down syndrome, otopalatodigital syndrome, primary acro-osteolysis and congenital disorders affecting the neural tube development [7,10,11].

The incidence of wormian bone in the literature is widely varying from 9% to 73%. The highest incidence was reported in the south Indian population (73.1%). In contrast, the lowest was reported in the Turkey population (9%) [7,12]. Incidence of multiple wormian bones was reported higher in the West Anatolia population (39.3%) [6]. The maximum and minimum rates of sutures that had Wormian Bones were found in left lambdoid 40.7% and right occipitomastoid 1.3% sutures, respectively. There was only a significant difference between the rate of right and left squamous sutures ($P = 0.04$). A study conducted on the Eastern Indian population reported 45% of skull having wormian bones, out of which 30% contains multiple wormian bones [5]. In the present study, we observed 53.3% of skulls with wormian bones. In line with the previous literature, Lambdoid suture observed to have a higher incidence of wormian bone and bregma not having any wormian bone in the present study.

Even though wormian bones are present in normal individuals, its incidence is increased in the pathological condition that shows its pathological and diagnostic implications [13]. The detailed description of the incidence, size, distribution in the normal individuals helps us to differentiate it from the wormian bones in the pathological condition [14]. Several authors hypothesized that the wormian bone should be arranged more than ten in number and each should be more than 4 to 6mm in size to label it as a pathological [14]. Wormian bones were commonly seen in the pathological condition where there is raised intracranial pressure or premature closure of sutures like hydrocephalus and cleidocranial dysplasia respectively [9,15]. Rarely in cases of decreased ossification of the skull, cranial sutures and fontanelles to be wider and for the skull to remain in a malleable state for a longer period of time [9]. The knowledge about the sutural bone is necessary for the physician for the early diagnosis and timely management of disorders associated with it. We hope the present study helpful to radiologists and surgeons in successfully differentiating a skull fracture from normal suture with Wormian bones by using modern-day imaging techniques.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Difficult Airway Assessment Score for Prediction of Difficult Intubation in Pre-Operative Assessment

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Abstract

Background: Maintaining a patent airway in anesthetized patients undergoing any procedure or surgery is very important for an anaesthesiologist. The vast majority of the airway-related events, especially inability to maintain patent airway, involve brain damage or death. Several independent bedside tests have been designed to predict a difficult airway or intubation but many have not gained popularity due to practical difficulties. So a new scoring system of Difficult Airway Assessment score based on ratio of patient's height to thyromental distance, upper lip bite test, head and neck movements, modified mallampati test and neck circumference was developed. **Objectives:** To determine the diagnostic validity of Difficult Airway Assessment score in predicting difficult intubation defined by Intubation difficulty scale. **Methods:** This prospective study was conducted among 300 patients aged between 18 and 65 years with ASA physical status I, II and III who underwent elective surgeries under general anaesthesia with endotracheal intubation at a tertiary care centre. Patients with history of burns, trauma or surgeries to airway, any obvious airway anomalies, inability to sit, edentulous or need awake intubation were excluded from the study. The Difficulty Airway Assessment Scoring system was devised with the airway parameters of Modified Mallampati test, Upper Lip Bite Test, Ratio of Height to Thyromental Distance, Neck Circumference and Head and Neck Movements. Each airway parameter was assessed pre-operatively and assigned a score of 0, 1, 2 depending on the severity and summated all the individual scores. Wilson score was also calculated for all the subjects. The difficulty in intubation was assessed with Intubation difficulty scale. **Results:** Out of 300 patients, the incidence of difficult intubation was 12%. Modified Mallampati test had the highest sensitivity (61.1%) and head and neck movements had the highest specificity (95.5%). Upper lip bite test and head and neck movements had highest Positive predictive value (42.9%) and likelihood ratio (5.5). Accuracy was highest for head and neck movements followed by Upper Lip Bite Test and RHTMD. Difficult airway assessment score with cut off ≥ 3 had a sensitivity of 88.9%, specificity of 82.6%, PPV of 41%, NPV of 98%, likelihood ratio of 5.1 and the accuracy was 83.3%. **Conclusion:** Difficult airway assessment score constructed using Modified Mallampati test, Upper Lip Bite Test, Ratio of Height to Thyromental Distance, Neck Circumference and Head and Neck Movements has a good predictive accuracy and was very much better compared to individual parameters.

Keywords: Difficult airway assessment score, difficult intubation, Modified Mallampati test, Upper Lip Bite Test, Thyromental Distance, Neck Circumference.

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Introduction

Maintaining a patent airway in anesthetized patients undergoing any procedure or surgery is very important for an anaesthesiologist. If securing the airway was failed or there is any hindrance in gas exchange, for even a few minutes, can reflect out in dangerous outcomes such as brain damage or even death. Closed claim analysis found that under anesthesia the vast majority of the airway-related events, especially inability to maintain patent airway, involve brain damage or death.[1] The Mallampati classification has been used for a long time for predicting difficult endotracheal intubation. It was reported that Mallampati class III and IV have a significant correlation with predicting difficult endotracheal intubation.[2] Mallampati classification is based on observation of the pharyngeal

structures with the mouth fully open and tongue maximally protruded. Khan et al. introduced upper lip bite test (ULBT) as a simple and effective method for predicting difficult intubations in 2003.[3] If the patient has a receding mandible, or a buck teeth, or if the patient cannot open his/her mouth very well, the ULBT class appears high and signifies difficult intubation. It is used especially in emergency patients where detailed airway evaluation cannot be done prior to surgery in the operating room before anaesthesia. Thyromental distance (TMD) is a measure of mandibular space and helps in determining how readily the laryngeal axis will fall in line with the pharyngeal axis when the atlanto – occipital joint is extended. measurement of TMD originated as a quantitative assessment of “receding jaw”. [4] The Ratio of height to thyromental distance (RHTMD) has been shown to be a more specific predictor for difficult intubation than TMD. Schmitt et al suggested that the RHTMD has a better accuracy in predicting a difficult laryngoscopy than the thyromental distance (TMD) alone.[5] Lavi et al classified its study population into normal and obese according to their BMI and they found that the intubation difficulty scale (IDS) was significantly

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Seeralan et al

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higher in obese patients.[6] Further studies have shown that it is the amount of tissue in the neck which correlates more with difficult intubation than BMI.[7] [8] Neck Circumference (NC) roughly correlates with the amount of tissue in the neck and is a useful and easily performed bedside test that helps the anaesthesiologist in the assessment of airway. Several independent bedside tests have been designed to predict a difficult airway or intubation. A recent Cochrane review concluded that none of the common bedside screening tests were well suited for detecting unanticipated difficult airway.[9] Several scores, which are a combination of the independent tests, have been described to assess the airway. But the scores have not gained popularity as bedside tests because they have been perceived to be cumbersome to perform at the bedside. Also, studies have proved that there is varying degree of inter-observer variability in pre-operative airway tests. Wilson score is one of the bedside test which was accepted widely due to its validity and easy administration.[10] Adnet et al. created an intubation difficulty scale (IDS) which had objective categories on difficulty of an endotracheal intubation after it was performed avoiding subjective variations.[11] In this prospective study, a new scoring system of Difficult Airway Assessment score based on ratio of patient's height to thyromental distance, upper lip bite test, head and neck movements, modified mallampati test and neck circumference was developed. The diagnostic validity of Difficult Airway Assessment score in predicting difficult intubation was assessed by comparing with Wilson score and Intubation difficulty scale.

Materials and Methods

Study Setting & Participants

The prospective study was conducted among patients who underwent elective surgeries under general anaesthesia with endotracheal intubation at a tertiary care centre, Trichy. Patients with age between 18 and 65 years with American Society of Anaesthesiologists (ASA) physical status I, II and III were included in the study. Patients with history of burns, trauma or surgeries to airway, any obvious airway anomalies, inability to sit, edentulous or need awake intubation were excluded from the study. After obtaining approval from Institutional Ethics Committee, the study was conducted after getting written and informed consent from each participant.

Sample Size & Sampling

According to Bhavdip Patel et al [12] study, the sensitivity of various parameters for assessing difficult intubation ranged widely from 28.6% to 100% and the incidence of difficult intubation was 8.1%. So considering an average estimated sensitivity for difficult intubation and airway assessment (Sn) as 50% with a precision (d) of 20% and 95% confidence interval ($Z_{1-\alpha/2} = 1.96$) and prevalence of difficult intubation (p) as 8.1%, the sample size is calculated as $N = Z_{1-\alpha/2}^2 * Sn * (1 - Sn) / p * d^2 = 303.45$. Rounded down, the sample size required was taken as 300. Systematic sampling of subjects was done to select them randomly. Every 5th patient attending pre-operative assessment for elective surgery with general anaesthesia was selected.

Study Procedure

Pre-Operative assessment

Preoperative assessment was done by the principal investigator in the pre anaesthesia check-up room. A thorough preoperative evaluation was done on the day before surgery to select patients satisfying the inclusion criteria. Routine preoperative investigations were done. The following parameters were assessed in the pre-operative assessment.

1. Height & Weight

The height of the subject was measured with the patient erect and barefoot on a flat surface against a solid wall and the height is measured with a metal tape to exact cm. The weight of the subject is measured with a bathroom weighing scale to nearest 0.5 kg.

2. Modified Mallampati Test Score

Modified Mallampati Test (MMT) was performed with patient sitting, head in neutral position and patient asked to open mouth maximally and to protrude the tongue without phonation. The visibility of the faucial pillars, soft palate and uvula noted. MMT score was assessed by the investigator at eye to eye level with the patient according to the following categories

Class I: Soft Palate, faucial pillars and uvula are visualised

Class II: Soft Palate, Fauces, uvula visible.

Class III: Soft Palate, base of uvula visible.

Class IV: Soft Palate is not visible.

3. Ratio of Height to Thyromental distance (RHTMD)

Thyromental distance (TMD) was measured as the distance from the thyroid notch to the end of the chin, using a scale, when the patient extended his/her neck. Then RHTMD was calculated as $RHTMD = \text{Height (cms)} / TMD \text{ (cms)}$

4. Head and neck movement range (HNMR)

It was measured as described in the study by Wilson et al by making the patient extend their neck as much as possible. Then, while holding a pen vertically to the patient's forehead, a notepad held against the side of the patient's face parallel to the pen. Then the patient's neck is flexed as much as possible. If the pencil was parallel to the bottom side of the notepad, it was recorded as 90°. If the pencil was lower than the bottom side of the notepad, it was recorded as more than 90°, if pencil was higher than the bottom side, it was recorded as less than 90°

5. Upper lip bite test (ULBT)

The upper lip bite test (ULBT) was performed as described by Khan et al. The patient is observed in the sitting position and asked to take a bite of the upper lip with the lower incisors as far as possible and classified according to the following criteria:

Class I-lower incisors can bite upper lip above the vermilion line

Class II-lower incisors can bite upper lip below the vermilion line

Class III-lower incisors cannot bite the upper lip

6. Subluxation of the mandible (SLux)

The patient was made to protrude the lower incisors as forward as possible. If the lower incisors were anterior to the upper incisors, then $SLux > 0$; if the lower incisors were equal to the upper incisors, then $SLux = 0$; and if the lower incisors failed to reach the upper incisors and remain posterior, then $SLux < 0$.

7. Inter incisor gap (IG)

Each patient was made to maximally open their mouth and the distance between the upper and lower incisors was measured. In the edentulous patient the distance between upper and lower gingiva was measured.

8. Receding mandible

The severity of receding mandible was estimated on subjective three-point scale (0 = normal; 1 = moderate; 2 = severe).

9. Buck teeth

The severity of buck teeth (long upper incisors) was also estimated on a subjective three-point scale (0 = normal, 1 = moderate, 2 = severe).

10. Neck circumference (NC)

It was measured with a tape at the level of thyroid cartilage, with head in neutral position.

Preoperative preparation

The patients were kept nil per oral for 8 hours. They were given orally Ranitidine 150mg, Metoclopramide 10mg and Alprazolam 0.25mg at 10pm on the night prior to surgery. Written informed consent was obtained from all patients who participate in the study.

Anaesthetic technique

After shifting to the operation theatre, pre induction monitors were connected. Datex Ohmeda Cardiocap monitor was used for monitoring which consisted of non-invasive blood pressure (NIBP)

monitor, 5 lead Electro-cardiogram (ECG) and Pulse oximeter. Iv cannula of appropriate gauge was secured in non-dominant hand under local anaesthesia and fluid was given according to Holliday Segar formula. Pre oxygenation was done with 100% oxygen with a fitting facemask at a flow rate of 6 litre/minute for 3 minutes and premeditated with fentanyl 2 µg/kg, midazolam 0.03mg/kg and glycopyrrolate 10 µg/kg iv before induction. Anaesthesia was induced with iv propofol, dose titrated according to loss of verbal response. After loss of response to verbal commands and ensuring adequate mask ventilation, neuromuscular blockade was achieved with vecuronium 0.1mg/kg iv and lungs were ventilated with bag and mask. Preservative free iv lignocaine 1.5 mg/kg was given 90 seconds before intubation. After ventilating with mask with sevoflurane 2% and oxygen for 3 minutes after induction, direct laryngoscopy was performed with head in sniffing position, using Macintosh blade size ¾ by an anesthesiologist (secondary

investigator) who was blinded to pre-operative airway assessment done by the primary investigator. Endotracheal intubation was confirmed using capnography and auscultation of bilateral lung fields. Post intubation monitoring included end tidal carbon dioxide and respiratory gas analyser. Case was continued and anaesthesia was maintained by the secondary investigator. The primary investigator was not a part of laryngoscopy. The whole intubation process was scored by the second investigator using 7 measuring variables of the Intubation Difficulty Scale (IDS).

Operational Definitions

Difficulty Airway Assessment Scoring system

Each airway parameter assessed in pre-operative assessment was assigned a score of 0, 1, 2 depending on the severity. The Difficulty Airway Assessment Scoring system was devised as show in the fig. and summated all the individual scores.

Table 1: Difficulty Airway Assessment Scoring system with individual parameters

| Airway Assessment factors | Score | | |
|--------------------------------|----------|----------|--------------|
| | 0 | 1 | 2 |
| Modified Mallampati Test Score | Class I | Class II | Class III-IV |
| RHTMD | < 23.5cm | > 23.5cm | |
| Upper lip bite test | Class I | Class II | Class III |
| Head and neck movements | > 90° | 90° | < 90° |
| Neck circumference | < 43cm | > 43cm | |

Wilson Score

For all the subjects, Wilson score was also calculated using the following parameters and scored 0,1 or 2 according to their grading. The total score ranged from 0 – 10.

Table 2: Wilson scoring system with individual parameters

| Criterion | Score | | |
|-------------------------|----------------------|----------------------|-----------------------|
| | 0 | 1 | 2 |
| Weight | < 90 | 90-110 | > 110 |
| Head and neck movements | > 90° | 90° | < 90° |
| Jaw movement | IG > 5cm or SLux > 0 | IG < 5cm and SLux= 0 | IG < 5cm and SLux < 0 |
| Receding Mandible | Normal | Moderate | Severe |
| Buck teeth | Normal | None | Severe |

Intubation Difficulty Scale (IDS)

The difficulty in intubation was assessed by the Intubation Difficulty Scale and was considered gold standard for assessing the validity of new scoring system of Difficulty Airway Assessment Score. IDS score is calculated as N1 to N7 as shown in the fig.1. and summated all scores of N1 to N7. The total score > 5 is considered as difficult intubation.

Statistical Analysis

Data was entered in MS excel sheet and analysed using SPSS software version 21. Continuous variables were represented in mean and standard deviation and categorical variables were represented in frequencies and percentages. The validity of the screening test was represented as sensitivity, specificity, positive predictive value and negative predictive value. The cut off value of the screening test for predicting the outcome variable is determined using ROC curve. p-values less than 0.05 were considered statistically significant.

| Calculating method | |
|--------------------|--|
| N ₁ | Every additional attempt adds 1 point |
| N ₂ | Each additional operator adds 1 point |
| N ₃ | Each alternative technique adds 1 point: repositioning of the patient, change of materials (blade, ET tube, addition of a stylette), change in approach (nasotracheal/orotracheal) or use of another technique (fibroscopy, intubation through a laryngeal mask) |
| N ₄ | Apply Cormack grade for 1st oral attempt. For successful blind intubation: N ₄ = 0 |
| N ₅ | Increased lifting force during laryngoscopy adds 1 point. For normal lifting force: N ₅ = 0 |
| N ₆ | External laryngeal pressure to improve glottic exposure adds 1 point |
| N ₇ | Position of vocal cords during laryngoscopy (abduction: N ₇ = 0, adduction: N ₇ = 1) |

Fig 1: Intubation Difficulty Scale (IDS)

Results

Out of 300 patients included in the study, 113 (37.7%) were males and 187 (62.3%) were females. The mean age of the study population

was 37.2 (± 10) years ranged from 18 to 64 years with majority (45%) in 31 – 40 years age group. 99 (33%) of the individuals were in class III & IV Mallampati class, 48 (16%) had >=23.5cm

RHTMD, 49 (16%) were in class III of Upper lip bite test, 21 (7%) had $\leq 90^\circ$ range of head and neck movements, 126 (42%) had ≥ 43 cm neck circumference. 36 (12%) had incidence of difficult intubation according to intubation difficulty scale. (Table 3)

The diagnostic validity of all the five parameters constituting the difficult airway assessment score namely Modified Mallampati test, Upper Lip Bite Test, Ratio of Height to Thyromental Distance, Neck

Circumference and Head and Neck Movements and their comparison had been shown in table 4.

The diagnostic validity of difficult airway assessment score for predicting difficult intubation with cut off at scores 2,3 and 4 were calculated and depicted in table 5 and compared with the diagnostic validity of Wilson score with cut off more than or equal to 2.

Table 3: Demographic characteristics of the study population

| | Frequency / Mean (\pm S.D.) | Percentage / Range |
|----------------------------------|--------------------------------|--------------------|
| Age | | |
| ≤ 30 years | 59 | 19.7% |
| 31- 40 years | 135 | 45.0% |
| 41- 50 years | 73 | 24.3% |
| > 50 years | 33 | 11.0% |
| Overall (years) | 37.2 (\pm 10) | 18 - 64 |
| Sex | | |
| Male | 113 | 37.7% |
| Female | 187 | 62.3% |
| BMI | | |
| <25 | 66 | 22% |
| 25-29.9 | 70 | 23.3% |
| >30 | 164 | 54.7% |
| Overall | 30.1 (\pm 5.6) | 22.4 - 38.5 |
| Modified Mallampati Score | | |
| III & IV | 99 | 33% |
| I & II | 201 | 67% |
| RHTMD | | |
| ≥ 23.5 | 48 | 16% |
| < 23.5 | 252 | 84% |
| Upper lip bite test | | |
| III | 49 | 16% |
| I & II | 251 | 84% |
| Head and neck movements | | |
| $\leq 90^\circ$ | 21 | 7% |
| $> 90^\circ$ | 279 | 93% |
| Neck circumference | | |
| ≥ 43 cm | 126 | 42% |
| < 43 cm | 174 | 58% |
| IDS Score | | |
| > 5 | 36 | 12% |
| ≤ 5 | 264 | 88% |

Table 4: Diagnostic validity of individual airway assessment factors

| | MMT | ULBT | HNM | NC | RHTMD | Hierarchy |
|-------------|------|------|------|-----|-------|-----------------------|
| Sensitivity | 61.1 | 58.3 | 25 | 44 | 55.6 | MMT>ULBT>RHTMD>NC>HNM |
| Specificity | 70.8 | 89.4 | 95.5 | 58 | 89.4 | HNM>ULBT=RHTMD>MMT>NC |
| PPV | 22.2 | 42.9 | 42.9 | 13 | 41.7 | ULBT=HNM=RHTMD>MMT>NC |
| NPV | 93 | 94 | 90.3 | 89 | 93.7 | ULBT>RHTMD>MMT>HNM>NC |
| PLR | 2.1 | 5.5 | 5.5 | 1.1 | 5.2 | ULBT=HNM>RHTMD>MMT>NC |
| NLR | 0.5 | 0.5 | 0.8 | 1 | 0.5 | NC>HNM>MMT=ULBT=RHTMD |
| Accuracy | 69.7 | 85.7 | 87 | 57 | 85.3 | HNM>ULBT>RHTMD>MMT>NC |

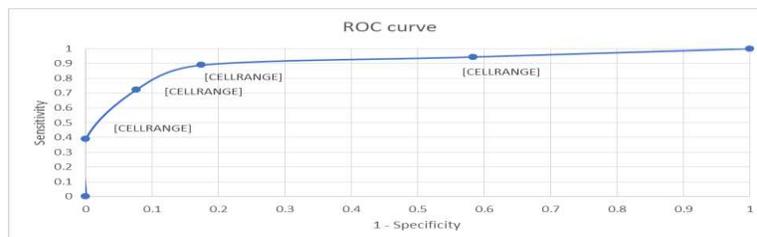


Fig 2: ROC curve for determining cut off of Difficult airway assessment score for predicting difficult intubation

Table 5: Diagnostic validity of difficult airway assessment score with various cut off and comparison with Wilson score

| Difficult Airway Assessment Score | IDS Score | | Sensitivity | Specificity | PPV | NPV | Likelihood ratio | Accuracy |
|-----------------------------------|----------------------------|------------------------|-------------|-------------|--------|--------|------------------|----------|
| | > 5 (Difficult intubation) | <= 5 (Easy Intubation) | | | | | | |
| Cut off score as 2 | | | | | | | | |
| >=2 | 34 | 154 | 94.4% | 41.7% | 18.1% | 98.2% | 1.6 | 48% |
| < 2 | 2 | 110 | | | | | | |
| Cut off score as 3 | | | | | | | | |
| >=3 | 32 | 46 | 88.90% | 82.60% | 41% | 98.20% | 5.1 | 83.30% |
| < 3 | 4 | 218 | | | | | | |
| Cut off score as 4 | | | | | | | | |
| >=4 | 26 | 20 | 72.20% | 92.40% | 56.50% | 96.10% | 9.5 | 90% |
| < 4 | 10 | 244 | | | | | | |
| Wilson score | | | | | | | | |
| >=2 | 16 | 77 | 44.40% | 70.80% | 17.20% | 90.30% | 1.52 | 67.67% |
| <2 | 20 | 187 | | | | | | |

Discussion

Difficult airway is one of the strenuous situation encountered by anaesthesiologists. Though many clinical bed side tests have been proposed preoperatively for detecting patients who may end up with difficult laryngoscopy, unfortunately, there is still no test or group of tests that can accurately predict difficult laryngoscopy. Predictive test for difficult intubation can be grouped into individual indices and scoring systems. Preoperative airway assessment test should be highly sensitive to predict maximum number of patients with difficult laryngoscopy correctly, and highly specific to predict easy laryngoscopy also.

The reported incidence of difficult airway varies from 1.3 to 18% in general population. In the present study, out of 300 patients, 36 had difficult intubation and the incidence of difficult intubation was 12% which is comparable to that observed by earlier studies. Shah et al [13] showed almost similar result of 13.95% difficult intubation, Patel et al [12] with a slightly reduced incidence of 8.1%, Vidhya et al [14] depicted 16% difficult intubation and Seo et al [15] showed 11.8% incidence.

The present study elucidated that Modified Mallampati Test (MMT) had a sensitivity of 61.1%, specificity of 70.8%, PPV of 22.2%, NPV of 93%, likelihood ratio of 2.1 and accuracy of 69.7%. These results were similar to the results shown by Shah et al [13]. It presented MMT with sensitivity of 70.15%, specificity of 61.02%, PPV of 22.6%, NPV of 92.65%. Bhavdip Patel et al [12] studied difficult intubation with MMT and depicted a sensitivity of 28.6%, specificity of 93%, PPV of 18.2%, NPV of 96% and accuracy of 89.6%. Various other studies also show very low sensitivity and PPV with moderate and high specificity and NPV values. Hence MMT alone can't be used to predict difficult airway.

Our study revealed ULBT as a predicting test with sensitivity 58.3%, specificity 89.4%, PPV 42.9%, NPV 94%, Positive LR 5.5 and accuracy 85.7%. The results were slightly different from the studies by Khan et al [3] which showed higher predictive values of Sensitivity 76.5%, specificity 88.7%, PPV 28.9%, NPV 98.4%, likelihood ratio 6.76 and accuracy of 88%. Other studies show a low sensitivity for ULBT similar to the current study. Eberhart et al [16] deduced the predictive values of ULBT with sensitivity, specificity, PPV, NPV, likelihood ratio, accuracy of 28.2%, 92.5%, 33.6%, 90.6%, 3.78 and 84.9% respectively. Hester et al [17] assessed ULBT as a predictive test for difficult intubation and showed sensitivity, specificity, PPV and accuracy of ULBT as 55%, 97%, 83%, 90% respectively. Shah et al [13] elucidated the predictive parameters of ULBT namely sensitivity, specificity, PPV, NPV and likelihood ratio of 74.63%, 91.53%, 58.82%, 95.7% and 31.76.

RHTMD, introduced by Schmitt et al [5] has better predictive value in predicting difficult laryngoscopy than TMD as it allows for individual's body proportions which are not allowed in TMD. In our

study RHTMD yielded a sensitivity of 55.6% and a specificity of 89.4% with positive and negative predictive value of 41.7% and 93.7% respectively. Compared to other studies, the sensitivity was slightly lower and specificity was higher with predictive values almost similar. Azim Honarmand et al [18] depicted predictive power of RHTMD with sensitivity, specificity, PPV, NPV and likelihood ratio of 64.7%, 82.42%, 38.8%, 93.2%, 3.68 respectively. The sensitivity, specificity, PPV, NPV and likelihood ratio of RHTMD deduced by Krobbuaban et al [19] was 77%, 66%, 24%, 95% and 2.26 respectively. Shah et al [13] shown RHTMD as a good predictive test for difficult intubation with sensitivity, specificity, PPV, NPV and likelihood ratio of 71.64%, 92.01%, 59.26%, 95.24% and 8.96 respectively. The RHTMD has some limitations because it depends on accurate measurement of patients TMD and height that lessens the simplicity of this method. Also, the cut-off point of RHTMD for prediction of difficult laryngoscopy is race dependent, we consider RHTMD ≥ 23.5 cm as a cut off for difficult intubation suggested by Krobbuaban et al.

Head and neck movements, in predicting difficult intubation, had a sensitivity of 25% and specificity of 95.5% with PPV 42.9%, NPV 90.3%, likelihood ratio 5.5 and accuracy of 87% which almost matches with Seo et al [15] which shows 25%, 94.4%, 37.5%, 90.39%, 86.22% of sensitivity, specificity, PPV, NPV and accuracy respectively. Shah et al [13], with slight difference matches with the present study findings, depicts sensitivity, specificity, PPV and NPV of 7.46%, 93.95%, 16.67% and 86.22% respectively. In the present study, the patients with obvious airway anomalies had been excluded which included obvious limitation of neck extension. Many patients belonged to obese category with short neck which might have resulted in falsely identifying patients as having limited head and neck movements. We assessed head and neck movements based on the method described in the study by Wilson et al. Accounts measurement can be done with a goniometer only. All these reasons might have contributed to lower sensitivity for HNM in our study.

Brodsky et al [20] studied morbidly obese patients and found Neck circumference as a significant predictor of difficult intubation. The study depicted that the probability of a difficult intubation was approximately 5% for neck circumference of 40 cm. while the probability increased up to 35% at a neck circumference of 60 cm. Gonzalez et al [7] compared obese and lean individuals for difficult intubation and determined neck circumference as more than 43 cm as a cut off for predicting difficult intubation with sensitivity, specificity, PPV and NPV of 92%, 84%, 37% and 99%. The present study predicted difficult intubation with neck circumference more than or equal to 43 cm as a cut off and the sensitivity, specificity, PPV and NPV of 44.4%, 58.3%, 13% and 88.5% respectively. It is not only the neck circumference but also the amount of per tracheal soft tissue that matters, as demonstrated in obese patients by the use

of ultrasound.[8] Gender related anatomic difference may also be significant. In current study, men had a significantly larger neck circumference than women and similar findings have been reported by Brodsky et al. Comparing the individual parameters in difficult airway assessment scoring, Modified Mallampati test had the highest sensitivity (61.1%) and head and neck movements had the highest specificity (95.5%). Predictive value and likelihood ratio were higher for upper lip bite test and head and neck movements had an equal score. Accuracy was highest for head and neck movements followed by Upper Lip Bite Test and RHTMD. This shows that no single test can be better in predicting difficult intubation. Various studies have also shown that a scoring system is better than individual parameters in predicting difficult intubation.[21] Hence a new scoring system for assessing difficult airway and intubation was developed by the authors. The new score, Difficult Airway Assessment score was designed with Modified Mallampati test, upper lip bite test, neck circumference, RHTMD (ratio of height to thyromental distance) and head and neck movements. The individual parameter scores were calculated in three categories as 0, 1 and 2 and the total score ranges from a minimum of 0 to a maximum of 8.

Difficult airway assessment score in predicting difficult intubation was assessed with various cut off scores. When the cut off was taken more than or equal to 2, the sensitivity was 94.4% but the specificity was low as 41.7%. PPV was very low of 18% with a high NPV of 98%. The likelihood ratio was 1.6 and the accuracy was moderate with 83.3%. This shows cut off ≥ 2 can identify almost all difficult intubations but many false positives are the drawback. When the cut off was taken more than or equal to 4, the sensitivity was moderate as 72.2% but the specificity was good of 92.4%. PPV was average with 56.5% with a high NPV of 96%. The likelihood ratio was very high of 9.5 and the accuracy was high with 90%. Thus with a cut off ≥ 4 can eliminate easy intubations as much as possible but with high false negatives. When the cut off was taken more than or equal to 3, the sensitivity and specificity was good with 88.9% and 82.6%. PPV was average with 41% with a high NPV of 98%. The likelihood ratio was good with 5.1 and the accuracy was 83.3%. Thus with a cut off ≥ 3 can identify difficult intubations as well as eliminate easy intubations to a maximum possible extent with least false negatives and false positives. ROC curve for determining the cut off score for predicting difficult intubation has been shown in the fig. which shows ≥ 3 is the best cut off in difficult airway assessment score for predicting difficult intubation with maximum sensitivity and specificity. The present study also evaluated the Wilson score for the same population. On comparison of Difficult airway assessment score (DAAS) with cut off of ≥ 3 with Wilson score, sensitivity of DAAS was very high 88.9% compared with 44.4% in Wilson score and specificity was also high with 82.6% compared to 70.8% in Wilson score. Positive predictive value was only 17.2% in Wilson score whereas it was 41% in DAAS. 98.2% NPV in DAAS score almost matches with the 90.3% NPV of Wilson score. The accuracy and likelihood ratio of DAAS outperformed Wilson score. Accuracy was 83.3% in DAAS compared to 67.67% in Wilson score. Likelihood ratio was 5.1 in DAAS compared to 1.52 in Wilson score. Thus it is very evident that the validity of the Difficult airway assessment score with cut off more than or equal to 3 is much better than Wilson score. Seo et al [15] designed a new score for predicting difficult intubation named Total Airway Score (TAS) which included the following factors: Mallampati classification, the thyromental distance, the head & neck movement, BMI, the severity of buck teeth, the inter incisor gap, and the ULBT. The predictive accuracy of TAS was 94.1% which was slightly higher than DAAS (83.3%). The sensitivity of TAS was low (69.4%) compared to DAAS but specificity was higher (97.4%). PPV was higher in TAS (78%) compared to 41% in DAAS and NPV almost matches between the two scores. Thus DAAS score has almost similar validity in predicting difficult intubation compared to TAS score. The five individual parameters taken in DAAS score had their own level of

predictive validity but when the 5 parameters are combined to form a Difficult Airway Assessment scoring system, and cut off score taken as ≥ 3 , it turned out to be a highly sensitive and specific predictor of difficult intubation and superior to Wilson score.

Conclusion

Modified Mallampati test, Upper Lip Bite Test, Ratio of Height to Thyromental Distance, Neck Circumference and Head and Neck Movements when used as an independent predictor for difficult intubation had its own predictive validity but failed to meet the criteria for an ideal predictive test. When these parameters were combined to derive Difficulty airway assessment score, the predictive accuracy was very much better compared to individual parameters.

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Effects of Intramuscular Dexmedetomidine as Premedication in Elective Laparoscopic Surgeries - A Prospective Randomized Controlled Study in Trichy, Tamilnadu

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ABSTRACT

BACKGROUND

The major concern of laparoscopic surgery is intra-operative hypercapnia induced stress response such as increase in heart rate (HR), increase in blood pressure (BP), increased stress hormones. The major concern of anaesthetist is to reduce stress response perioperatively. Drugs like clonidine, dexmedetomidine, nitroglycerine and esmolol are used to control the hemodynamic response associated with pneumoperitoneum in laparoscopic surgeries. Dexmedetomidine has been found to have hemodynamic stability with good analgesic effect. Dexmedetomidine is a highly selective α_2 agonist with sedative, analgesic and sympatholytic properties. Here in this prospective randomized controlled study, we evaluate the effects of intramuscular dexmedetomidine as a premedication in laparoscopic cholecystectomy.

METHODS

This is a randomized controlled study. Forty patients aged 20 to 50 years, both sexes, with American society of anaesthesiology (ASA) grade I & II planned for elective laparoscopic cholecystectomy were randomly assigned into two groups, Group DS : (N - 20) Received 2 mcg/kg of dexmedetomidine with normal saline (total 2 ml) Group CS : (N - 20) Received 2 ml of normal saline as intramuscular injection in the deltoid region 60 minutes before induction. We compared the hemodynamic parameters like pulse rate, mean arterial pressure (MAP) in baseline, preinduction, during intubation, before and after carbon dioxide insufflation, post extubation, visual analog score (VAS) and the analgesic requirements in both groups.

RESULTS

Compared to control group, intramuscular dexmedetomidine group had statistically significant reduction in pulse rate, mean arterial pressure perioperatively during intubation, before and after carbon dioxide insufflation, during surgery and post extubation ($P < 0.001$) and also found to decrease the analgesic requirement post operatively.

CONCLUSIONS

2 mcg/kg intramuscular dexmedetomidine premedication produces better hemodynamic stability, reduced perioperative analgesic requirement and hence could be a better alternative to other premedicant agents.

KEYWORDS

Dexmedetomidine, IM Premedication, Laparoscopy, Stress Response

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BACKGROUND

Nowadays many advanced techniques have been emerging in the field of surgery, from conventional procedure to minimal or less invasive surgeries like laparoscopy, arthroscopy, and endoscopic spine surgery. One among the best advances is laparoscopic surgery. Advantages are less blood loss, better wound healing, rapid return of gastrointestinal function, less post-operative respiratory complication, lesser duration of hospital stay and early mobilisation. Most of the hospitals are using carbon dioxide for creating pneumoperitoneum, which has its own advantage and disadvantage. The main advantages are it does not support combustion, and absorbed carbon dioxide is eliminated via respiration from the blood. The major disadvantage is hypercapnia, which can stimulate sympathetic nervous system causing hemodynamic changes like tachycardia, increased systemic and pulmonary vascular resistance and hypertension. It is of major concern in elderly people and patient with compromised cardiac status.

The hemodynamic disturbances in laparoscopic surgeries are not only due to pneumoperitoneum, but also with patient positioning, anaesthetic technique and hypercapnia. Higher intra-abdominal pressure will worsen the hemodynamic changes. The safest and most commonly used anaesthetic technique for laparoscopic surgery is general anaesthesia with controlled ventilation. Our aim is to reduce both the intubation and extubation stress response, as well as to alleviate the hemodynamic consequences of hypercapnia, pneumoperitoneum with the added advantage of post-operative pain relief. Drugs like clonidine, dexmedetomidine, nitroglycerine and esmolol are used to control the hemodynamic response associated with pneumoperitoneum in laparoscopic surgeries.

Dexmedetomidine is a highly selective α_2 agonist. It is 8 to 10 times more selective towards α_2 receptors than Clonidine. Stimulation of α_{2A} receptors in locus ceruleus inhibits nociceptive neurotransmission in the descending medullospinal noradrenergic pathway and causes analgesia.¹ Stimulation of post synaptic activation of α_{2A} receptors in the CNS causes sympatholytic effect leading to hypotension and bradycardia. Activation of α_{2C} receptor causes anxiolytic effect.² Dexmedetomidine also reduces the shivering threshold by 2°C .³

Many studies have been done using IV dexmedetomidine as premedicant in laparoscopic surgeries to attenuate the stress response associated with pneumoperitoneum and carbon dioxide induced hypercapnia and they have reported frequent episodes of bradycardia with IV dexmedetomidine.⁴⁻⁷ Hence, we formulated a new study to evaluate the hemodynamic effects of intramuscular dexmedetomidine.

Objectives

1. To determine the effects of intramuscular dexmedetomidine as a premedication in patients undergoing laparoscopic surgery on perioperative haemodynamics

2. To compare the perioperative analgesic requirements and pain score among patients undergoing laparoscopic surgery with intramuscular dexmedetomidine premedication and controls.

METHODS

The study was conducted in Trichy SRM Medical College Hospital and Research Centre, Irungalur, Trichy, Tamilnadu, India from June 2018 to February 2020. This study was a single centre, prospective, randomized, comparative and observer blinded study. We obtained the ethical committee approval from our institution and the reference number is 407 / TSRMMCH & RC / IEC – No 135. Written informed consent was obtained from the patients in their own language. Forty patients aged between 20 - 50 years with American society of anaesthesiology Grade I & II undergoing elective laparoscopic cholecystectomy were included in this study. Patients were excluded from the study if they had difficult airway, baseline heart rate less than 60 per minute, diabetes mellitus, cardiac disease, respiratory disease, neurological disorder, impairment of hepatic and renal functions.

All the study participants were shifted to the pre-operative room where the baseline pulse rate, blood pressure and saturation were noted. The patients were randomly allocated into two groups according to computer generated random numbers and group allocation was done using sealed envelope technique. Participants in Group DS received 2 mcg/kg dexmedetomidine with normal saline (total 2 ml) intramuscularly, and the participants in Group CS received 2 ml normal saline intramuscularly in the deltoid region 60 minutes before induction. To ensure blinding, the medical staff administering injection and the attending anaesthesiologist was unaware of the content of the drug.

Patients were preloaded with 500 ml Ringers lactate. After entering into operation theatre, ECG, pulse oximeter, non-invasive BP monitors were connected. Before induction pulse rate, systolic blood pressure, diastolic blood pressure and saturation were recorded. After preoxygenation with 100 percent oxygen for 3 minutes, patients in both groups were induced with inj. fentanyl 2 mcg/kg IV and inj. propofol 2 mg/kg IV slowly. After confirming the ability to mask ventilate, patients were paralysed with inj. vecuronium (0.08 mg/kg). Pulse rate, systolic blood pressure, diastolic blood pressure and saturation were again documented as post induction hemodynamic parameters. 3 minutes after giving vecuronium, tracheal intubation proceeded with the appropriate size endotracheal tube. ET tube position was confirmed with ETCO₂, equal bilateral air entry. Pulse rate, mean arterial blood pressure, saturation were noted 1 minute and 5 minutes after intubation.

Patients were ventilated with tidal volume of 8 ml per kg of ideal body weight and respiratory rate at 12 to 14 per minute. Anaesthesia was maintained with isoflurane (0.8 %) with nitrous oxide and oxygen mixture as 2 : 1 and inj. vecuronium 0.01 mg/kg intravenous intermittently. Intra-abdominal pressure was maintained less than 12 mmHg.

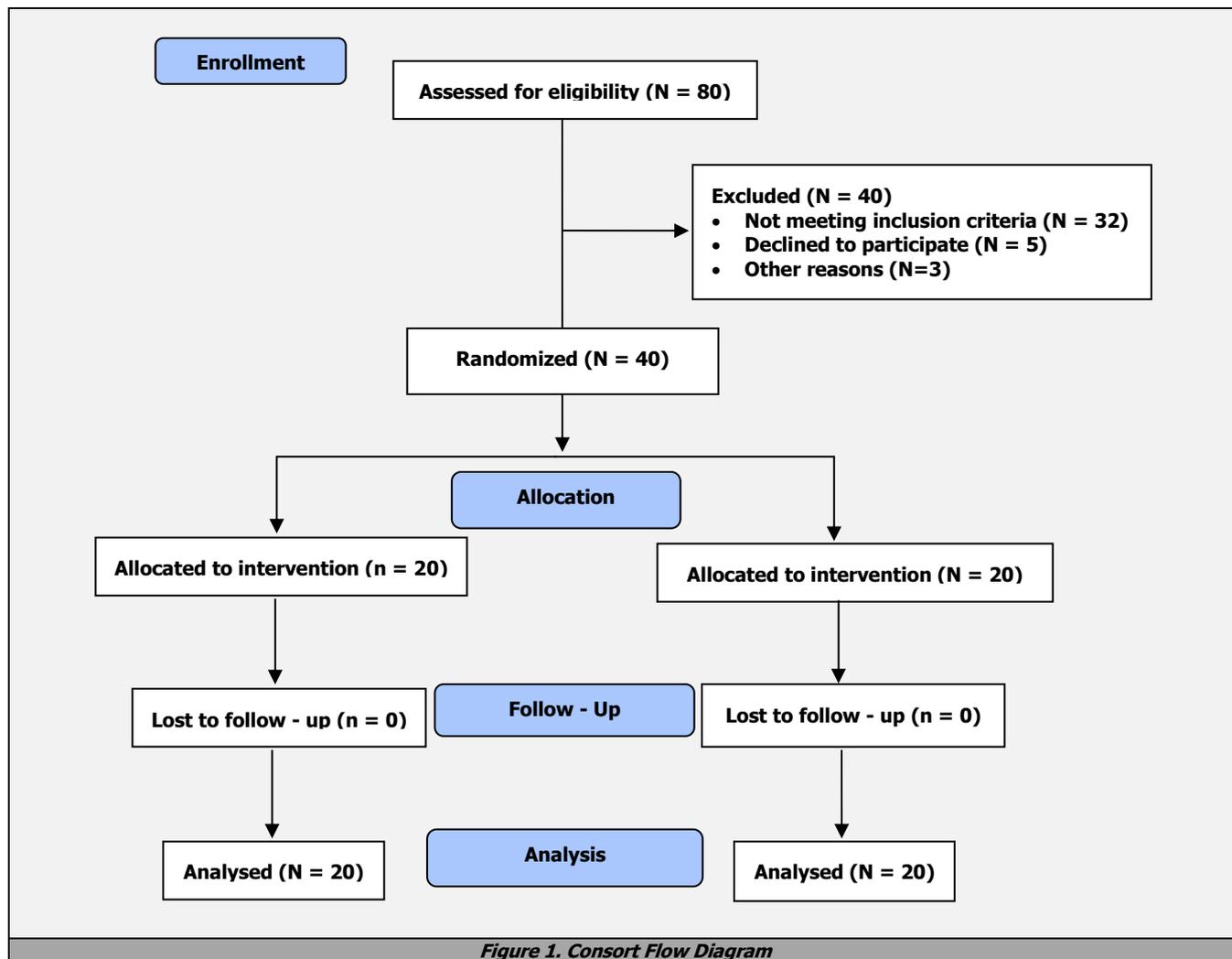


Figure 1. Consort Flow Diagram

Again, the pulse rate, mean arterial pressure were documented just before pneumoperitoneum, 5 min, 10 min, 20 min, 30 min, 45 min after pneumoperitoneum. Signs of inadequate anaesthesia (HR > 20 % and MAP > 20 % of preinduction values) were managed with inj. propofol 20 mg in titrated dose and bradycardia (< 50/min) was treated with Inj. glycopyrrolate 0.2 mg.

Other parameters such as requirement of propofol boluses, post-operative analgesic requirement, dryness of mouth, post-operative shivering were recorded. At the end of the surgery residual neuromuscular block was reversed with inj. neostigmine 50 mcg/kg with inj. glycopyrrolate 10 mcg/kg IV. After regaining protective reflexes and spontaneous respiratory effort, patient was extubated. Again, the pulse rate, mean arterial pressure was recorded 1 min, 5 min 15 min & 30 min after extubation.

Pain was assessed with visual analogue scoring system at the end of the surgery, 30 min, 60 min, 90 min, 2nd hour, 4th hour after surgery. Inj. paracetamol 15 mg/kg IV was given for the patients with VAS more than 5.

Statistical Analysis

According to Kalpana S Vora et al. study, considering a mean difference in reduction of heart rate between dexmedetomidine and saline group as 10 beats per minute ($\mu_1 - \mu_2$) and an average standard deviation of reduction of

heart rate in both groups as 10 beats per minute (σ), at 95 % confidence interval ($Z_{1-\alpha/2} = 1.96$) with 80 % power ($Z_{1-\beta} = 0.84$), the sample size is calculated as $N = Z_{1-\alpha/2} + Z_{1-\beta}^2 * 2 * \sigma^2 / (\mu_1 - \mu_2)^2 = (1.96 + 0.84)^2 * 2 * 10^2 / 10^2 = 16.8$. The sample size is rounded off to 20 per group and the total sample size is 40. Data was analyzed with SPSS version 21.

All data were expressed in mean \pm SD. The data were analysed by chi - square test and student's t - test. A P - value of < 0.05 was considered statistically significant.

RESULTS

| | Group DS | | Group CS | | P Value |
|-------------------------------|----------|-----------|-----------|-------|---------|
| | Mean | SD | Mean | SD | |
| Age (years) | 25.75 | 3.508 | 25.6 | 5.220 | 0.894 |
| Sex | Male | 12 (60 %) | 11 (55 %) | | |
| | Female | 8 (40 %) | 9 (45 %) | | |
| Weight (kg) | 53.9 | 3.575 | 53.55 | 5.196 | 0.833 |
| Duration of surgery (min) | 57.7 | 6.658 | 58.50 | 6.581 | 0.654 |
| Duration of anaesthesia (min) | 74.1 | 7.833 | 74.60 | 7.437 | 0.892 |

Table 1. Demographic and Descriptive Data

The distribution of age, sex, weight, duration of surgery and anaesthesia between the two groups were statistically not significant. (P >0.05) (Table 1) The duration of surgery is considered as the time between skin incision for port entry to skin closure in minutes. The duration of anaesthesia is

considered as the time between induction to extubation in minutes.

| Pulse Rate | Group DS | | Group CS | | P Value |
|-------------------------------|----------|-------|----------|-------|---------|
| | Mean | SD | Mean | SD | |
| Baseline | 82.85 | 5.102 | 83.45 | 4.430 | 0.712 |
| Pre induction | 71.50 | 4.425 | 89.90 | 1.940 | 0.001 |
| Post induction | 71.50 | 2.395 | 92.10 | 2.340 | 0.001 |
| 1 min after intubation | 87.60 | 2.644 | 120.45 | 6.262 | 0.001 |
| 5 min after intubation | 87.25 | 2.531 | 117.50 | 3.940 | 0.001 |
| Before pneumoperitoneum | 79.85 | 3.066 | 113.65 | 6.243 | 0.001 |
| 5 mins after pneumoperitoneum | 77.95 | 4.395 | 96.70 | 5.253 | 0.001 |
| 10 min after pneumoperitoneum | 75.05 | 4.651 | 92.10 | 2.337 | 0.001 |
| 20 min after pneumoperitoneum | 72.45 | 2.212 | 96.25 | 1.916 | 0.001 |
| 30 min after pneumoperitoneum | 71.80 | 1.852 | 95.55 | 1.852 | 0.001 |
| 45 min after pneumoperitoneum | 72.20 | 1.240 | 96.65 | 2.084 | 0.001 |
| 1 min after extubation | 92.05 | 3.663 | 120.50 | 3.317 | 0.001 |
| 5 min after extubation | 78.95 | 3.980 | 107.10 | 3.144 | 0.001 |
| 15 min after extubation | 75.25 | 2.074 | 104.31 | 5.868 | 0.001 |
| 30 min after extubation | 71.70 | 8.720 | 89.90 | 1.944 | 0.001 |

Table 2. Comparison of Perioperative Pulse Rate between the Two Groups

| MAP | Group DS | | Group CS | | P Value |
|-------------------------------|----------|-------|----------|-------|---------|
| | Mean | SD | Mean | SD | |
| Baseline | 89.45 | 4.499 | 94.45 | 3.901 | 0.902 |
| Pre induction | 95.25 | 5.385 | 99.10 | 1.210 | 0.038 |
| Post induction | 91.00 | 3.878 | 122.15 | 3.951 | 0.001 |
| 1 min after intubation | 90.65 | 5.410 | 118.00 | 3.554 | 0.001 |
| 5 min after intubation | 92.80 | 5.324 | 115.60 | 3.775 | 0.001 |
| Before pneumoperitoneum | 94.60 | 5.540 | 119.95 | 3.017 | 0.001 |
| 5 mins after pneumoperitoneum | 92.40 | 5.093 | 115.70 | 2.296 | 0.001 |
| 10 min after pneumoperitoneum | 91.25 | 5.500 | 111.20 | 1.436 | 0.001 |
| 20 min after pneumoperitoneum | 94.75 | 5.428 | 110.75 | 1.743 | 0.001 |
| 30 min after pneumoperitoneum | 103.00 | 5.437 | 112.80 | 5.167 | 0.001 |
| 45 min after pneumoperitoneum | 93.00 | 2.513 | 126.30 | 2.179 | 0.001 |
| 1 min after extubation | 87.40 | 5.331 | 107.02 | 1.170 | 0.001 |
| 5 min after extubation | 84.35 | 4.135 | 100.90 | 1.212 | 0.001 |
| 15 min after extubation | 89.45 | 3.083 | 99.10 | 1.210 | 0.001 |
| 30 min after extubation | 88.97 | 3.146 | 98.16 | 1.631 | 0.001 |

Table 3. Comparison of Perioperative Mean Arterial Pressure between the Two Groups

The pre-operative baseline hemodynamic variables of pulse rate and mean arterial pressure among the two groups were statistically not significant (P value - 0.902) (Table 2 & 3). Compared to control group intramuscular dexmedetomidine group had statistically significant reduction in heart rate, after premedication, preinduction (P – value - 0.001), post induction (P – value - 0.001), 1 minute (P – value - 0.001) and 5 minutes (P – value - 0.001) after intubation (P – value - 0.001), before pneumoperitoneum (P – value - 0.001), 5 min, 10 min, 20 min, 30 min, 45 min, after pneumoperitoneum (P – value - 0.001) and 1 min, 5 min, 15 min, 30 min after extubation (P – value - 0.001) and also 1st hour, 2nd hour, 4th hour in the post-operative period (P – value - 0.001). (Table 2 & 4)

Compared to control group, intramuscular dexmedetomidine group had statistically significant reduction in mean arterial pressure, after premedication, pre induction (P – value < 0.05), post induction (P – value - 0.001), 1 minute (P – value - 0.001) and 5 minutes (P – value - 0.001) after intubation (P – value - 0.001), before pneumoperitoneum (P – value - 0.001), 5 min, 10 min, 20 min, 30 min, 45 min, after pneumoperitoneum (P - value -

0.001) and 1 min, 5 min, 15 min, 30 min after extubation (P - value - 0.001) and also 1st hour, 2nd hour, 4th hour in the post-operative period (P – value - 0.001). (Table 3 & 5)

| Pulse Rate | Group DS | | Group CS | | P Value |
|----------------|----------|-------|----------|-------|---------|
| | Mean | SD | Mean | SD | |
| Post op 1 hour | 75.15 | 1.663 | 86.90 | 1.210 | 0.001 |
| 2 hours | 76.45 | 3.052 | 85.00 | 1.170 | 0.001 |
| 4 hours | 78.70 | 4.669 | 86.90 | 1.483 | 0.001 |

Table 4. Comparison of Post-Operative Pulse Rate between the Two Groups

| Mean Arterial Pressure | Group DS | | Group CS | | P - Value |
|------------------------|----------|-------|----------|-------|-----------|
| | Mean | SD | Mean | SD | |
| Post op 1 hour | 91.85 | 5.214 | 97.20 | 0.951 | 0.001 |
| 2 hours | 92.30 | 5.079 | 96.85 | 1.424 | 0.001 |
| 4 hours | 92.45 | 5.624 | 96.20 | 0.951 | 0.005 |

Table 5. Comparison of Post-Operative Mean Arterial Pressure between the Two Groups

| VAS Score | Group DS | | Group CS | | P - Value |
|----------------|----------|-------|----------|-------|-----------|
| | Mean | SD | Mean | SD | |
| End of surgery | 1.50 | 0.513 | 4.0 | 0.918 | 0.001 |
| 30 minutes | 3.70 | 1.129 | 5.35 | 1.268 | 0.001 |
| 60 minutes | 5.25 | 0.786 | 7.0 | 1.169 | 0.001 |
| 90 minutes | 6.10 | 1.124 | 6.8 | 0.768 | 0.027 |
| 2 hours | 6.56 | 0.843 | 6.86 | 0.610 | 0.205 |
| 4 hours | 6.72 | 1.104 | 6.75 | 0.842 | 0.923 |

Table 6. Comparison of Visual Analog Scale between the Two Groups

The visual analog score between dexmedetomidine group (Group DS) and control group (Group CS) was calculated, and it was found that dexmedetomidine group was statistically significant at the end of surgery, 30 min, 60 min, and 90 min postoperatively (P – value - 0.001). The visual analog score at 2nd hour and 4th hour post operatively was statistically not significant (P value > 0.05).

The frequency of rescue analgesic requirement was significantly higher in group CS. (Table - 6). In group CS, all patients required propofol bolus to control haemodynamics, but in Group DS, only 2 patients required propofol bolus. Four patients in the control group and none in the dexmedetomidine group experienced shivering postoperatively. All patients in group DS had significant dryness of mouth. In group DS, three patients had vomiting postoperatively, in group CS one patient had vomiting.

DISCUSSION

Dexmedetomidine is an alpha₂ - agonist that received FDA (Food and drug administration) approval in 1999. Dexmedetomidine is the dextro isomer of medetomidine. It produces good sedation without respiratory depressant effect. Dexmedetomidine is more selective alpha₂ – adrenoceptor agonist than clonidine. It is shorter-acting drug than clonidine. It has an elimination half-life of 2 to 3 hours and highly protein bound > 90 %. Dexmedetomidine is a weak inhibitor of cytochrome p450 enzyme. Because of this effect, when we use dexmedetomidine with opioids, it increases the plasma concentration of opioids. Dexmedetomidine’s sedative and cardiovascular effects are effectively reversed by atipamezole which is a selective and specific alpha 2 antagonist.

The mechanism for the sedative effect of dexmedetomidine differs from the sedative effects of drugs which acts on GABA receptors (such as barbiturates, benzodiazepines, propofol). Dexmedetomidine acts on alpha 2 receptors, reduces the sympathetic nervous system outflow and reduces the arousal response. So, the patient will be calm and easily arousable to full conscious state. But the intravenous anaesthetic agents like barbiturates, benzodiazepines, propofol activates the GABA receptors, increase the chloride influx which will produce the hyperpolarisation of post synaptic membrane. It makes the post synaptic membrane more resistant to excitation, results in anxiolysis, sedation, anticonvulsant effect and anterograde amnesia. So, during recovery it produces clouding of consciousness and patient may be in agitated state. It also causes tolerance and dependence. Post operatively, after extubation dexmedetomidine was found to keeps the patients calm and relaxed with no residual ventilatory depressant effect.

When used as premedication in general anaesthesia, dexmedetomidine reduces intubation stress response, reduces intraoperative anaesthetic requirements of inhaled anaesthetics and opioids, reduces the plasma concentrations of stress hormones.

Severe bradycardia and cardiac arrest were reported in a patient receiving intravenous dexmedetomidine infusion while giving general anaesthesia. Benzodiazepines has to be used cautiously with opioids, because it aggravates the ventilatory depressant effect of opioids especially in patients with poor pulmonary reserve. In that situation we can use dexmedetomidine as an alternative for them. It is used in perioperative period as sedative, analgesic, premedication, and as an anaesthetic adjunct for general as well as regional anaesthesia and also for post-operative sedative and analgesia.

So, we used dexmedetomidine, as an adjuvant for premedication since this drug possess sedative, anxiolytic, analgesic and sympatholytic properties with stable hemodynamic profile. It potentiates the anaesthetic effects of all intra-operatively used anaesthetics (intravenous, volatile or regional block). Since bradycardia and cardiac arrest were reported in some patients, receiving intravenous dexmedetomidine infusion, we preferred intramuscular route.

Cengiz kaya et al. found that 1 mcg/kg of intra muscular dexmedetomidine premedication reduced opioid requirement at induction and post-operative period without any changes in hemodynamic parameters.⁹ In our study 2 mcg/kg intramuscular dexmedetomidine premedication reduced anaesthetic and analgesic requirement in perioperative period.

Aho, M. scheini et al. compared the effects of three different doses of intramuscular dexmedetomidine (0.6, 1.2, 2.4 mcg/kg) with placebo in patients undergoing gynaecologic diagnostic laparoscopy. They found that maximum MAP and HR after intubation were lower in 2.4 mcg/kg group than placebo group.¹⁰

M.T. Taittonen et al. studied the effects of clonidine 4 mcg/kg, dexmedetomidine 2.5 mcg/kg intramuscular premedication in elective plastic surgical procedures. They

found that dexmedetomidine group had 18 % maximum reduction in HR, 11 % maximum reduction in systolic arterial pressure, and 15 % maximum reduction in diastolic arterial pressure.¹¹ Aantaa R, et al. compared the effects of Dexmedetomidine 1 mcg/kg intramuscular and inj. midazolam 0.08 mg/kg intramuscular premedication for minor gynaecological surgery. They found that dexmedetomidine group had marked reduction in MAP (20 %) and heart rate (15 %).¹²

Virkkila et al. compared the five different doses of 0.25, 0.5, 0.75, 1.0, 1.5 mcg/kg dexmedetomidine intramuscularly in cataract surgery under regional block. They detected 1 mcg/kg of intramuscular dexmedetomidine produced a 32 % reduction of intra ocular pressure without significant hemodynamic change.¹³

MC Jaakola et al. demonstrated the intubation stress response with intramuscular dexmedetomidine 2.5 mcg/kg and saline placebo intravenously with inj. midazolam 0.08 mg/kg intramuscularly with inj. fentanyl intravenously. They found that hemodynamic changes following intubation was statistically not significant between the two groups.¹⁴

In our study intramuscular dexmedetomidine premedication 2 mcg/kg reduces the stress response, significant reduction in heart rate, mean arterial pressure during and after intubation, also during extubation, during the pneumoperitoneum and also in the post-operative period.

CONCLUSIONS

2 mcg/kg of intramuscular dexmedetomidine appears to be an effective premedicant to attenuate the stress induced haemodynamic changes, and is also effective in decreasing the intraoperative anaesthetic and post-operative analgesic requirements and hence it could be a better alternative to other premedicant agents.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

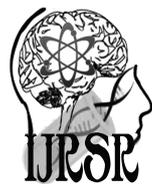
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Research Article

EFFICACY OF PREOPERATIVE GABAPENTIN IN PRODUCING POST-OPERATIVE ANALGESIA IN PATIENTS UNDERGOING ELECTIVE SURGERY UNDER GENERAL ANESTHESIA: A RANDOMIZED DOUBLE BLINDED TRIAL

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ABSTRACT

Gabapentin is an adjuvant antiepileptic agent with analgesic properties used for the treatment of postoperative pain. The primary objective of this study is to assess and compare the analgesic effects of administering Gabapentin and Paracetamol preoperatively, in patients undergoing elective surgery, under General Anesthesia. The study was a single-center, randomized and double-blind trial. A total of 60 patients of either sex, age >18 years, with American Society of Anesthesiology (ASA) status I/II undergoing elective ENT surgery, under General Anesthesia at a tertiary care hospital. The patients were randomized into two groups (each 30) - Group 1 received oral Paracetamol and Group 2 received oral Gabapentin, preoperatively. In this study, comparison of the duration of analgesia provided by a single dose of 600mg of Gabapentin over 650mg of Paracetamol, consumption of postoperative rescue analgesia and the incidence of side effects between both groups were assessed. The mean duration of analgesia produced by Paracetamol and Gabapentin was 83 and 74.5 minutes respectively. The mean analgesic consumption of Paracetamol and Gabapentin was 53.0 and 51.7 mg of Tramadol respectively. The incidence of adverse effects like nausea, vomiting, dizziness and headache were comparable but statistically not significant. Both Paracetamol and Gabapentin were comparable in terms of potency as pre-emptive analgesic. This study has shown that the time to first rescue analgesia, total amount of rescue analgesia between the two groups in the first 6 hours following surgery were not statistically significant.

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INTRODUCTION

Inadequate pain relief in the post-operative phase is a well-known problem world-wide. The incidence of post-operative pain has been found to be between 25%-76%¹. From review of literature it is seen that many patients still suffer from moderate to severe postoperative pain despite an increased focus on multimodal pain management¹. Aside from the suffering caused by insufficient pain relief, this is an issue with potential adverse physiological and psychological consequences for patients².

Patients may anticipate future medical interventions with greater anxiety if pain has not been managed effectively in the past³. In the earlier periods analgesia was restricted to surgical and postoperative period. However, this was associated with lots of morbidity to the patient in terms of surgical stress and increased requirements for analgesics in the post-operative

period which were associated with various adverse effects⁴. Pre-emptive analgesia is a treatment that is initiated before and is operational during the surgical procedure in order to reduce the physiological consequences of nociceptive transmission provoked by the procedure.

Owing to this protective effect on the nociceptive pathways, pre-emptive analgesia has the potential to be more effective than a similar analgesic treatment initiated after surgery⁵. Consequently, immediate postoperative pain may be reduced and the development of chronic pain may be prevented⁶. Gabapentin is an analogue of GABA (gamma amino butyric acid) which was initially used as an anti-epileptic, and later it was tried out in diabetic neuropathy and chronic pain⁷. Of late there is increased evidence suggesting their efficacy as an anxiolytic, for attenuation of pressor response during laryngoscopy and intubation, postoperative analgesia, and for prevention of postoperative nausea and vomiting⁸.

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Paracetamol is a well-established analgesic agent used as breakthrough analgesic⁹. Various studies have been done regarding its potency as a preemptive analgesic. The purpose of our study was to analyze the pre-emptive analgesic efficacy of gabapentin compared to that of Paracetamol. Several dosage regime of gabapentin has been tried in different studies and found to be of varying effect in postoperative analgesia. We have compared the efficacy of 600mg of gabapentin with 650 mg of Paracetamol. To assess the efficacy of preoperative gabapentin in providing adequate postoperative analgesia compared to preoperative paracetamol.

MATERIALS AND METHODS

This study is a randomized double-blind study, which was conducted at the department of Anesthesiology, Tertiary care teaching hospital, South India. After the approval of Institutional Ethical Committee and obtaining informed consent, the study was conducted on 60 patients, divided into two groups of both gender, aged >18 years, with Grading of American Society of Anesthesiology (ASA) status I/II undergoing elective ENT surgery under general anaesthesia, in this Institution were included in this study. The patients with ASA III, IV, V and Emergency surgeries, renal dysfunction, known allergy and prior usage of Gabapentin were excluded from this study. The patients were randomized into two groups of 30 patients each by simple random sampling method. They were also explained about Visual Analogue Scale for the assessment of pain the day before the surgery.

Blinding

The study medications were numbered according to random number table method. Participants was also assigned numbers according to random number table method and added to their group. One group was given Tab. Gabapentin 600mg 1 hour before surgery and the other group was given Tab. Paracetamol 650mg 1hour before surgery. All patients were premedicated with lorazepam 1mg on the night before and morning of surgery. On table they were medicated with Inj. Fentanyl 1.5mcg/kg, inj. Glycopyrolate 10mcg/kg and induced with inj. Thiopental 3- 5mg/kg iv and inj. Vecuromium 0.1 mg/kg iv, and maintained with O₂, N₂O and volatile anaesthetics and Vecuronium. About 20 minutes prior to extubation inj. Ondansetron 4mg iv was given. After the surgery they were reversed with inj. Neostigmine 60mcg/kg iv and Inj. Glycopyrolate 10mcg/kg iv. The patients were then shifted to recovery room.

Parameters studied

Post operatively the patients were examined at half hourly interval for first 6 hours for the presence of pain and pain was graded using visual analogue scale. If pain grading >4 or if moderate, inj. Tramadol 1mg/kg IV was given as rescue analgesic and noted. Any adverse effect such as dizziness, vomiting, nausea was also noted at half hourly interval for first six hours following surgery. Total dosage and frequency of tramadol in the 6-hour period was also calculated. Use of antiemetics was noted.

RESULTS AND DISCUSSION

In our study, we have compared the postoperative analgesic efficacy of 300mg of oral gabapentin given one hour prior to surgery compared with 650mg of oral paracetamol given one hour prior to surgery. Study population consists of 60 patients who underwent surgery under general anaesthesia. Group 1 had 30 patients who were given oral Paracetamol one hour prior to surgery. Group 2 had 30 patients who were given oral gabapentin one hour prior to surgery.

Patient demographic details including age, gender, weight and ASA grades were also recorded and depicted as not-significant (Table 1). The mean duration of surgery between the groups and mean duration of the analgesia produced by paracetamol and gabapentin were found to be in same range, and the P value is statistically insignificant.

Table 1 Mean demographic details of the study groups

| Characteristic module | Group 1 – Paracetamol (n=30) - Mean±SD | Group 2 – Gabapentin (n=30) - Mean±SD | p value |
|------------------------------------|--|---------------------------------------|-----------|
| Age (in years) | 40.53±15.6 | 36.2±14.9 | 0.95 (NS) |
| Gender - Male | 17 (56.7) | 17 (56.7) | - |
| Gender - Female | 13 (43.3) | 13 (43.3) | - |
| Weight (in kgs) | 56.1±7.4 | 54.9±8.1 | 0.56 (NS) |
| Duration of analgesia (in minutes) | 83±43.6 | 74.5±46.7 | |
| Duration of surgery (in minutes) | 148.5±31.0 | 154.8±35.9 | 0.32 (NS) |

[Figure in parenthesis denoted percentage; NS – Not-significant]

The types of surgery done were also recorded and impregnated in figure 1. The analgesic consumption between paracetamol and gabapentin were found to be in similar range and not statistically significant (Table 2).

Table 2 Comparison of analgesic consumption between the groups

| Group description | Analgesic consumption (mg) Tramadol IV - Mean±SD | p value |
|------------------------------|--|---------|
| Group 1 – Paracetamol (n=30) | 53.0±7.0 | 0.627 |
| Group 2 – Gabapentin (n=30) | 51.7±7.5 | (NS) |

Preoperatively patient's heart rate and blood pressure were recorded. In the postoperative period, all the patients were subjected for clinical observation and thereby they all were examined at half hourly interval for first six hours for pain, nausea, vomiting, headache and dizziness (Figure 2). Pain was graded with VAS and if more than or equal to 4, rescue analgesia was given. Post-operative VAS between both the groups were comparable during the first 6 hours. In case of antiemetic consumption (to control nausea and vomiting), Ondansetron 4mg was given intravenously (Table 3).

Table 3 Comparison of Ondansetron consumption among the groups

| Drug group | Ondansetron | | p value |
|---------------------|-------------|----|---------|
| | Yes | No | |
| Group I Paracetamol | 8 | 22 | 0.76 |
| Group II Gabapentin | 7 | 23 | |

Post-operative pain is a major cause of morbidity in the postoperative period. Pain control in the post-operative period is essential for improving gastrointestinal, respiratory function, psychological outcome of a patient and also patient's anticipation of future surgeries³. Preemptive analgesia helps to prevent the sensitization of neurons both peripherally and centrally. This not only helps in better analgesia but also helps to prevent unwanted adverse effects associated with commonly used breakthrough analgesics such as opioids and NSAIDs. Post-operative pain increases stress hormone release and cause negative nitrogen balance which eventually weakens the immune system⁶.

This study was designed with the aim of assessing the preemptive analgesic efficacy of oral gabapentin in post-operative period compared with Paracetamol which is an established preemptive and breakthrough analgesic. We also studied the adverse effects of both groups. Opioids form the mainstay of post-operative pain management, but they have adverse effects like respiratory depression, pruritis, nausea, vomiting, constipation, tolerance. Gabapentin is a GABA analogue earlier used as an antiepileptic, later found to be beneficial in neuropathic pain. Despite its functional similarities with GABA, they don't act via mechanisms related to GABA. Their exact mechanism of action is not understood. Proposed mechanisms include ability to increase concentration and rate of synthesis of GABA in brain, binding with high affinity to alpha binding site in brain tissues associated with voltage sensitive calcium channels. It has antihyperalgesic and antiallodynia properties¹⁰. Paracetamol is a centrally acting inhibitor of COX. The drug was first found to be associated with suppression of prostaglandin synthesis through inhibition of cyclooxygenase enzyme. Along with this, it also inhibits the descending serotonergic pain pathway¹¹.

Duration of analgesia

The mean duration of analgesia produced by paracetamol was 83 minutes; whereas the mean duration of analgesia produced by gabapentin was 74.48 minutes. This was not found to be statistically significant between the groups. The duration of first breakthrough analgesia for preoperative gabapentin in a study by syal *et al* where 1200mg of gabapentin was used was 332 minutes. The duration of analgesia has not been studied previously with 600mg in any trial.

The difference in the number of rescue analgesic consumption was very significantly reduced in patients who consumed Gabapentin (Group 2) and the duration of analgesia produced by preoperative Paracetamol was consistent¹²

Mean analgesic consumption

The mean analgesic consumption of group 1 which received Paracetamol was 53.0 mg of tramadol, whereas the mean analgesic consumption of group 2 which received gabapentin was 51.66 mg of Tramadol. This finding is not consistent with the previous studies comparing the analgesic consumption between Gabapentin and Paracetamol.

Incidence of adverse effects

The incidence of headache in Paracetamol group is 1 among 30 patients which is negligible. And the incidence of headache in gabapentin group is 4 among 30. This effect has not been

studied in the previous studies. The incidence of dizziness among patients who received Paracetamol is 1 out of 30 and incidence among patients who received gabapentin is 5 out of 30. There is a mildly significant difference in the incidence of dizziness which is comparable with another study¹³ where 1200mg of gabapentin was given one hour prior to surgery and the incidence of dizziness was 6 among 25 patients.

The incidence of nausea in Paracetamol group is 8 among 30 and incidence in gabapentin group is 7 among 30. This result was consistent with the study by Dirks *et al* where the incidence was 5 among 25 patients who received 1200mg of gabapentin one hour prior to surgery. The incidence of vomiting in Paracetamol group is 2 among 30 patients and in gabapentin group is 3 among 30 patients. This result is comparable with another study where the incidence of vomiting was 1 among 25 patients who received 1200mg of gabapentin one hour prior to surgery^{14,15}.

The incidence of antiemetic use in Paracetamol group is 8 among 30 and with gabapentin group are 7 among 30. There hasn't been much difference between the groups in our study which is comparable with another study^{16,17} which had an incidence of 9 among 25 patients.

CONCLUSION

Both Paracetamol and Gabapentin had comparable potency as pre-emptive analgesic. Our study has shown that the time to first rescue analgesia, total amount of rescue analgesia between the two groups in the first 6 hours following surgery were not statistically significant. Moreover, the incidence of adverse effects like nausea, vomiting, dizziness and headache were comparable also comparable between two groups.

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A Study on C-reactive Protein and Liver Function Tests in Laboratory RT-PCR Positive Covid-19 Patients in a Tertiary Care Centre – A Retrospective Study

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ABSTRACT

Background: COVID-19 pandemic has been one of the greatest challenges to the global healthcare system. Although the respiratory system is the main target of SARS-CoV-2 infection; other organs, exposure to the viral infection might also be a concern for COVID-19 affected patients especially the cardiovascular system and liver.

Objective: To know the status of C-reactive protein (CRP) and Liver Function Tests (LFT) in Covid-19 positive patients before initiating any treatment in a tertiary care hospital.

Methods: Age and sex-matched 40 cases were taken for the study who were hospitalized and COVID-19 infection had been confirmed by real-time RT PCR for COVID-19. Patients with a previous history of liver illness, renal disorders, chronic inflammatory conditions, malignancy and autoimmune disorders were excluded from the study.

Results: Almost all the liver enzymes were higher than the normal levels as seen in aspartate transaminase (35%), alanine transaminase (22.5%), alkaline phosphatase (20%), and gamma-glutamyl transaminase (35%). And whenever the protein, especially albumin was low there was an increased value of CRP and correspondingly with increased total and direct bilirubin levels.

Conclusion: In our study liver function test was altered even before starting any treatment for SARS-CoV-2 indicates that LFT can be a tool to assess multiorgan involvement whenever the patient is going for complication or cytokine storm by doing serial measurements of liver function.

Key Words: C-reactive protein (CRP), Liver Function Tests (LFT), Severe acute respiratory symptom (SARS-CoV-2)

INTRODUCTION

COVID-19 pandemic has been one of the greatest challenges to the global healthcare system. People with Diabetes, Hypertension, coronary artery disease and the older population get affected more and the course of illness also severe.^{1,2} Although the respiratory system is the main target of SARS-CoV-2 infection; damages also may occur in other multiple organs. The liver is an important organ in the body and its exposure to viral infection might also be a concern for COVID-19 affected patients. Up to now, there are a few pieces of

evidence that the hepatic cells are exposed to SARS-CoV-2 in severe cases also it remains unclear that at what extent liver diseases are considerable risk factors of severity and mortality.

As it has been observed with a similar coronavirus, SARS, liver derangement is also an emerging concern with COVID-19. According to many studies with respiratory viral infection in the past, about 60% of patients had a liver impairment as was shown by liver biopsy.³⁻⁵ Almost all the studies researchers noted that it might have been the result of drug-induced liver

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damage, especially of antibiotics, hepatotoxic antiviral drugs and steroids are given to the majority of the patients. The liver because of ACE2 expression in this site is prone to injury as a result of infection with SARS-CoV-2.

There were many studies have reported that laboratory investigations, results and clinical features suggestive of liver dysfunction in patients with COVID-19 infection.⁶ But in most of these studies, the pre-existing liver conditions have not been listed out and the pre-existing liver disease with COVID-19 has not been investigated thoroughly and underlying causes of liver injury in the severe liver disease, which are major limitations. However, it has been stated that decreased levels of albumin and Platelet count and increased levels of alanine aminotransferase (ALT) showed an association with higher mortality rates in COVID-19 patients.^{2,7,8}

It is still unknown that whether pre-existing liver diseases in severe patients, these laboratory tests are an indicator of liver failure caused by the SARS-CoV-2 itself or there is an over-reaction of the immune system which may cause progression and lead to hepatic injury.^{1,9-11} Our study is to know the status of C-Reactive Protein (CRP) and Liver Function Tests in Covid-19 laboratory positive patients before initiating any treatment in a tertiary care hospital.

MATERIALS AND METHODS

It was a retrospective observational study. After ethical clearance by our Institutional Review Board and Institutional Ethical Committee. Age and sex-matched 40 cases were taken for the study who were hospitalized and COVID-19 infection had been confirmed by TrueNat testing by real-time RT PCR for COVID-19. Patients with a previous history of liver illness, renal disorders, chronic inflammatory conditions, malignancy and autoimmune disorders were excluded from the study. The first blood sample was collected as a routine before starting treatment for the analysis of liver function and C-Reactive Protein along with other tests, was done in Cobas c-311 auto analyzer and the estimated values were recorded taken for the study.

Statistical Analysis

Data was entered into the MS Excel 2007 version and further analyzed using the SPSS version 20. For descriptive analyses, the categorical variables were analyzed by using percentages and the continuous variables by calculating mean \pm Standard Deviation. Regression analysis was applied to numerical data. $p < 0.05$ was considered as statistically significant.

RESULTS

In our study population >85% were more than 35 years with male predominance (>70%). Almost all the liver enzymes were

higher than the normal levels as seen in aspartate transaminase (AST- 35%), alanine transaminase (ALT-22.5%), alkaline phosphatase (ALP-20%), and gamma-glutamyl transaminase (GGT-35%). And whenever the protein, especially Albumin was low there was an increased value of CRP showing the liver injury and also correspondingly with total and direct bilirubin levels (Tables 1-3).

DISCUSSION

COVID-19 infection predominantly causes pulmonary symptoms, but simultaneously affects other organs too, such as Cardiac, Renal and Liver. 14-53% of COVID-19 patients developed hepatic dysfunction, particularly those with severe COVID-19. In critically ill patients hepatic dysfunction was significantly higher and has been associated with poor outcome. Liver dysfunction can be assessed for liver cellular integrity (e.g. ALT and AST), synthetic function (e.g. albumin), or biliary canaliculi (e.g. ALP and GGT).¹² Recent studies on SARS-CoV-2 clinical features have revealed that the liver enzyme abnormalities are common, but not as the prominent feature of this illness.¹³

Many researchers reported that increased CRP is an observed clinical characteristic of most patients with COVID-19 infection. CRP levels were positively correlated with the severity of the disease according to the diameter of lung involvement.¹³ Through the recent pandemic of SARS-CoV-2, liver dysfunction in many varieties has been reported in many cases. Liver test abnormality at the admission time can be used as a predictor for the severity of the disease.^{14,15}

Hepatotoxicity is a common adverse event that could occur during clinical practice because of the systemic toxicity of drugs and chemicals.¹⁶ Drugs that are used for the treatment of COVID-19 patients, like antivirals, steroids, and antibiotics, potentially damage the liver, but still was not evident.¹⁷ Cai Q et al observed in their study that an increase of liver enzymes could be due to drugs used for the treatment for Covid-19 patients, sepsis, and shock.¹⁵ However, for patients who were hospitalized, needed more attention for any drug-induced liver damage, hence our study was conducted in patients who got admitted and the first sample was sent for the analysis to assess the status of inflammation and liver insult before initiating any treatment after real-time RT-PCR confirmation of COVID-19.

Incidence of liver damage in severe cases of COVID-19 with higher levels of total bilirubin ALT, AST, LDH, CRP, D-dimer and lower albumin indicating the severity of the disease is reported.¹⁸ Moreover, a study by Yang F, et al. on deceased cases of COVID-19 with liver abnormalities, it was reported that patients with severe complications enzymes like ALT, AST, and also Bilirubin, total and conjugated bilirubin, which is consistent with our study also the albumin levels

were also reported lower as in our study.¹⁹ Hypoalbuminemia could be a result of inadequate nutritional intake and over-consumption during hospitalization²⁰ but in our study, it is evident that even during admission there is lower albumin levels and albumin globulin ratio, which was statistically significant ($p < 0.001$). It has been observed that in our study whenever there is an increase of 1 unit of conjugated bilirubin (direct) there was a 45.6 units rise of CRP indicating that the severity of illness and liver insult was even before starting any treatment likewise 1 unit increase of protein -5.8 unit decrease in CRP (down trending levels).

CONCLUSION

The liver because of ACE2 expression prone to cholangial tissue injury as a result of infection with SARS-CoV-2 and its mediators of inflammation. In our study liver function test was altered even before starting any treatment for SARS-CoV-2 indicates that LFT can be a tool to assess multiorgan involvement whenever the patient is going for complication or cytokine storm by doing serial measurements of liver function. We suggest from our observation that LFT may be performed along with the other markers to know the severity as well as the prognosis and for a better outcome of the patients.

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Limitations: Small sample size and liver function test panel before covid infection not available to compare.

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Table 1: Frequency distribution of characteristics of the patients with covid-19

| Indicators Variable | Frequency (N) | Percentage (%) | |
|----------------------------------|---------------------|----------------|------|
| Age | <35 years | 5 | 12.5 |
| | >35 years | 35 | 87.5 |
| Sex | Male | 29 | 72.5 |
| | Female | 11 | 27.5 |
| Serum total bilirubin(mg/dl) | Low (<0.3) | 6 | 15 |
| | Normal (0.3 to 1.3) | 33 | 82.5 |
| | High (>1.3) | 1 | 2.5 |
| Serum Direct bilirubin (mg/dl) | Low (<0.1) | 7 | 17.5 |
| | Normal (0.1 to 0.4) | 28 | 70 |
| | High(>0.4) | 5 | 12.5 |
| Serum Indirect bilirubin (mg/dl) | Low (<0.2) | 12 | 30 |
| | Normal (0.2 to 0.9) | 27 | 67.5 |
| | High (>0.9) | 1 | 2.5 |
| Serum AST (U/L) | Low (<12) | 0 | 0% |
| | Normal (12 to 38) | 26 | 65 |
| | High (>38) | 14 | 35 |
| Serum ALT (U/L) | Low (<7) | 0 | 0% |
| | Normal (7 to 41) | 31 | 77.5 |
| | High (>41) | 9 | 22.5 |
| Serum ALP (U/L) | Low (<33) | 1 | 2.5 |
| | Normal (33 to 96) | 31 | 77.5 |
| | High (>96) | 8 | 20 |
| Serum GGT (U/L) | Low (<9) | 0 | 0% |
| | Normal (9 to 58) | 26 | 65 |
| | High (>58) | 14 | 35 |

Table 2: Bilirubin and Protein with CRP (applying Regression analysis)

| Variable | CRP |
|----------------------------|-----------------------------|
| 1 unit increase in TB | 4.6 units increase in CRP |
| 1 unit increase in DB | 45.6 units increase in CRP |
| 1 unit increase in IB | -5.6 units decrease in CRP |
| 1 unit increase in PROTEIN | - 5.8 units decrease in CRP |

Table 3: CRP withAlbumin, globulin, AG ratio and AST (Applying Regression analysis)

| Variable | Regression Co-efficient value | 95% Confidence intervals | | 'p' Value |
|------------|-------------------------------|--------------------------|--------|-----------|
| | | Lower | Upper | |
| CRP | | | | |
| ALBUMIN | -116.3 | -175.4 | -57.1 | *<0.001 |
| GLOBULIN | 53.8 | 4.6 | 103.1 | *0.033 |
| AG | -282.8 | -405.8 | -159.9 | *<0.001 |
| AST | -0.2 | -0.8 | 0.5 | 0.620 |

(*p<0.05 is Statistically Significant)

Short communication

Association of serum adenosine deaminase level with atherosclerotic index in type 2 diabetes mellitus patientsRasheed Khan M.¹, Vinod Babu S.², Kuzhandai Velu V.³¹Assistant Professor, Department of Biochemistry, Trichy SRM Medical College, Hospital and Research Centre, Irungalur, Trichy, 621105, Tamil Nadu, India²Associate Professor, ³Assistant Professor, Department of Biochemistry, Mahatma Gandhi Medical College and Research Institute, SBV, Pillaiyarkuppam, 607 402, Pondicherry, India*(Received: October 2020 Revised: February 2021 Accepted: February 2021)*Corresponding author: **Vinod Babu S.** Email: drvinodbabu@gmail.com**ABSTRACT**

Introduction and Aim: The atherosclerosis is the major cause of morbidity and mortality among diabetes population. Diabetes mellitus can accelerate atherosclerotic processes. Adenosine deaminase (ADA) plays a significant role in both glucose and lipid metabolism through adenosine. This study aimed to correlate the atherosclerotic index with adenosine deaminase levels in Type 2 diabetes mellitus patients. The aim of the study is to find the association between serum ADA levels with atherosclerotic index.

Materials and Methods: A cross sectional study conducted in 100 subjects (50 control and 50 T2DM patients). The following biochemical parameter were estimated: total cholesterol, triacylglycerol, HDL- C and ADA. VLDL, LDL and other atherosclerotic index were calculated using formulae. Statistical analysis such as Student's 't' test and Pearson's correlation were performed.

Results: We found significant increase (p value <0.001) in lipid profile, Non-HDL-C and lipid ratio when compared to T2DM with control group. The correlation of serum ADA with lipid profile and lipid ratio did not show any correlation.

Conclusion: Serum ADA used as a biomarker for evaluation of glycemic status. ADA was insignificant, when correlated with dyslipidemia and atherosclerotic index.

Keywords: ADA; atherosclerotic index; T2DM; lipid profile.

INTRODUCTION

Diabetes is a disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production (1). Prevalence of Type 2 Diabetes Mellitus (T2DM) is a global public health threat (2-4). The metabolic disturbance causes secondary pathophysiologic changes in multiple organ systems. This imposes a tremendous burden on the individual as well as health care system of both developed and developing countries.

Hyperlipidemia characterized by hypertriglyceridemia, increased LDL, decreased HDL, high apolipoprotein B and low apolipoprotein A-1 (5). Literature indicates that lipid accumulation also represents a chronic inflammatory process (6,7).

Adenosine acts on glucose and lipid metabolism, will be similar to insulin action. By reduces free fatty acid levels and increases sensitivity of insulin. As ADA enzyme regulate bioactivity of insulin and decreasing glucose uptake by inhibiting adenosine (8-10). ADA increases lipolysis that leading to insulin resistance. We found there is association of adenosine with insulin and lipid metabolism this study was undertaken to evaluate the role of ADA in T2DM and to examine its correlation with lipid profile that will

help us in early detection of cardiovascular complication.

MATERIALS AND METHODS

A cross-sectional study conducted for a period of one year from 2013- '14, in the Department of Internal Medicine and Department of Biochemistry at Narayana General Hospital, Nellore. 100 Subjects were selected with the age group between 35 -70 years, divided into two groups, 50 subjects diagnosed with T2DM and 50 healthy subjects served as control.

They were included based on the following criteria: Known diabetic patients for at least one-year duration with normal hepatic function. Subjects are excluded based on the following criteria: any chronic diseases and any associated complications with diabetes. Patients taking drugs for thyroid disorder or any corticosteroids, lipid-lowering drugs, oral contraceptives, aspirin, sulphonamides and pregnant women were also excluded.

Five ml blood sample was collected in fasting condition from each subject, and it was transferred into the grey tube for glucose analysis, lavender tube for HBA1c and plain red tube for ADA and Lipid Profile (TC, TG, HDL).

For statistical analysis data was collected and entered

using Microsoft Excel™. The mean and standard deviation were calculated for all the Biochemical parameters. The significance between the groups was determined using Student t-test for equality of mean. The p value of < 0.05 was considered significant.

RESULTS

The mean age was approximately 51 year in both the groups. Out of 100 subjects, 43 (86%) males and 7 (14%) females were in T2DM group and 39 (78%) and 11 (22%) were in control group. There was a significant increase (p value < 0.001) in glycemc

index (FBS, PPBS, and HbA1c), as shown in Table 1.

We found significant increase (p value <0.001) in lipid profile (TC, TGL, HDL, LDL, and VLDL), Non-HDL-C and lipid ratio (LDL/HDL, TC/TG, TG/HDL and Non-HDL/HDL) when compare T2DM with control group which has been depicted in Table 1. The correlation of serum ADA with lipid profile and lipid ratio (Table. 2) didn't show any correlation within the groups of T2DM and control groups.

Table 1: Base line and biochemistry characteristics of study and control group

| Variables | T2DM | Control | p Value |
|-----------------|---------------|---------------|---------------------|
| Age (years) | 52.96±7.951 | 50.34±8.494 | 0.115 |
| FBS (mg/dL) | 193.02±56.008 | 90.52±11.682 | < .001 ^a |
| PPBS (mg/dL) | 243.5±40.991 | 137.44±7.234 | < .001 ^a |
| HbA1c (%) | 8.334±1.952 | 4.764±0.406 | < .001 ^a |
| ADA (U/L) | 34.92±2.522 | 14.1±2.613 | < .001 ^a |
| TC (mg/dL) | 223.94±39.991 | 183.72±40.501 | < .001 ^a |
| TG (mg/dL) | 182.86±56.272 | 115.88±36.976 | < .001 ^a |
| HDL (mg/dL) | 42.62±8.696 | 51.86±10.359 | < .001 ^a |
| LDL (mg/dL) | 145.32±40.851 | 108.5±32.222 | < .001 ^a |
| VLDL (mg/dL) | 36.572±11.254 | 23.176±7.395 | < .001 ^a |
| Non-HDL (mg/dL) | 181.32±42.681 | 131.86±34.675 | < .001 ^a |
| LDL/HDL | 3.637±1.597 | 2.123±0.607 | < .001 ^a |
| TC/TGL | 1.319±0.392 | 1.723±0.581 | < .001 ^a |
| TG/HDL | 4.449±1.467 | 2.286±0.769 | < .001 ^a |
| Non-HDL/HDL | 4.512±1.737 | 2.584±0.656 | < .001 ^a |

Note: Student's t-test. ^a Levene's test is significant (p < .05), suggesting a violation of the equal variance assumption

Table 2: Pearson's correlation between serum ADA vs Non-HDL and Lipid ratios

| Pearson's Correlation | ADA | | | |
|-----------------------|---------|---------|---------|---------|
| | T2DM | | Control | |
| | r value | P value | r value | P value |
| LDL/HDL | -0.074 | 0.609 | 0.109 | 0.452 |
| T Chol/TGL | -0.075 | 0.603 | -0.166 | 0.251 |
| TGL/HDL | 0.027 | 0.852 | 0.241 | 0.092 |
| Non-HDL/HDL | -0.063 | 0.662 | 0.172 | 0.233 |
| Non-HDL | -0.056 | 0.701 | 0.172 | 0.233 |

* p < .05, ** p < .01, *** p < .001

DISCUSSION

Diabetes mellitus is a common metabolic disorder of the endocrine system and is a leading cause of death globally. It is characterized by increased levels of plasma blood glucose due to multiple factors that may be hereditary, environmental impact mainly diet or associated insulin resistance (11, 12). It may lead to several complications in due course of time which could lead to disability and morbidity in patients (13). The incidence of patients with type 2 diabetes mellitus has been increasing rapidly in the past 2-3 decades and there is notably higher prevalence of associated with cardiometabolic risk factors (14).

Present study found significant increased serum Adenosine deaminase (ADA) activity in T2DM subjects when compared with control group, as shown in Table.1. Hoshino *et al.*, also documented increased levels of ADA activity and don't show any significant correlation with lipid profile (15). Increased ADA activity inhibits the action of adenosine which mimic the action of insulin in the adipose tissue and skeletal muscles through GLUT-4 receptors. Depletion of adenosine leads to insulin resistant, subsequent cause's hyperglycemia.(15,16) Lee *et al.*, and Shiva Prakash *et al.*, reported that ADA activity is comparatively reduced in T2DM patients who are under good glycemc control (13,

16).

Inhibition of adenosine increases CAMP, this directed to increase in circulation of free fatty acids. These FFA are converted into triglycerides and get deposited in the adipose tissue (17). This excess storage of triglyceride stimulated pro-inflammatory responses such as interleukins and another factor. Based on the above mechanism, we tried to find association of ADA activity with lipid profile and atherosclerotic index. We found significant increase (p value <0.001) in lipid profile (TC, TGL, HDL, LDL, and VLDL), Non-HDL-C and lipid ratio (LDL/HDL, TC/TG, TG/HDL and Non-HDL/HDL) when compare T2DM with control group which has been depicted in Table 1. The correlation of serum ADA with lipid profile and lipid ratio (Table 2) did not show any correlation within the groups of T2DM and control groups.

ADA alongside other immune modulating enzymes acts as a deconstructive oxidative marker in diabetes and plays an important role in progression in its complications.(16) The indication of increase in ADA may serve as a glycemic marker in of T2DM. Role of ADA activity along with lipid profile and atherosclerotic index score was not statistically significant.

CONCLUSION

Assessment of serum ADA level is found to be a cost-effective biomarker and its efficient use may help in assessing cell-mediated immunity in diabetic individuals. Thus, we conclude that increased ADA activity may be an indicator in the immunopathogenesis of T2DM and can also be implicated as a biomarker for predicting glycemic control individuals with T2DM, but there were no significant findings noted in the assessment of dyslipidemia and atherosclerotic index. Further studies are required, especially the elucidation of the role of ADA levels on the lipid profile of T2DM patients with fair to poor control.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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Study of Calcium, Magnesium and Phosphorus Levels among Hypothyroid Patients in Trichy, Tamil Nadu

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ABSTRACT

BACKGROUND

Mineral metabolism is frequently disturbed in thyroid dysfunctions. Among thyroid dysfunctions, hypothyroidism is one of the most common form resulting from the deficiency of thyroid hormones. Studies done on serum calcium, phosphorus and magnesium in hypothyroid patients earlier have conflicting results. Hence the present study has been undertaken to study the levels of serum calcium, phosphorus, and magnesium among hypothyroid patients and analyse their correlation with thyroid stimulating hormone (TSH).

METHODS

The case control study was conducted in the Department of Biochemistry in Trichy SRM Medical College Hospital and Research Centre for a period of 6 months from 2017 January to July 2017. The study was undertaken involving 50 hypothyroid cases and 50 healthy volunteers as controls after proper ethical clearance and informed consent of all the study subjects. Serum calcium, phosphorus and magnesium were measured along with tri-iodothyronine (FT3), tetra-iodothyronine (FT4) and TSH among all study subjects. Statistical analysis of data was done using statistical package for social sciences (SPSS) software.

RESULTS

The mean value of serum total calcium and total magnesium was lower among hypothyroid cases and phosphorus value was increased as compared to controls. ($P < 0.001$) Statistically significant negative correlation was observed between serum calcium, magnesium and TSH among hypothyroid cases. Statistically significant positive correlation was observed between serum phosphorus and TSH among hypothyroid cases.

CONCLUSIONS

Among hypothyroid patients the values of serum calcium, magnesium and phosphorus is significantly altered. Thyroid disorders have an important role in bone and mineral metabolism. Thus, monitoring of these minerals among hypothyroid patients in regular follow up may effectively improve the clinical manifestations in them. Hence, monitoring of mineral status of the hypothyroid patients on follow-up will be of benefit to the patients.

KEYWORDS

Hypothyroidism, Calcium, Phosphorus, Magnesium, Minerals

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BACKGROUND

Optimal health maintenance requires adequate and balanced amount of macronutrients and micronutrients. Macro-minerals have a definite role in various biological functions by influencing enzymatic activity, protein function and cell membrane permeability. Hormones influence mineral metabolism at several levels of action, including transport and excretion of minerals.¹ Thyroid gland produces tri-iodothyronine and tetra-iodothyronine which are commonly known as T3 and T4 respectively. T3 is biochemically more active form of thyroid hormone and is produced by deiodination of T4 by the enzyme 1,5' deiodinase in the peripheral tissues. These hormones are in turn regulated by thyroid stimulating hormone and thyroid releasing hormone (TRH) secreted by pituitary gland and hypothalamus respectively. Thyroid hormones play a major role in the cell differentiation, in fetal development and also to maintain thermogenic, metabolic and mineral homeostasis in adults. Hypothyroidism in the sub-normal activity of thyroid gland that leads to many metabolic processes to slow down causing clinical, psychological and biochemical alterations. According to the recent studies the prevalence of hypothyroidism in India varies from 4 – 11 %, more prevalent in women and elderly age group.²

Mineral metabolism is frequently disturbed in thyroid dysfunctions. Among thyroid dysfunctions, hypothyroidism is one of the most common form resulting from the deficiency of thyroid hormones. Studies done on serum calcium, phosphorus and magnesium in hypothyroid patients earlier have conflicting results. Anatomical abnormalities and dysfunction of thyroid are the most common diseases of endocrine glands. Hypothyroidism and hyperthyroidism are two primary pathological conditions involving the thyroid glands.³ Among thyroid dysfunctions, hypothyroidism is one of the most common form resulting from the deficiency of thyroid hormones or from their impaired activity.⁴ Hypothyroidism is ten times more common in women than men and its prevalence increases with age.⁵ Hyper secretion of pituitary TSH occurs by the decrease in T3 and T4 concentration and causes amplified increase in TSH levels. It plays a key laboratory finding in diagnosis of hypothyroidism.⁶ Thyroid hormones play a role in the metabolic functions by regulating the lipid, carbohydrate, protein and mineral metabolism.⁷

Thyroid hormones are essential for normal growth and maturation of skeletal system. Calcium and phosphorus homeostasis is frequently altered in thyroid dysfunction. Hence thyroid disorders are important causes of secondary osteoporosis. Serum calcium levels can be fairly used as an index of bone resorption.⁸ The depressed turnover due to impaired mobilization of calcium into the bone in hypothyroidism leads to decrease in blood calcium level. In hypothyroidism the increased production of calcitonin can promote tubular reabsorption of phosphate and tubular excretion of calcium.⁹ The renal phosphate reabsorption is elevated by FT3 and hence elevates the serum phosphorus levels in rats. Animal studies propose thyroid hormones as long term regulators for phosphate metabolism.¹⁰ Normal calcium and phosphorus have been shown in few studies^{11,12}

while other studies have shown decreased levels in hypothyroidism.^{13,14} Even though the changes in calcium and phosphorus may be slight in thyroid disorders, these disturbances will be important for the patient in long run.¹⁵ Studies show that metabolic disorders, hypertension and cardiovascular diseases are related to defects in metabolism of divalent cations such as calcium and magnesium.^{16,17} Hence the present study was undertaken to study the levels of serum calcium, phosphorus and magnesium among hypothyroid patients and to analyse their correlation with TSH.

METHODS

The case control study was conducted in the Department of Biochemistry in Trichy SRM Medical College Hospital and Research Centre for a period of 6 months from 2017 January to July 2017. The study was conducted after approval from ethical committee [IEC NO: 47 24 / 11 / 2016] and informed consent was taken from all patients. Study participants of age group 20 - 50 years were selected from patients who have underwent thyroid profile. Among 100 participants, 50 were selected as cases and 50 as controls based on their thyroid profile report.¹⁸ The reference range of thyroid profile according to Tietz textbook¹⁹ was followed and patients were categorized as controls and hypothyroid cases based on the report. The reference ranges are Free T3: 2.1 - 4.4 (pg/ml), Free T4: 0.8 - 2.7 (ng/dl) and TSH: 0.4 - 4.2 (mIU/ml).

Exclusion Criteria

Patients suffering from infectious diseases, renal diseases, hepatic diseases, pituitary diseases, bone diseases, diabetes mellitus, alcoholism and patients on mineral supplementation were excluded from the study.

Under aseptic precaution 5 ml venous blood was collected from the ante-cubital fossa for all the patients. Serum was separated by centrifugation and analysis was done. Routine IQC was run and after confirmation the samples were analysed for biochemical parameters. Serum was analysed for FT3, FT4 and TSH by eCLIA method in Cobas e411 auto-analyser followed by calcium (Arsenazo III method), phosphorus (phosphomolybdate UV method) and magnesium (xylydyl blue method) in MindrayBS420 auto-analyser in the biochemistry section of SRM college laboratory.

Statistical Analysis

Data was entered in Microsoft Excel and analysed using SPSS software. Descriptive statistics such as mean and standard deviation (SD) were computed for the data respectively. Pearson's correlation was done to correlate the relationship between FT3, FT4, TSH and minerals (calcium, phosphorus, and magnesium)

RESULTS

The results of the study are presented in the tables. There were a total number of 42 females and 8 males in controls and 33 females and 17 males in hypothyroid cases. The mean age of controls was 38.8 ± 12.45 (years) and the mean age of cases was 37.0 ± 10.06 (years). The mean TSH values were 2.15 ± 0.81 μ IU/L among controls and 61.49 ± 34.82 μ IU/L among hypothyroid cases. There was a statistically significant increase in TSH among cases as compared to the controls. The mean FT3 values were 2.84 ± 0.44 pg/ml among controls and 2.52 ± 0.91 pg/ml among hypothyroid cases. FT3 values were significantly decreased in cases as compared to the controls (2.52 ± 0.91 vs 2.84 ± 0.44 pg/ml). The mean FT4 values were 1.28 ± 0.18 ng/ml among controls and 0.85 ± 0.36 ng/ml among hypothyroid cases. Similarly, FT4 values were also decreased among cases as compared to the controls (0.85 ± 0.36 vs 1.28 ± 0.18 ng ml).

The study observed a significant decrease level of calcium among cases 8.01 ± 0.17 (mg/dl) as compared to the controls 10.16 ± 1.47 (mg/dl) and also magnesium of 2.25 ± 0.20 (mg/dl) among cases as compared to controls of 2.49 ± 0.07 (mg/dl). But the serum phosphorus values were increased among cases as compared to the controls (4.81 ± 0.88 vs 3.83 ± 0.40 mg/dl). The mean calcium values were 10.16 ± 1.47 mg/dl among controls and 8.01 ± 0.17 mg/dl among hypothyroid cases. The mean phosphorus values were 3.83 ± 0.40 mg/dl among controls and 4.81 ± 0.88 mg/dl among hypothyroid cases. The mean magnesium values were 2.49 ± 0.07 mg/dl among controls and 2.25 ± 0.20 mg/dl among hypothyroid cases. The mean calcium, and magnesium levels were significantly lower in

hypothyroidism cases compared to healthy controls. ($P < 0.001$). Similarly, significant increase in serum phosphorus concentration was found among hypothyroidism patients ($P < 0.001$) when compared to controls. Table 1 shows the mean & SD values of parameters.

| Parameters | Cases | Controls | P Value |
|--------------------|-------------------|------------------|--------------|
| Age [Years] | 37.0 ± 10.06 | 38.8 ± 12.45 | -- |
| Sex [M / F] | 17/33 | 8/42 | -- |
| FT3 [pg/ml] | 2.52 ± 0.91 | 2.84 ± 0.44 | $< 0.0001^*$ |
| FT4 [ng/dl] | 0.85 ± 0.36 | 1.28 ± 0.18 | $< 0.0001^*$ |
| TSH [μ IU/L] | 61.49 ± 34.82 | 2.15 ± 0.81 | $< 0.0001^*$ |
| Calcium [mg/dl] | 8.01 ± 0.17 | 10.16 ± 1.47 | $< 0.0001^*$ |
| Phosphorus [mg/dl] | 4.81 ± 0.88 | 3.83 ± 0.40 | $< 0.0001^*$ |
| Magnesium [mg/dl] | 2.25 ± 0.20 | 2.49 ± 0.07 | $< 0.0001^*$ |

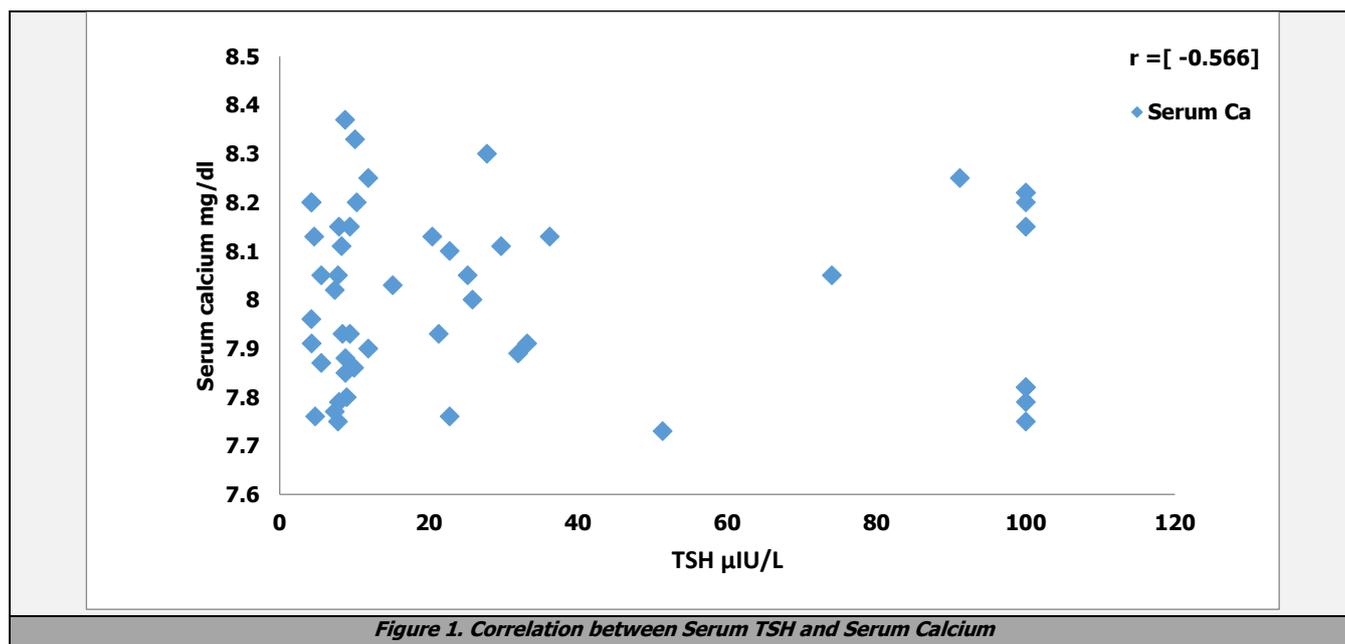
Table 1. Mean & SD Values of Parameters

P value < 0.05 is statistically significant (* is highly significant)

The correlation coefficient between TSH and calcium was $r = [-0.566]$ and correlation coefficient between TSH and phosphorus was $r = [0.376]$. The correlation coefficient between TSH and magnesium was $r = [-0.479]$. The serum TSH values of hypothyroid patients were studied in relation to serum calcium phosphorus and magnesium levels. On statistically analysing the values, a significant negative correlation was observed between serum TSH with serum total calcium and total magnesium. Also, a statistically significant positive correlation was observed between serum phosphorus levels and TSH. Table 2 shows correlation between TSH and other parameters. Figure 1, 2 and 3 represent the correlation between serum TSH and calcium, phosphorus and magnesium respectively.

| Parameters | Correlation Coefficient [r Value] | P Value |
|-------------------|------------------------------------|---------|
| TSH VS Calcium | $r = [-0.566]$ | 0.001 |
| TSH VS Phosphorus | $r = [0.376]$ | 0.01 |
| TSH VS Magnesium | $r = [-0.479]$ | 0.003 |

Table 2. Correlation between TSH and Other Parameters



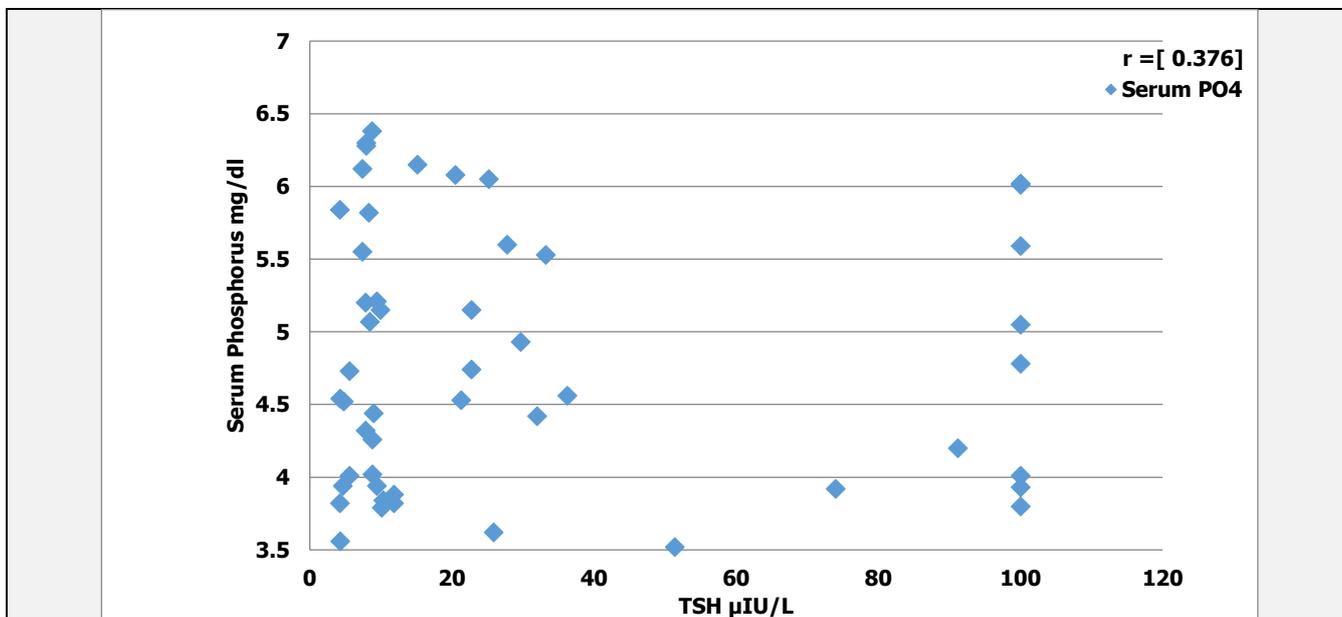


Figure 2. Correlation between Serum TSH and Serum Phosphorus

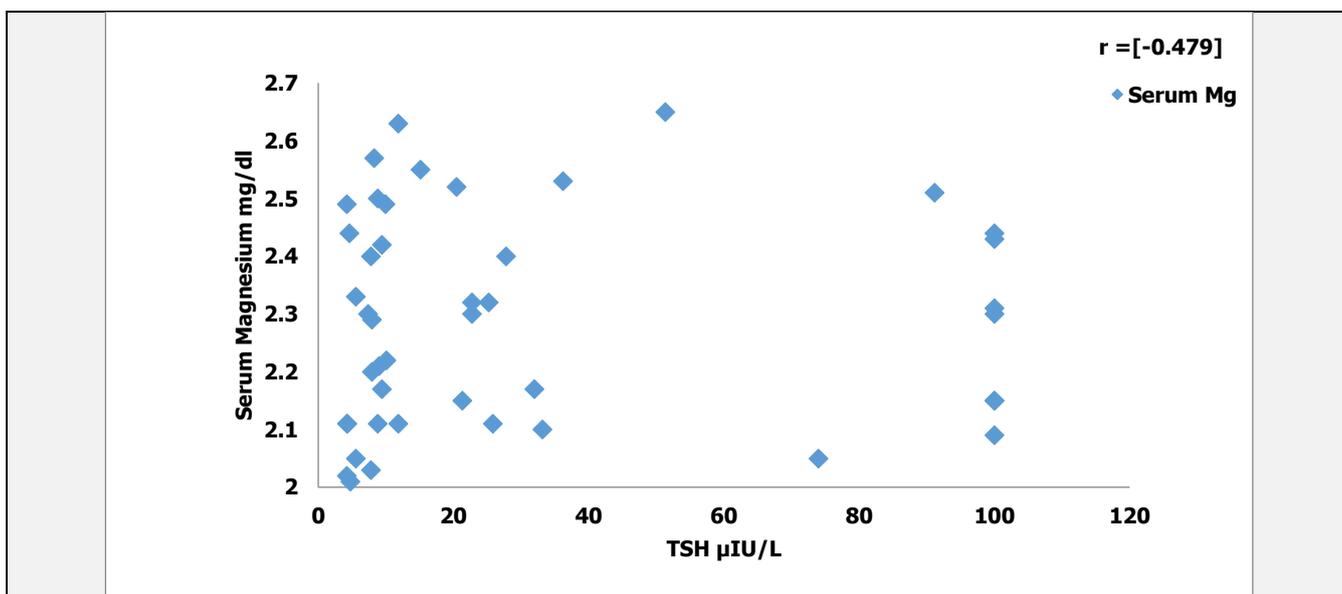


Figure 3. Correlation between Serum TSH and Serum Magnesium

DISCUSSION

Normal growth and maturation of skeletal system needs the thyroid hormones. In thyroid dysfunction, calcium and phosphorus homeostasis is frequently altered. Hence secondary osteoporosis is commonly seen in thyroid disorders. Serum calcium levels can be fairly used as an index of bone resorption. The depressed turnover due to impaired mobilization of calcium into the bone in hypothyroidism leads to decrease in blood calcium level. The increased production of calcitonin in hypothyroidism can promote tubular reabsorption of phosphate and tubular excretion of calcium. The renal phosphate reabsorption is elevated by FT3 and hence elevates the serum phosphorus levels in rats. Animal studies propose thyroid hormones as long-term regulators for phosphate metabolism. Normal

calcium and phosphorus levels have been shown in few studies while other studies have shown decreased levels in hypothyroidism. Even though the changes in calcium and phosphorus may be slight in thyroid disorders, these disturbances will be important for the patient in long run.

Thyroid hormones have a direct effect on the calcium and phosphorus resorption by altering the blood flow and glomerular filtration rate (GFR).²⁰ Thyroid hormones play a role as the central regulator of body haemodynamics, thermoregulation and metabolism. It has an influence on renal haemodynamics, glomerular filtration and electrolyte handling.²¹ Mineral pool in the blood is determined by the thyroid hormones by influencing their mobilization into the blood, and also their clearance through effect on glomerular filtration rate. There is an increased renal blood flow leading to increased clearance of calcium as well as decreased extracellular release of calcium in hypothyroidism.²² Our

study has shown significant low levels of serum calcium in cases than controls. The serum phosphorus levels were markedly increased among hypothyroid patients in the present study compared to the controls. Serum magnesium levels were found to be significantly lower in hypothyroid cases. There was a significant positive correlation between serum phosphorus and TSH and negative correlation between serum calcium, magnesium and TSH.

Normal growth and maturation of the skeleton needs thyroid hormones. In hypothyroidism there is a decreased turnover due to impaired mobilization of calcium into the bones, which leads to the decrease in blood calcium. In hypothyroidism there is also an increased production of calcitonin which promotes the tubular reabsorption of phosphate and favours the tubular excretion of calcium which leads to hypocalcaemia and hyperphosphatemia. Thyroxin regulates blood calcium level by releasing calcium from cells, but in hypothyroidism less thyroxin enters the cells and hence less calcium is released leading to hypocalcemia.²³ In hypothyroidism there is hypomagnesaemia because of urinary output and fractional excretion of magnesium through urine. Hypothyroidism is six times more common in women than in men has been suggested by earlier statistics. The higher prevalence of thyroid diseases in women shows that oestrogen might be involved in the pathophysiology of thyroid dysfunction. Estradiol has an antagonistic effect on thyroid hormones as estradiol competes with the thyroid hormone binding sites on the receptor proteins. Estradiol also limits the thermogenic action of thyroid hormones and promotes the storage of fat.²⁴ Gantus MA et al. studied the effects of oestrogen on a homogeneous stromal cell population of rat thyroid gland. Their results point to the cytokine transforming factor brta 1/transcription factors mad-2 signalling pathway as a putative target of oestrogen actions on a thyroid stromal cell.²⁵

Roopa M et al. & Jaikiran K et al. studied the changes in electrolyte profile in patients with hypothyroidism and reported that calcium level is significantly reduced and magnesium and phosphorus level is increased in patients with hypothyroidism. It was also found that there was a significant negative correlation between serum calcium and TSH, positive correlation between serum TSH and magnesium and phosphorus. But in our study, we observed a negative correlation between magnesium and TSH.^{23,26} The animal study done by Kumar and Prasad et al. have concluded that renal calcium excretion was increased in rats with high TSH levels.²⁷

Suneel B et al. studied mineral status in patients of thyroid disorders and found decreased calcium and increased phosphorus in hypothyroidism mainly due to influence of parathyroid hormone (PTH) and calcitonin. Magnesium level is decreased due to influence on GFR and decreased clearance. In hypothyroidism, the renal blood flow is increased leading to high clearance of magnesium from kidneys. So it will be causing hypomagnesemia which is matching our study findings of decreased magnesium in hypothyroid patients.²⁸ Khadem H had also shown reduced total and ionized magnesium in patients with hypothyroidism along with study of lipid profile.²⁹ Few studies have shown

decreased levels of serum magnesium levels in hypothyroid patients as compared to the controls and show a negative correlation with thyroid hormones which is matching with our study finding of decreased magnesium in cases of hypothyroid as compared to the controls.³⁰ Abbas MM et al. study also indicated elevated phosphate and decreased calcium levels in hypothyroid patients which are in accordance with our study. But the magnesium levels increased in hypothyroid patients is not matching with our study finding as we have found decreased levels of magnesium among cases as compared to the controls of healthy volunteers.³¹

Asamaik AS et al. have observed that phosphorus levels are significantly increased in subclinical hypothyroid cases (SCH) and overt hypothyroid cases (OH) which is in accordance with our study finding of increased phosphate among hypothyroid cases.³² In another study by AITonsi, found altered phosphate concentrations in hypothyroid patients. The increased serum phosphate levels in hypothyroid cases is in accordance with our study.³³ Schwars C et al. study of 9012 patients found a significant positive correlation between TSH and phosphate levels. Phosphate levels are higher in cases with elevated TSH than in controls which is also matching with our present study.³⁴ Magnesium, on the other hand, is an important cation that ameliorates atherosclerosis and hypertension, promotes coronary vasodilatation and unloading of the heart causing an increase in its efficiency. Low serum magnesium is often associated with arrhythmias, coronary vasospasm and high blood pressure.³⁵ A significant decrease of serum magnesium in hypothyroid patients was observed which is in congruence with the results reported in humans³⁶ and in experimental animals.³⁷ A study done by Mamatah et al. have shown a statistically significant increase in serum magnesium levels and a positive correlation between serum magnesium and TSH which are not in accordance with our study, but we had a decrease in serum magnesium levels in hypothyroid cases and a negative correlation between serum magnesium and TSH levels.³⁸ As per the study conducted by McCaffrey and Quamme, magnesium retention increased by 15 – 30 % from kidneys due to increased reabsorption in renal tubules as the thyroid hormones have direct effect on renal tubules as well.³⁹ Sussanna et al. showed decrease in serum magnesium in hypothyroid patients with negative correlation with TSH which is correlating to our study findings.⁴⁰ But in a study by Frizel et al. they have observed increased plasma ionized and total magnesium levels among hypothyroid patients which is not in accordance with our study.⁴¹ Thyroid hormones determine the mineral pool in the blood by influencing the mobilization of minerals like calcium and phosphorus, in the blood by influencing their clearance through urinary excretion by its effect on GFR or renal plasma flow. Low levels of calcium in hypothyroid cases reflect poor metabolism of calcium. Low levels of magnesium reflect poor influence of thyroid hormones on GFR and thereby clearance of these minerals by filtration. The treatment modalities can also be framed while treating the hypothyroid patients keeping in view of altered mineral metabolism. The decreased magnesium levels will be influencing the action of thyrotrophic hormone on the

thyroid gland through the formation of cyclic amp involved in the activation of adenylyl cyclases and stimulates cyclic 3, 5 nucleotide phosphodiesterase disturbing various metabolism in the body that has been shown in the in vitro studies.⁴²

CONCLUSIONS

Our study demonstrated that hypothyroid patients show low serum total calcium, total magnesium and increased serum phosphorus levels compared to the healthy controls. Hence monitoring of mineral status of the hypothyroid patients on follow-up will be of benefit to the patients. Supplements can be initiated based on these values to avoid the effects resulting from changes in the mineral levels mainly related to bone metabolism.

Limitations of Our Study

Our study is limited by the limited number of patients. In addition, the list of confounders for the mineral metabolism disturbances is long which needs to be studied in details. The other markers related to mineral metabolism like vitamin D, PTH and calcitonin can also be studied for better understanding. Prospective study can also be planned to study the improvement on the clinical manifestations on initiating the supplements for these minerals among hypothyroid cases.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

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Original Research Article

TyG index as a cardiovascular risk factor, with reference to anthropometry in first year medical students - A cross sectional study

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ABSTRACT

Background: Triglyceride-Glucose (TyG) index is an independent predictor of cardiovascular risk in several pathological conditions such as T2DM, Metabolic syndrome (MetS), CVD, and CAD. Early studies which were done on TyG index focus on insulin resistance. High TGL level induces production of small density LDL particle. All these proposed theories, explains why TyG index used as an independent factor of cardiovascular risk.

Materials and Methods: 150 First year medical students were included and their anthropometric measures done using standard scale. Fasting blood glucose estimated by GOD POD method. Total cholesterol, triglyceride, High density lipoprotein and direct low density lipoprotein were estimated by IFCC approved methods. Statistical analysis was performed using IBM SPSS version 20.

Results: Results 26% were obese and 5.33% were overweight. Lipid profile found to be significant different among the group. Atherosclerotic indices were found to have mild to moderate correlation with anthropometric measures ($p < 0.05$).

Conclusion: Present study showed a high prevalence of overweight to obesity ratio in first year medical students. With TyG index we observed an independent association of cardio-metabolic risk with BMI in young adult.

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1. Introduction

Studies have shown that Triglyceride-Glucose (TyG) index is an independent predictor of cardiovascular risk in several pathological conditions such as T2DM, Metabolic syndrome (MetS), CVD, CAD, etc.^{1,2} Sanchez et al. documented the association of TyG index with hypertension in health individuals.³ Early studies which were done on TyG index focus on insulin resistance.⁴ Triglyceride-glucose (TyG) index, product of triglyceride and fasting plasma glucose (FPG) is a novel tool that has been found to correlate with surrogate and direct measures of IR.⁵

Later research found that TyG index was an independent predictor of cardiovascular risk in MetS. Since TyG index was calculated based on TGL and glucose, both compounds are responsible for adverse cardiovascular events.^{6,7} It has been evident that high TGL levels elevate the lipoprotein and cause CVD. High TGL level induces production of small density LDL particle. All these proposed theory, explains why TyG index used as an independent predicate of cardiovascular risk.³

Currently about 10% of first year medical students affected by MetS.⁸ Major factor for increase in MetS was overweight and obesity. In 2015, ICMR documented in one of its study that 11.8% to 31.5% and 16.9 to 36.3% of young adults have obesity and central obesity.⁹ Numerous

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studies have discussed the predictive ability of obesity and lipid-related indices in identifying metabolic abnormalities. Body mass index (BMI) is a simple measurement of obesity status, while waist circumference (WC) reflects abdominal adiposity and may represent visceral adiposity better than BMI.¹⁰

Increase in overweight and obesity was due to life style modification. Stress found to be important factor for obesity, along with improper diet and reduced physical activity. Many studies have documented increased stress level in First year medical students.^{11–13}

In this study we have tried to evaluate the role of TyG index as a risk factor for cardiovascular disease, with reference to BMI, in first year medical students.

2. Materials and Methods

A cross sectional study was done on medical students at Trichy SRM Medical College hospital and research centre. They were included in the study after obtaining informed consent. The study was approved by the institutional research and human ethics committee. Total number of students participating in the study was 150 based on the following inclusion and exclusion criteria. The duration of the study was about three months from November 2018 to January 2019. Students were sub-grouped into Underweight, Normal, Overweight and Obesity based on their BMI respectively.

2.1. Inclusion criteria

Healthy volunteers between the age group 18 – 25 were included.

2.2. Exclusion criteria

Students who were not willing, had severe illness and those who are regular medication were excluded.

2.3. Anthropometric measures

For all the students, height and weight were measured using a standard scale respectively. Body mass index was calculated by formula using, weight in Kg divided by height in meter square [kg/m²].¹⁴ Waist circumference, measured to the nearest 0.1 cm at the midpoint between tip of iliac crest and last costal margin in the back and at umbilicus in the front using a non-stretchable tape at the end of normal expiration with subject standing erect in a relaxed position.¹⁵ Waist-hip ratio and waist-height ratio were calculated.

2.4. Sample collection

5ml of fasting venous blood sample was collected under aseptic precautions. 3ml was added in red tube for analyzing lipid profile and 2ml was added in fluoride tube for glucose

estimation. Both parameters were analyzed on the same day.

2.5. Biochemical investigation

Fasting blood glucose was estimated by GOD POD method. In lipid profile that includes total cholesterol, triglyceride, high density lipoprotein and direct low density lipoprotein were estimated by IFCC approved methods. Above mentioned biochemical parameters were analyzed in Roche C311 autoanalyzer using kits from Roche diagnostics, Germany. Other atherosclerotic index such as non-LDL-c, TC/ HDL-c ratio, LDL-c/ HDL-c ratio, TGL/ HDL-c ratio, non-HDL-c/ HDL-c ratio and TyG index were calculated.

2.6. Statistical analysis

Anthropometric measurements and lipid profile were the primary variable. Atherosclerotic index variables along with WHR and hip height ratio were secondary variable. Descriptive analysis was carried out and values were expressed as mean \pm SD, median with inter-quartile range for quantitative variables, frequency and proportion for categorical variables. Independent t test was performed to assess statistically significant for quantitative variable. Pearson's correlation was used to assess the association between two variables. Chi-square test was used to assess the statistical significant for categorical variable. Data was entered in Microsoft office excel and statistical analysis was performed using IBM SPSS version 20. For all the statistical analysis $p < 0.05$ considered as significant.

3. Result

Totally 150 students were included in the study based on inclusion and exclusion criteria, out of which 55 [36%] were male and 95 [64%] were female. We measured the anthropometric index of cardiovascular risk factor BMI and waist hip ratio. Results showed 60% of students were in normal BMI, 8.67% were underweight, 26% were obese and remaining 5.33% were only overweight.

In waist hip ratio 64% were found to be low, 35% were normal and 19% were high. Both measurements are depicted in Table 1. Present study estimated a mean weight to height ratio of 0.43 with SD 0.15, which is an emerging anthropometric measure in assessing the cardiometabolic risk factor in obesity.

The results of lipid profile and fasting blood sugar are shown in Table 2. Using lipid value, atherosclerotic indexes such as non-HDL-c, TC/ HDL, LDL/ HDL ratio, TGL/ HDL ratio and Non-HDL/ HDL were calculated using standard formulae. TyG index was calculated with above values, since it's an emerging cardiovascular risk factor assessment tool, using the formula [(TGL x Fasting Glucose)/2].¹⁶ To find significance of biochemical parameters in the study population, subjects were sub-grouped based on the BMI

Table 1: Gender and anthropometrics measurement of first year MBBS students

| Parameters | N (%) |
|------------------------|------------|
| Gender | |
| Male | 55 (36.67) |
| Female | 95 (63.33) |
| BMI | |
| Underweight | 13 (8.67) |
| Normal | 90 (60) |
| Overweight | 39 (26) |
| Obesity | 8 (5.33) |
| Waist Hip Ratio | |
| Low | 96 (64) |
| Moderate | 35 (23.33) |
| High | 19 (12.67) |

Table 2: Association of biochemical parameter with body mass index (n=150)

| Parameter | Overall | Underweight (n = 13) | Normal (n = 90) | Overweight (n = 39) | Obesity (n = 8) | p Value |
|--------------------------|---------------|-------------------------|--------------------|------------------------|--------------------|---------|
| FBS | 85.07 ± 9.81 | 81.92 ± 5.8 | 84.07 ± 9.82 | 87.79 ± 10.27 | 88.25 ± 10.44 | 0.104 |
| Cholesterolc | 146.85 ± 25.8 | 146 ± 20.75 | 142.34 ± 25.63 | 157.38 ± 26.46 | 147.63 ± 19.96 | 0.024 |
| TGL ^{a,c} | 83.03 ± 35.89 | 64.38 ± 25.41 | 76.17 ± 24.99 | 105.23 ± 50.12 | 82.38 ± 27.07 | 0.000 |
| HDL ^{a,b,c} | 47.43 ± 9.91 | 55.85 ± 11.84 | 48.46 ± 8.99 | 43.18 ± 9.5 | 43 ± 8 | 0.000 |
| LDL ^c | 96.11 ± 23.91 | 91.31 ± 15.85 | 92.12 ± 23.79 | 106.41 ± 24.18 | 98.63 ± 22.68 | 0.014 |
| Non-HDL | 99.42 ± 25.54 | 90.15 ± 17.19 | 93.89 ± 23.08 | 114.21 ± 28.15 | 104.63 ± 22.42 | 0.000 |
| TC/HDL ratio | 3.21 ± 0.84 | 2.68 ± 0.49 | 3 ± 0.62 | 3.8 ± 1.03 | 3.55 ± 0.9 | 0.000 |
| LDL/HDL ratio | 2.12 ± 0.75 | 1.71 ± 0.51 | 1.95 ± 0.57 | 2.6 ± 0.9 | 2.41 ± 0.88 | 0.000 |
| TGL/HDL ratio | 1.9 ± 1.19 | 1.19 ± 0.52 | 1.65 ± 0.8 | 2.68 ± 1.69 | 2.05 ± 1.03 | 0.000 |
| Non-HDL/HDL ratio | 2.21 ± 0.84 | 1.68 ± 0.49 | 2 ± 0.62 | 2.8 ± 1.03 | 2.55 ± 0.9 | 0.000 |
| TyG index ^{b,c} | 4.39 ± 0.19 | 4.26 ± 0.18 | 4.36 ± 0.17 | 4.51 ± 0.22 | 4.42 ± 0.18 | 0.000 |

a - The mean difference is significant at the 0.05 level between underweight and overweight

b - The mean difference is significant at the 0.05 level between underweight and Obesity

c - The mean difference is significant at the 0.05 level between Normal and overweight

Table 3: Correlation between anthropometric measurements and lipid profile (n=150)

| Parameters | Body Mass Index r | Waist Hip Ratio R | Weight Height Ratio r |
|-------------------|----------------------|----------------------|--------------------------|
| FBS | 0.177* | 0.112 | 0.015 |
| Cholesterol | 0.213** | 0.074 | 0.088 |
| TGL | 0.317** | 0.239** | 0.214** |
| HDL | -0.348** | -0.209* | -0.154 |
| LDL | 0.275** | 0.158 | 0.127 |
| Non-HDL | 0.351** | 0.156 | 0.149 |
| TC/HDL ratio | 0.447** | 0.244** | 0.208* |
| LDL/HDL ratio | 0.420** | 0.263** | 0.210** |
| TGL/HDL ratio | 0.363** | 0.274** | 0.226** |
| Non-HDL/HDL ratio | 0.447** | 0.244** | 0.208* |
| TGL glucose index | 0.374** | 0.216** | 0.257** |

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level(2-tailed).

following which one way ANOVA was performed. Glucose didn't show any statistical significance among the group. In lipid profile, there was a statistical significance between the estimated and calculated values among the groups, which has been depicted in Table 2.

In Table 3 lipid profile, atherosclerotic and TGL glucose index was correlated with anthropometric measurement which included weight to height ratio. All atherosclerotic indexes were found to have mild to moderate correlation with anthropometric measures ($p < 0.05$). BMI had the highest 'r' value of 0.447 with TC/HDL ratio and Non-HDL/HDL ratio. But HDL showed negative correlation.

4. Discussion

In the present study, primary data showed that 26% was overweight and 5.33% was obesity, based on WHO guidelines for BMI.¹⁴ Nearly 9% of student found to be underweight and 60% were observed to be normal. Similar result was found in Boo NY et al¹⁷ with overall 30% found to be both overweight and obese, where 15% of students were in underweight group. The overweight/obesity ratio was 4.61% in first year medical students. In a study by Gupta et al.¹⁶ it's recorded 17.5% and 3.4% of prevalence of overweight and obesity in Midnapore medical college, India. Another study done by Chhabra et al.¹⁸ it was documented that there was an overall prevalence of 11.7% and 2% of overweight and obesity in medical students at Delhi. Studies conducted by Fernandez et al.¹⁹ and Gopalakrishnan et al.²⁰ in Pune and Malaysia respectively, obtained a high prevalence of overweight/obesity ratio in medical students.

Secondarily, We have investigated dyslipidaemia and patho-glycemia along with atherosclerotic index and TyG index which has been emerging as an independent factor causing cardiovascular disease in many pathological conditions.²¹

Our results have shown very high significance with TyG index as a risk factor in obese and overweight individuals which correlates well with the studies reported by Locateli et al.²² and Gurrola et al.²³

We have noticed TyG index and other atherosclerotic indices showing significant correlation with anthropometric measurements in this study which goes well with the study reported by Kim et al.²⁴

In this study, we also found significant correlation between TyG and other anthropometric measures, which also aligned with the study by Manjareeka et al.²⁵

5. Conclusion

Present study showed a high prevalence of overweight to obesity ratio in first year medical students. With TyG index we observed an independent association of cardio-metabolic risk with reference to BMI in young adults. Thereby focussing on individuals' risk of getting any cardiovascular

disease seems to be higher in association to TyG index and BMI.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Evaluation Of Serum Cholinesterase Levels Among Farm Workers Exposed To Chemical Pesticides In Rural Population Of Tiruchirapalli

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Abstract

In this study, we compared the SChE levels between exposed and unexposed subjects of pesticides. This cross-sectional study was conducted in the tertiary care teaching hospital. A total of fifty-two farmers were exposed to mixture of pesticides. Fifty non exposed controls from same geographical area, who had no history of exposure to chemicals were randomly selected for this study. A detailed history, including the personal and occupational details, list of pesticides employed, duration of exposure and the number of usages were recorded among agricultural workers with the help of questionnaire. Among the exposed group, 88.46% were males and 11.53 % were females. The mean \pm S.D of

SChE levels in males those who exposed to OP and non-exposed group were 5864 ± 1208 and 7335 ± 1581 respectively. The mean \pm S.D of SChE levels in females those who exposed to OP and non-exposed group were 5203 ± 1141 and 6608 ± 1611 respectively. In our study, the farm workers had complaints of headache (18.2%), dizziness (15.5%), eye irritation (22.4%) and excessive sweating (15.6%) and were associated with decreased serum cholinesterase levels with significant ‘p’ value. It is recommended that the farm worker’s cholinesterase level should be assessed periodically. There is mounting evidence that chronic moderate pesticide exposure is

always lethal. Further studies are needed to substantiate these findings.

Keywords: Chemical pesticides - farm workers - serum cholinesterase - rural population.

Introduction

Agriculture workers are at high-risk group for exposure to pesticides. The contamination of pesticides may happen in several ways during storing, application in fields, warehouses, and wrong use by peoples. Pesticide exposure often induces acute and chronic neurological toxicity and dysfunctional lipid, protein, and carbohydrate metabolism [1]. In India, 76% of the pesticide used is insecticide, as against 44% globally [2,3]. In India, more than 1 billion people are engaged in agricultural activities and increased amount of pesticides is used to protect their crop against pests to get more yields [4].

Organophosphates (OP) were commonly employed for killing insects on farms, resulting in several adverse effects which affect the health and cause environmental pollution. OP inhibit the activity of cholinesterase (ChE) enzymes in muscle and nerves, [4,5] which results in the accumulation of the neurotransmitter acetylcholine (ACh) in the nervous system. OP exposure may result in acute and chronic cholinergic poisoning effects. Acute toxic effects of OP pesticides are due to inhibition of acetyl cholinesterase (AChE) in the nervous system, which can cause myocardial, respiratory and neuromuscular transmission impairment [4].

Immediate effects of exposure to large doses of OP produce a wide range of neurological symptoms and it can be observed by clinical signs and symptoms and inhibition of acetyl cholinesterase activity. Low or moderate level of exposure remains ambiguous and begins to suspect their adverse health effects. Chronic

effects of OP exposures are not well reported; however, several current studies elucidate that certain birth outcomes (e.g., decreased gestational age, decreased birth length) [6] and abnormal reflex functions in newborn [4,7] may be linked with low level environmental exposures to OP pesticides. People are directly exposed to these pesticides through dermal contact and inhalation, and indirectly through the food chain.

Few studies suggested that a low level of exposure to OP can also induce oxidative stress and damage strand break in Deoxyribose nucleic acid (DNA), which poses an increased threat for chronic diseases, like cancer and neurodegenerative diseases [8]. Significant studies have reported cytogenetic damage in agricultural workers, floriculturists, vineyard cultivators, cotton field workers, and others exposed to different types of pesticides. The chlorinated pesticides remain in the environment for very prolonged periods, undergo bioaccumulation, biomagnifications and hence, impart toxicity to non-target organisms including human beings. Pesticides are generally considered as active, evident, and economical solution for controlling weeds and insect pests in urban landscapes.

Serum cholinesterase (SChE) has been used as an exposure index, to assess low-level, chronic residue exposures among field workers [9]. Moreover, AChE is the primary target of organophosphorus and carbamate pesticides, the most commonly used classes of insecticides worldwide. Appropriate legislations and pesticides control, particularly OP, which are the most commonly-used pesticides, are recommended for the developing countries, especially those with poor regulations and controls. Complete understanding of the mechanism of specific pesticide will help to prevent the risk of such exposure.

Although some attempts have been made in this regard, more comprehensive studies are required to find a preventive approach from the hazardous effects of pesticides. Apart from their lethal acute toxicity at high doses, the extensive use and availability of OP in agricultural and domestic applications raise questions about the safety of long-term exposure at the currently approved levels. Serum cholinesterase levels estimation indicates whether the person has been exposed to pesticide exposure or not. It is recommended that the farm worker's cholinesterase level should be assessed periodically. The main objective of the study is to find out the serum cholinesterase levels in agricultural workers related to symptoms and duration of exposure.

Materials and Methods

This is a prospective cross-sectional study and was conducted in tertiary care teaching hospital in the rural area of southern part of Tamilnadu. The study was conducted over a period of three months at tertiary care teaching hospital and research Centre during the months of August to October 2019. A total of 52 farm workers aged 18 to 60 years were randomly selected and included as cases. Additionally, 50 apparently healthy people who were not exposed to pesticides (Profession other than farming) were selected as control.

Agricultural workers exposed to pesticides residing in the rural area were included in this study. Known liver disease, pregnancy, malignancy and those who are not willing to participate were excluded from the study. A detailed demography, working pattern, type of pesticides used, and duration of exposure, exposure frequency and usage of personnel protective equipment (PPEs) was recorded by contacting them personally. The health paradigm related to chemical exposure was explained to the subjects included and further all are requested to attend medicine OPD of the

study centre for further clinical and laboratory investigations. Ethical clearance was obtained from Institutional ethical committee (Ref: No. 636/TSRMMCH&RC/ME-1/2019-IEC No.012 dated 17.07.2019) and the present research work has been carried out according to the guidelines issued by the Institutional human ethical committee.

Under strict aseptic precautions, 3ml of venous blood was collected from the study population after getting informed written consent. Serum cholinesterase level was measured by kinetic photometric method in fully automated analyzer Mindray BS-420. The reference range of serum cholinesterase was 4620-11500U/L. Calibration of instruments and reagents were done before performing procedures.

After completing the *in vitro* biochemical procedures, all the data were analyzed statistically using SPSS software, version 21. Variables are expressed as mean \pm standard deviation. Student's *t*-test/chi-square test was used to compare the significance of the mean differences in ChE activity between exposed and control subjects. For all analyses, 'p' values < 0.05 was considered as significant.

Results

The study subjects of 52 individuals who were exposed to pesticides were recruited as cases and mean spraying time was 90 minutes ranging from 30 to 120 minutes. According to the period of exposure to pesticides, 35% had worked in agriculture for more than 5 years, 24.5% for 2 to 5 years and 40.5% for less than 2 years. Fifty eight percent of the farmers reported that frequency of spraying pesticides range from one to two times a week, 12% sprayed once or twice a month and 0.8% applied pesticides daily. The prevalence of individuals with abnormal cholinesterase levels were 19.5%.

Among the study group, 88.2% were males and 11.8% were females. The age group of the most of the participants was found among 46 to above 55 (Table 1).

Among the control groups, the age and gender matching were more or less equal and coherent.

Table 1: Age and Gender wise distribution of study population

| Age groups (in years) | Gender wise distribution | |
|--------------------------|--------------------------|-----------------|
| | Males (n=90) | Females (n=12)* |
| Cases (n=52) | | |
| 18 to 25 | 6 (6.7) | 1 (8.3) |
| 26 to 35 | 9 (10) | 1 (8.3) |
| 36 to 45 | 7 (7.8) | 1 (8.3) |
| 46 to 55 | 11 (12.2) | 1 (8.3) |
| Above 55 | 13 (14.4) | 2 (16.7) |
| Controls (n=50) | | |
| 18 to 25 | 4 (4.5) | 1 (8.3) |
| 26 to 35 | 8 (8.9) | 1 (8.3) |
| 36 to 45 | 9 (10) | 1 (8.3) |
| 46 to 55 | 11 (12.2) | 2 (16.7) |
| Above 55 | 12 (13.3) | 1 (8.3) |

[Figure in parentheses denoted percentage; *the percentage decimal cannot be 100]

SChE levels among exposed and non-exposed population were observed using student ‘t’ test. The mean ± S.D of SChE in farmers exposed to OP and control group is shown in Table 2. There was no significant difference observed among pesticide exposed and non-exposed

individuals. This study observed that the serum cholinesterase activity was decreased in pesticide users/applicators compared to non-exposed group in both males and females.

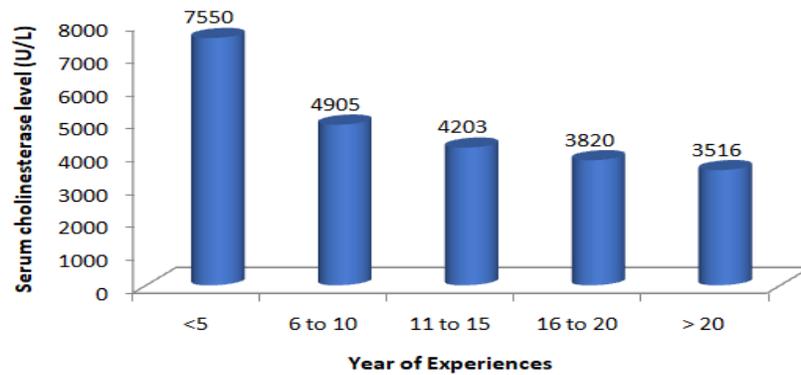
Table 2: Comparison of serum cholinesterase levels among cases and controls.

| Gender | Serum cholinesterase levels | | p value |
|---------|-----------------------------|----------------|---------|
| | Cases (n=52) | Control (n=50) | |
| Males | 5864±1208 | 7335±1581 | 0.06 |
| Females | 5203±1141 | 6608±1611 | 0.07 |

Comparison of serum cholinesterase levels according to duration of exposure of Organophosphates are well analyzed thereby the pesticide spraying individuals verses serum cholinesterase levels were

compared. The workers who sprayed pesticides were likely to have decreased serum cholinesterase levels (Figure 1).

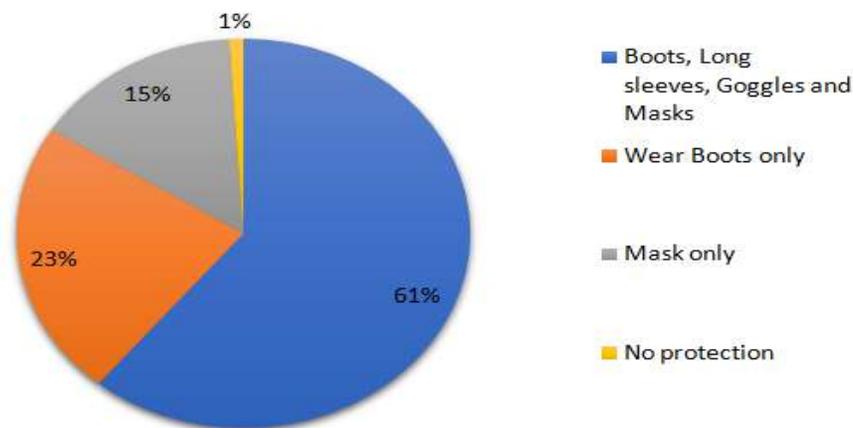
Figure 1: Comparison of serum cholinesterase levels according to duration of exposure of Organophosphates



Among the 52 farmers who sprayed pesticides, 34 to 56% practiced good protective behavior before, during, and after application. Among the sprayers, 61% wore

boots and longsleeved shirts, mask and during application, only 23% used goggles, whereas 15% wears mask alone. Usage of masks and goggles among farmers exposed to pesticides were given in Figure 2.

Figure 2: Usage of masks and goggles among farmers exposed to pesticides.



Self-reported pesticide-related symptoms among farm workers based on serum cholinesterase (SChE) levels (Table 3). The prevalence of clinical symptoms

like headache, dizziness, eye irritation and excessive sweating were associated with serum cholinesterase levels significantly.

Table 3: Self-reported pesticide-related symptoms among farm workers, by SChE levels

| Symptoms | Abnormal SChE (%) | Normal SChE (%) |
|-----------------------------|-------------------|-----------------|
| Headache | 18.2 | 6.6 |
| Dizziness | 15.5 | 4.5 |
| Nausea and vomiting | 4.5 | 3.6 |
| Fever | 1.2 | 0.6 |
| Fatigue | 2.8 | 3.5 |
| Eye irritation | 22.4 | 10.5 |
| Skin irritation/itching | 13.5 | 12.9 |
| Burning sensation on skin | 12.5 | 10.5 |
| Excessive sweating | 15.6 | 10.8 |
| Abdominal pain/ Diarrhea | 1.2 | 1.1 |

p < 0.01 - significant

Discussion

This study was proposed to categorize the serum cholinesterase levels of farm workers exposed to chemical pesticides in rural areas. Cholinesterase hydrolyzes acetylcholine (a neurotransmitter) into choline and acetic acid which is used in the proper functioning of the nervous systems of humans [8]. The RBC cholinesterase is found primarily in the blood and neural synapses while the pseudo-cholinesterase (BuChE) is normally found in the liver. The BuChE is generally used in reference to a clinical test that reflects levels of enzymes in blood which chemically interfere with the action of cholinesterase and become potent neurotoxins such as carbamate and organophosphate (OP) pesticides.

Among the exposed group, 88.2% and 11.8% were males and females respectively. Male workers are involved in pesticide mixing, loading, application and they spent majority of time in fielding when compared to females. The mean \pm S.D of SChE levels in males those who exposed to OP and non-exposed group were 5864 ± 1208 and 7335 ± 1581 respectively. The mean \pm S.D of SChE levels in females those who exposed to OP and non-exposed group were 5203 ± 1141 and 6608 ± 1611 respectively. There was no significant 'p' value observed among pesticide exposed and non-exposed individuals. This study observed that the serum cholinesterase activity was decreased in pesticide users/applicators compared to non-exposed group in both males and females. Males are spraying large hectare paddy field while females are spraying in small fields, particularly involved in vegetable planting. Hence, the decreased levels of SChE are reported in males than females and the decreased data also due to the awareness found among them and usage of PPEs.

The prevalence of abnormal SChE in our study was 19.5%. Chomthaisong et al. found that 66.2% of tomato growers for seed production and 48.1% of tomato growers for consumption were observed to have abnormal SChE activity [11,12]. The serum cholinesterase levels gradually declined as duration of exposure increased. Prolonged exposures without using proper PPEs resulted in increased risk of developing abnormal SChE levels and health hazards.

In our study, the farm workers had complaints of headache (18.2%), dizziness (15.5%), eye irritation (22.4%) and excessive sweating (15.6%) and were associated with decreased serum cholinesterase levels with significant 'p' value. These results were consistent with the study of Yassin et al [13]. The symptoms of headache, dizziness, fatigue and skin irritation were recorded as 35.2, 27.6, 20 and 11.4% respectively as given in the studies of Tunsaringkarn et al¹⁰. The factors like application method, use of personal protective equipment, work practices related to hygiene, spills, and attitudes toward risk may all influence the degree of pesticide exposure and the SChE values. Exposure to pesticides is related with increase in prevalence of many symptoms, having little evidence for specificity. The limitations of study are small sample size; symptoms relied on self-reports and examined clinically, may be influenced by recall bias or related to nonspecific symptoms and cross-sectional study predicts only causal association between exposure and SChE levels.

Conclusion

The mean \pm S.D of SChE levels in males those who exposed to OP and non-exposed group were 5864 ± 1208 and 7335 ± 1581 respectively. Serum Cholinesterase levels were low in farmers those who used pesticides in farming compared to non-exposed persons. The prevalence of clinical symptoms like

headache, dizziness, eye irritation and excessive sweating were associated with serum cholinesterase levels significantly. SChE were likely to be proportionately decreased in individuals with increased duration of exposure. These findings may warrant further studies and investigations in more detail.

From this study, it was suggested that appropriate training to the farmers is needed to reduce exposure to OP pesticide. Government can take certain necessary steps for screening of toxicity in the pesticides provided to the farmers; The farmers who have been supplied with these pesticides can be allowed to have a free access for routine checking of serum cholinesterase in primary health centres nearer to them, so that regular monitoring for blood cholinesterase to reduce pesticide exposure can prevent health effects. Both the farm workers and the non-working populations exposed through other modes of environmental contamination need control over the use of pesticides by developing monitoring and surveillance systems. Farmers can actually opt to organic manures, though possibly not easy to prepare, but it would prevent the ill-effects of these pesticides for both the farmers as well as consumers.

The special suggestion to the producers of the pesticides and the chemical authorities of various levels is to provide the PPEs as complementary material along with the pesticides so as the usage of PPEs may be increased among the end users.

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Review Article

India's COVID-19 vaccination programme: challenges and solutions

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ABSTRACT

Following January 2020, the SARS-COV-2 spread worldwide, ravaging health systems and causing widespread disruptions in the economic and social spheres. Successive waves of the COVID-19 pandemic in different countries concluded that the fight against the virus will be prolonged. Countries including India tried to reduce the spread of SARS-COV-2 by inducing restrictions on population mobility, high testing rates and dynamic algorithmic management but vaccination remained the only definitive answer against SARS COV-2. Though India started its vaccination program in a phased manner as early as January 2021, the second covid wave thwarted its gains with Indian facing a triple whammy of high incident cases, overburdened health infrastructure and inelastic vaccine supplies. In this review, we examined the strength, weakness, opportunities and threats (SWOT) of India's vaccination programme and also suggest solutions to augment its strengths, manage its weakness, consolidate on opportunities and neutralise threats with effective measures of priority vaccination in high-risk districts, war footing immunisation, emergency use authorisation of vaccines, expanding vaccination programme to include children above 12 years and countering vaccine hesitancy with fact and reason. The review concluded that by strategic immunisation of high priority/high-risk districts the upcoming third wave can be thwarted if not completely prevented at a lower economic and social cost.

Keywords: Vaccination, India, SWOT, SARS COV-2, COVID-19, Challenges

INTRODUCTION

The COVID-19 is an infectious disease caused by a newly discovered coronavirus SARS-CoV-2, which spread rapidly throughout the world from the Wuhan province of China from January 2020. In March 2020, the WHO declared the COVID-19 outbreak a pandemic. Following which successive waves of SARS-COV-2 infection occurred in different parts of the globe ravaging health systems, leading to economic catastrophe and affecting human development. Though several countries, including India, took strong measures to contain the spread of COVID-19 through measures like lockdown,

social distancing, high testing, containment and syndromic management, it is agreeable now that vaccinating a sufficiently large population will provide a lasting solution by enhancing immunity and containing the spread of the disease.¹

The vaccines which were under clinical evaluation in India require two doses to be administered four weeks to 12 weeks apart, through the intramuscular route.¹ Anticipating the COVID-19 vaccine roll out the government of India (GoI) was bestowed with the magnanimous task of expeditiously vaccinating its large population. One of the milestones in this direction was

the constitution of a national expert group on vaccine administration for COVID-19 (NEGVAC) a technical group of experts to guide on all aspects of the COVID-19 vaccination programme in India.¹

Countries like the US, UK started their vaccination programme earlier in early to mid-December.^{2,3} In India the COVID-19 vaccination programme was introduced on 16 January 2021 in three phases through prioritisation of beneficiaries in following order phase 1 included approximately 1 crore healthcare workers, phase 2 included 2 crore frontline workers and phase 3 included prioritised population of elderly and those with morbidities.¹

The successful introduction of the COVID-19 vaccination programme will largely depend upon the availability of effective vaccine commensurate with the demand, a dedicated and trained workforce, logistics, trained enumerators for beneficiary listing, the mobilisation of health functionaries for vaccination activities, social mobilisation through the involvement of local leaders, NGOs and community based organisations and finally advocacy of vaccination through mass media and social media.

In this context, the present communication attempts to do a situation analysis of the readiness of India's healthcare system for implementing the COVID-19 vaccination programme, identify the various bottlenecks and suggest possible solutions for the successful implementation of the programme.

CURRENT STATUS OF IMPLEMENTATION OF COVID-19 VACCINATION PROGRAMME IN INDIA

As on 31 May 2021, 16,86,13,371 (12.1%) people have been administered single dose of vaccine while 4,45,40,758 (3.2%) both the doses.⁴ The two vaccines that have been granted emergency use authorization by the central drugs standard control organization (CDSCO) in India are Covishield® (AstraZeneca's vaccine manufactured by Serum Institute of India) and Covaxin® (manufactured by Bharat Biotech Limited). The COVID-19 vaccine intelligence network (Co-WIN) system, a digital platform is being used to track the enlisted beneficiaries for vaccination and COVID-19 vaccines on a real-time basis.

According to the guidelines issued by the ministry of health and family welfare (MoHFW), people are advised to register on the Co-WIN app and receive the vaccine from government and private health facilities as notified, known as COVID vaccination centres (CVCs). Those who cannot get themselves registered online can contact their local government health workers, who will help the beneficiaries to the government CVC for on the spot registration, appointment, verification and vaccination on the same day.

Based on the potential availability of vaccines the government of India has selected the priority groups who will be vaccinated on priority as they are at higher risk. The first group includes healthcare and frontline workers. The second group to receive the COVID-19 vaccine will be persons over 60 years of age and persons between 45 and 59 years of age with comorbid conditions.⁵

Presently, as India desperately tries to control a deadly second wave of the novel coronavirus amid warnings of a third wave, many experts have said that fast vaccination is the only long-term solution for India. However, vaccination numbers have been falling of late because of inefficient planning as well as a dearth of supply.

India's daily COVID-19 shots have fallen sharply from an all-time high reached early last month as domestic companies struggle to boost supplies and imports are limited, even as the country fights the world's worst surge in infections. Daily inoculations have averaged 2.5 million since hitting a peak of 4.5 million on April 5. A quadrupling of coronavirus cases during the period has collapsed the public health system in many regions of the country.⁷

More than 13 million people aged 18-45 have registered for the jab, but states including central Madhya Pradesh and hard-hit Maharashtra have said they would not start vaccinating this age group on 1 May as planned due to supply problems. Many states have announced that they can't begin the vaccination drive as the manufacturers have not been able to keep up with demand.⁸ One estimate has suggested that at the present rate India will take 10.8 years to administer both doses to 70 percent of its population to attain herd immunity.⁹

Vaccine production in India is expected to benefit from the financial support announced by the Quad nations to increase COVID-19 vaccine manufacturing by 1 billion doses. The stock of items such as syringes and gloves for the frontline healthcare workers is to be aligned with the expected increase in vaccine production in India in the next two years.¹⁰

Currently, cold chain infrastructure is highly concentrated in urban areas. The inter-state disparity in the distribution of cold-chain points is another area that needs to be focused on to overcome challenges associated with vaccine distribution. With an increasing number of COVID-19 cases in several states due to the second wave of the virus, a wider vaccination program is becoming critical for controlling its spread.¹⁰

After considering these issues which may have a direct or indirect bearing on the effective implementation of the COVID-19 vaccination programme, here we attempted a SWOT analysis of the vaccination programme in India.

STRENGTHS

Robust primary health care system

The primary health care system in India has evolved since independence and there is an elaborate network of nearly 200,000 government primary health care facilities (GPHCFs), both in rural and urban areas.¹¹

Well-trained healthcare cadre

With over 25 lakh aanganwadi workers, 10 lakh ASHA workers, 27079 health assistants with 219326 ANM India has a dedicated cadre of grass-root works that form a backbone of India's public healthcare. Judicious utilisation of their services in the vaccination programme will be instrumental in achieving high vaccination coverage in the upcoming months.¹¹

Vaccine distribution, logistics are supply chains

One of the important factors which determine the timely supply of vaccines from the site of manufacture to different states is the logistics network availability. An adequate supply chain and logistics infrastructure, real-time visibility along the supply chain, micro-level planning to organize the administration of vaccines, effective planning and coordination among the agencies involved in vaccine administration are some of the factors expected to assist in overcoming vulnerabilities in the

vaccine supply chain. India's vaccine distribution is a robust network operated through four government medical store depots (GMSDs) in Karnal, Mumbai, Chennai and Kolkata which procure vaccines from the manufacturers. About 53 state vaccine stores get their supplies either from these GMSDs or directly from manufacturers.¹²

Strong administrative and legal machinery

The Union Ministry of Health and Family Welfare is responsible for the implementation of various programs on a national scale in the areas of health and family welfare. In addition, the ministry assists states in preventing and controlling the spread of seasonal disease outbreaks and epidemics through technical assistance.¹³ The epidemics act 1897 and the disaster management act of 2005 give a strong legislative framework for controlling the spread of diseases and streamlining roles of various government agencies during a disaster.

WEAKNESSES

Snail pace of vaccination

India's daily vaccine doses have fallen steadily over the month of April and May. As of May 15, India's 7 days moving average of vaccination is 18,73,005 doses for the preceding week.¹⁴ At such rate of vaccination India will reach only 35 to 40% immunised population by mid-November (Figure 1).

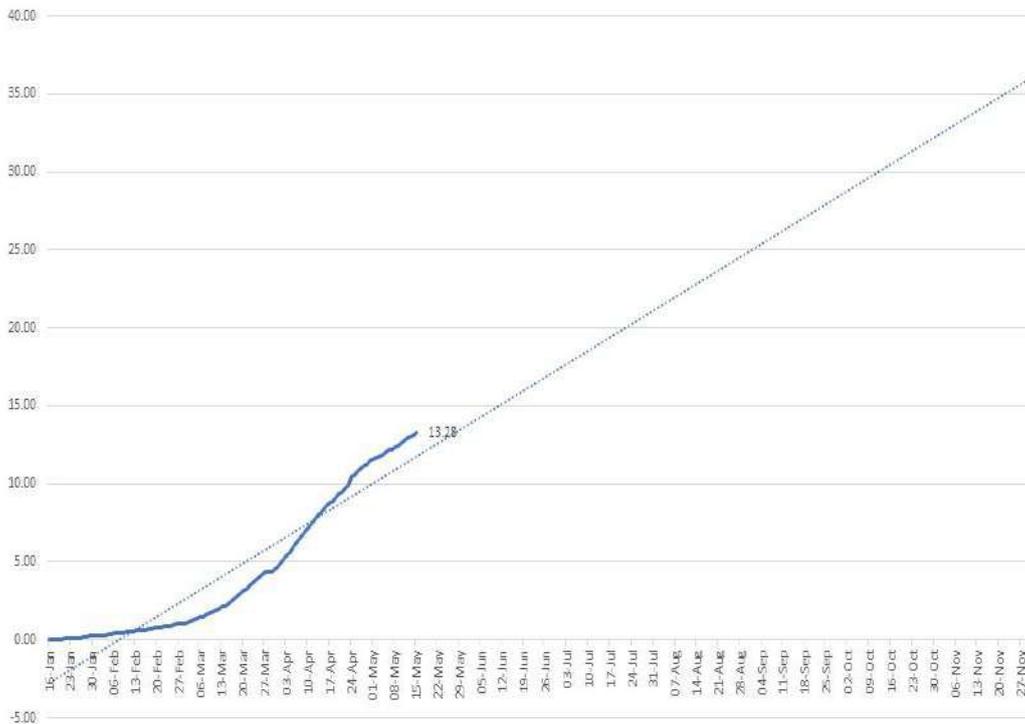


Figure 1: Linear projection of the population that will be vaccinated at the current pace of vaccination.

In elastic vaccine production capacities not being able to meet the demand

Presently, two private Indian vaccine manufacturers, Serum Institute of India (SII) and Bharat Biotech are struggling to meet India's domestic demand of 90 million doses every month besides their other contractual obligations. But they are producing about 70 million doses per month, which is considerably below the required quantity.¹⁵

Bureaucratic hurdles

The Indian bureaucracy considered bulwark of India's public administration has always delivered on the ideals of the constitution of India. But recently, there has been criticism of the bureaucracy regarding inordinate delay in decision making.¹⁶ This systemic fallibility has been brought to the fore during this pandemic which required fast-tracking of technical inputs to be transmitted more efficiently at ground level and eliciting better coordinator among different sectors.

Trade-off between lives and livelihood

Having to choose between life and livelihood is a grim situation for a country to be in. Governments can take care of livelihood concerns by getting food and money across to people, while they continue with a lockdown and use that time to add health facilities to brace for the rising tide of COVID-19 cases. It is important to stem that tide because it will hit at the heart of both life and livelihood.¹⁷

OPPORTUNITIES

Short term

India has successfully overcome the dreaded smallpox in the late 1970s and the crippling poliomyelitis in 2014 through mass vaccination programmes. It also runs the world's largest immunisation programme in the world through a dedicated network of well-coordinated immunisation centers which reaches the last person. If India is successful in vaccinating its population in a short period, it will be the greatest achievement in the history of public health and a role model to other nations.

Long term

The pandemic has also allowed strengthening the healthcare infrastructure and human resources through increased capital expenditure on health beyond the envisaged 2.5% of GDP by 2030. Healthcare spending on universal health coverage and social security measures will be yielding greater dividends if the majority of the population is covered with affordable health insurance programmes.

THREATS

An upcoming third wave of COVID-19 infection

According to the 'SUTRA model' created by experts at IIT-Kanpur it is projected that the third wave of COVID-19 cases is expected in 6-8 months. The impact of the third wave can be cushioned if an adequate number of people are vaccinated.¹⁸

Vaccine hesitancy among the population

The WHO defines vaccine hesitancy as a delay in acceptance or refusal of vaccines despite availability of vaccination services.¹⁹ It involves a complex mix of cultural, psychosocial, spiritual, political and cognitive factors.²⁰ A significant portion of the population with vaccine hesitancy for COVID-19 vaccine poses a significant threat to both the individual and their community, since exposure to a contagious disease like COVID-19 places the person at risk and individuals are far more likely to spread the disease to others if they do not get vaccinated.

A high migrant population difficult to track

As per the census, India had 45.6 crore migrants in 2011 (38% of the population) compared to 31.5 crore migrants in 2001 (31% of the population). Between 2001 and 2011, while the population grew by 18%, the number of migrants increased by 45%. In 2011, 99% of total migration was internal and immigrants (international migrants) comprised 1%.²¹ It is a humongous task to keep track of such large numbers of migrants for the COVID-19 vaccination programme especially with frequent lockdowns and unlocking of cities to tackle the pandemic.

Non-universal observance of COVID-appropriate behaviour

Central teams have reported non-adherence of COVID-19 appropriate behaviour in almost all of the 50 most affected districts in Maharashtra, Chhattisgarh and Punjab, the union health ministry said advising the states to more strictly enforce norms to prevent the spread of coronavirus. Of the 50 districts most affected by COVID-19, 30 are in Maharashtra, 11 in Chhattisgarh and nine in Punjab.²²

Fear of a replicating virus turning into a new mutation

Although it wasn't noticed at the time, the mutant B.1.617.2 had been sequenced and its genetic code deposited in the global database as early as October 2020. This new variant (designated as the delta strain) has spread fast, causing more than 60 percent of all coronavirus infections in the Indian state of Maharashtra alone.^{23,24} Fear of a new mutation that will escape vaccine efficacy and acquired immunity looms large if people are

not vaccinated faster enough to control the spread of infection.

SUGGESTED SOLUTIONS FOR EFFECTIVE IMPLEMENTATION OF THE COVID-19 VACCINATION PROGRAMME

Strategic immunisation

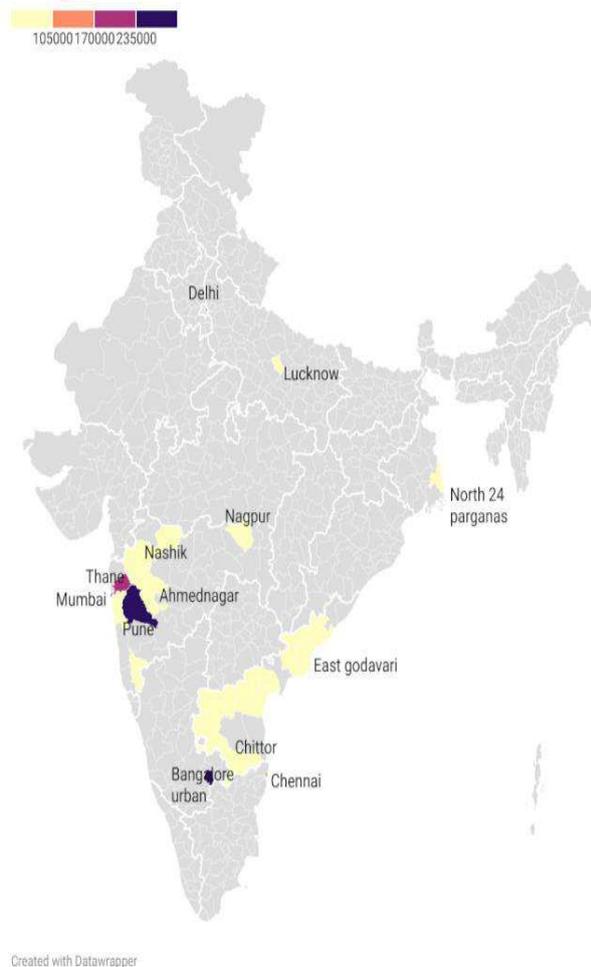
When the COVAX alliance co-led by CEPI, GAVI and WHO alongside key delivery partner UNICEF came into existence it aimed to accelerate the development and manufacture of COVID-19 vaccines and to guarantee every country in the world fair and equitable access to vaccines.²⁵ Unfortunately, the COVAX is severely underfunded and hampered by vaccine hoarding in high-income countries with only 1.1 billion doses of vaccine purchased by it as compared to the 4.6 billion doses bought by high-income nations.²⁶ India which believes in the idea of *vasudhaiva kutumbakam* (the world is one family) through its humanitarian and commercial

initiative of vaccine maitri has been instrumental in providing 64.5 million doses of COVID-19 vaccines to 95 countries around the world.²⁷ Following the second wave of COVID-19, India had to curb vaccine exports as cases surged and domestic vaccine shortfall was evident.

In event of such an inelastic vaccine supply, we suggested that strategic immunization should be carried out through prioritisation of vaccination in those districts which had the highest burden of cases and deaths in both the COVID-19 waves.

Figure 2 and 3 shows that in both the COVID-19 waves (24 March 2020 to 1 October 2020) and (1 March 2021 to 15 May 2021) similar districts were affected.¹⁴ Districts like Mumbai, Delhi, Chennai, Pune, Bengaluru urban continue to record the highest cumulative cases and deaths even in the second covid wave because of their previously well-researched factors like high population density, high mobility, high rural-urban migration and more interconnected social networks could well be turned high risk or hot spots districts.²⁸⁻³¹

Districts with Highest Cases (24 March 2020-1st October 2020)



Districts with Highest Cumulative cases (1st March 2021-15th May 2021)

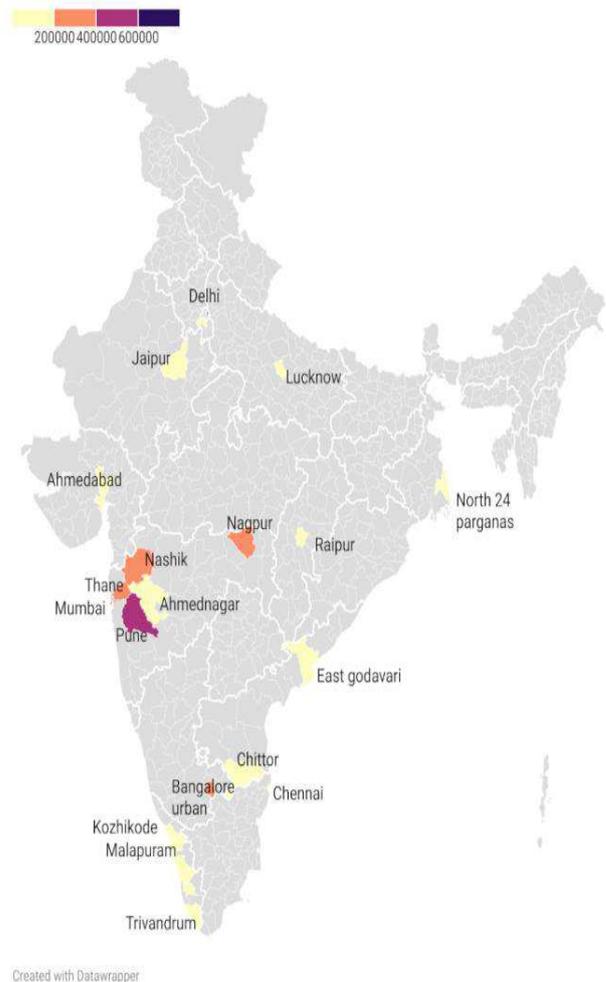
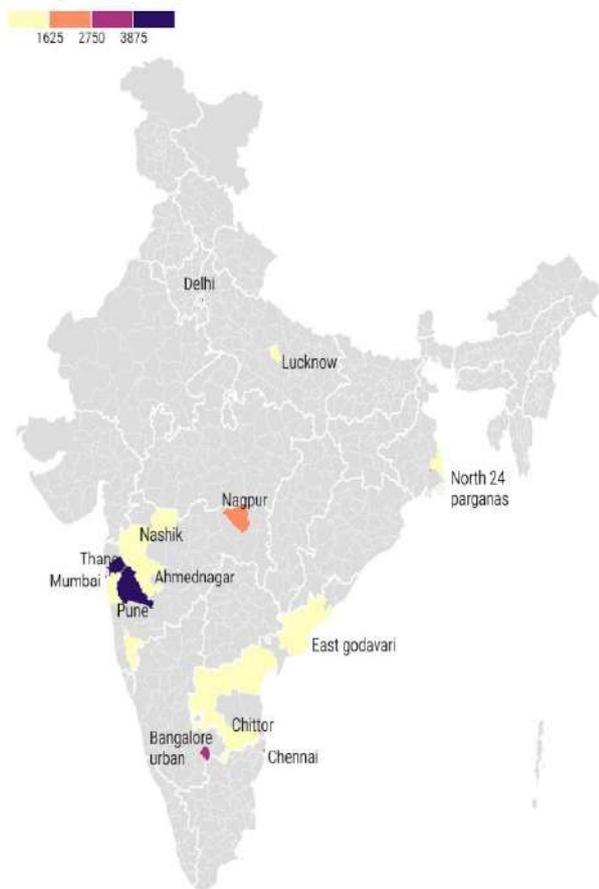


Figure 2: Similarity of districts reporting the highest number of SARS COV-2 in both waves.

Districts with Highest Deaths (24 March 2021-1st October 2020)



Districts with Highest death (1st March 2021-15th May 2021)

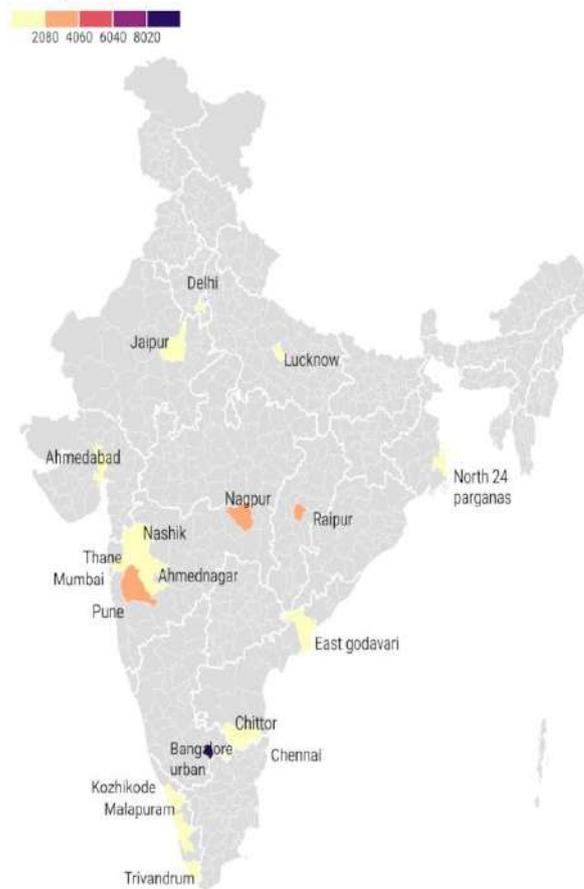


Figure 3: Similarity of districts reporting high COVID-19 related deaths in both the waves.

Table 1: Comparison of cases and deaths in both the COVID-19 waves in the highest 25 districts.¹⁴

| Comparison | 1st COVID wave (24 March to 1 October 2020) | 2nd COVID wave (1 March to 15 May 2021) |
|--|---|---|
| Cumulative cases in India | 6391480 | 13558915 |
| Cumulative cases of 25 districts | 2481248 | 4881679 |
| % of cases contributed by these 25 districts | 38.8 | 36.0 |
| Cumulative deaths in India | 99806 | 113033 |
| Cumulative deaths in 25 districts | 47667 | 43037 |
| % of deaths contributed by these 25 districts | 47.8 | 38.1 |

These 25 districts cumulatively contributed to 38.8 % of COVID-19 cases and 47.7 % of COVID-19 deaths in the first wave and 36% and 38% of cases and deaths respectively in the second wave (Table 1).

Secondly, these hot spot districts also serve as draining areas for the adjacent districts because of their employment opportunities. The resultant spill-over effect

of infections from high-risk districts is transmitted to these adjacent districts (satellite districts) which also continue to record higher infections in case of a rise in hot spots/high-risk districts. For example, the Thane district of Maharashtra is closely interconnected to the Mumbai district through well-developed transport networks. Also, Chengalpattu and Tiruvallur district in Tamil Nadu are in close transport proximity to Chennai report higher cases than other districts.¹⁴

So, if the inelastic vaccine supply continues and vaccine allocation is carried out proportionately according to the population, these high-risk districts will not get sufficient doses to achieve threshold herd immunity, leading to a high burden of the unimmunized population which are susceptible to infection and rapid spread of the disease.

The economic, social and psychological cost of SARS-COV-2 spread is higher for high-risk/hot spot districts than others.³² The report by Mumbai based care ratings states that potential loss of 40000 crores gross value added (GVA) took place in the phase of current lockdown since April 5 in the state of Maharashtra.³³ Once universal immunisation is carried out in these high-risk districts, these districts can go back to their pre-pandemic levels of livelihoods and economic growth.

Potential benefits of this strategy after a threshold population herd immunity is achieved include primarily a break in the chain of transmission, a faster reduction of daily incident cases and deaths, reduction in clustering of cases, reduce spillover effect in adjacent districts, reduce population pressure on health human resource and infrastructure, reduce the use of lockdown as a preventive measure to control the outbreak, faster resumption of economic activities and sustainable livelihoods, resumption of international travel, trade and tourism.

War footing immunisation

Data from the United States, United Kingdom, Israel and other countries suggests that vaccination with the high rate of vaccination infections and deaths related to COVID-19 can be brought down (Figure 4).³⁴

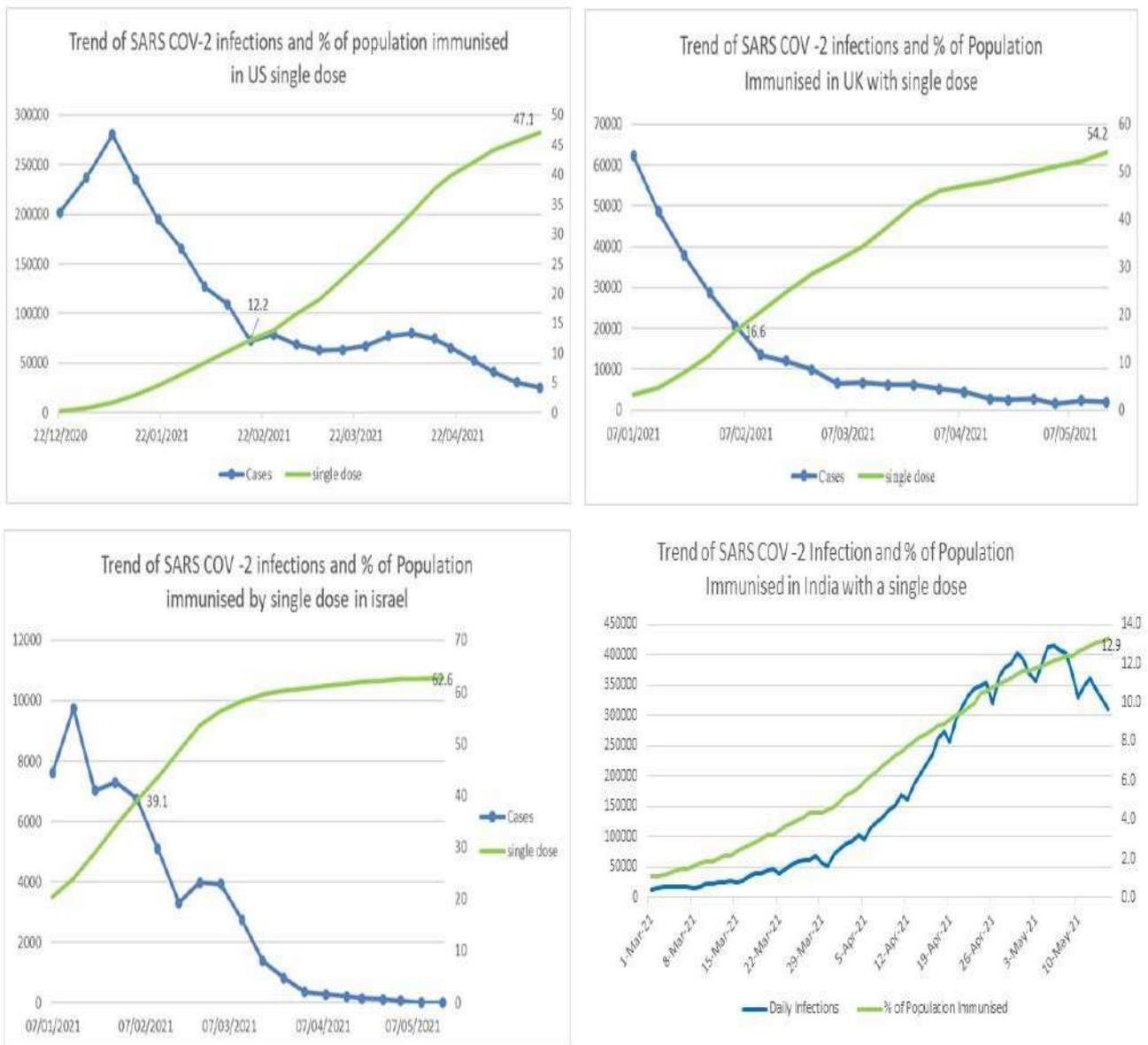


Figure 4: Trend of SARS COV-2 Daily cases and % of Population Immunised by a single dose.

The government should make vaccination of citizens the priority as a policy above anything else. This snail's pace of vaccination will hamper any attempt at acquiring threshold herd immunity. Vaccination programme can go 24×7 hours in major cities which do have adequately trained staff for this process.³⁵

In cities, there should be aggregated in 5-10 jumbo vaccination centers which can vaccinate 1 lakh people per day. This will save the cost of travel, logistics, maintain the cold chain and efficacy of the vaccine and streamline vaccine procedures.

The role of the private sector cannot be overemphasized here. Multinational companies can procure vaccines from the manufacture at agreed commercial costs and vaccinate their employees under corporate social responsibility (CSR) or even under their health insurance package.³⁶ Some states and cities have started mohalla or community vaccination which is a welcome step and will go a long way in achieving high coverage and reduce wastage.^{37,38}

Emergency use authorisation of vaccines with proved efficacy

The drug controller of India (DGCI) should consider emergency use authorisation (EUA) for more who listed vaccines like the Pfizer/Biontech, Astrazeneca-SK Bio, Serum Institute of India, Janssen and Moderna vaccines for emergency use as these are proved to be a success in bringing down the pandemic in US, UK, Israel and other countries.^{34,39} The focus should be on having more vaccines in the armour so that vaccine supply is commensurate to the demand. For example, if 5 crore doses of 5 different vaccine manufacturers are available every month, India will be able to immune 12.5 crore people and achieve threshold herd immunity of 67% (assuming the highest R0-3 for SARS-COV-2) in the next 6 months.

The mRNA vaccines which require storage below minus 18 degrees may not be feasible for administration in rural areas or district headquarters. These vaccines can be utilised in metropolitan cities where cold storage facilities and logistics are easily available and the population has purchasing power to access these vaccines.

Inclusion of population of between 12-18 years in the vaccination programme

The CDC recommends everyone 12 years and older should get a COVID-19 vaccination to help protect against COVID-19 as widespread vaccination is a critical tool to help stop the pandemic.⁴⁰ Countries like Dubai and Japan have started vaccinations.⁴¹ India cannot afford to leave 41% of the population less than 18 years of age unimmunised as it will not be able to achieve herd immunity and prone to repeated waves of infection.⁴²

Here we suggested the inclusion of the 12-18 year population under the COVID-19 vaccination programme.

Countering vaccine hesitancy with fact and reason

The state governments should take up the initiative of advocating the benefits of vaccines through building vaccine confidence by involving various stakeholders like the beneficiaries, public administrators, community-based organisations, socio-religious organisation, local political leaders, NGOs and most importantly women self-help groups. It can spread spell out vaccine success stories in other countries through effective mass media and social media communication. It can root in popular celebrities, politicians to advocate for vaccination to dispel the associated rumors and fear. We can encourage leaders in family, community or organizations to be vaccine champions which can help educate people about COVID-19 vaccines, how they are manufactured and monitored for safety.⁴³

Wastage

The national average of COVID-19 wastage is 6.5% on average. While vaccine shortage has hampered the COVID-19 vaccination program in several states, vaccine wastage is as high as 37.3% in Jharkhand (37.3%), 30.2% in Chhattisgarh and 15.5% in Tamil Nadu.⁴⁴ Wastage, in general, occurs at three levels, during transportation, during cold chain point and at a vaccination site-both at service and delivery levels. Daily vaccination drives must be well mobilized and planned, vials must be opened only after 10 beneficiaries arrive, adequate training must be given to healthcare workers on how to draw doses, rumors and misinformation about vaccines must be dealt with strictly and cold chain system must be maintained properly.

Compulsory vaccine certificate/passport to attend public gatherings and in-migration

Subsequent universal immunisation of a particular geographic area, the local governments should ensure that people attending a public gathering or in migrating should have vaccination certificate to prevent the outbreak of diseases.

CONCLUSION

History has taught us during smallpox eradication and polio elimination that a united response in a large and geographically diverse country like India is possible to overcome a common enemy.

Prioritizing high-risk districts for vaccination, the establishment of jumbo vaccination centers, enforcement of compulsory vaccination certificates, achieving involvement of the private sector will go a long way in increasing coverage of the vaccination programme. The governments need to take proactive decisions to

overcome vaccine hesitancy and wastage which at times might infringe upon individual autonomy but are essential in the interest of larger public good and which are well spelled out in Article 19 subclause 2(A) of the constitution of India.

Fast tracking of proven vaccines and import of vaccines from other countries cutting short the red-tapism is another key activity at the regulatory level which ensures quick availability of the vaccine to the target population. The SARS COV-2 virus is moving fast. We must move faster. Finally, we firmly believe in what the famous German philosopher G. W. F. Hegel said, 'The state is the march of God on Earth' and will be able to tide us over the present crisis.

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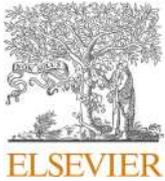
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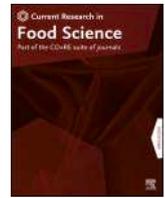
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Ultrasonic treatment: A cohort review on bioactive compounds, allergens and physico-chemical properties of food

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ABSTRACT

Implementation of ultrasonic for the extraction of bioactive compounds and retention of physico-chemical properties is an important technology. This technology applies physical and chemical phenomena for the extraction of compounds. Ultrasonic assisted extraction causes less damaging effect on quality properties of food as compared to the conventional extraction technique. The present review article focuses on the degradation of various bioactive compounds as a result of ultra-sonication which include vitamins, carotenoids and phenolic compounds. This review article also discusses the influence of ultrasonic extraction on the physico-chemical properties of extracted food products. In addition, the paper explores the effect of ultrasonication on food allergenicity through changes in solubility, hydrophobicity, molecular weight as well as conformational changes of the allergens, a direct result of increase in temperature and pressure during cavitation process.

1. Introduction

Post pandemic, there has been an enhanced consumer interest and attention towards healthy and nutritious food products. People are looking for food ingredients which not only provide nutrients but also enhance their wellbeing. In this regard, bioactive compounds, nutraceuticals and functional foods have captured widespread attention (Chemat et al., 2017). Several bioactive compound such as polyphenolics, flavones, vitamins and essential oils have been incorporated as supplements and ingredients by food manufacturers. These bioactive compounds play important roles as novel therapeutic agents and show significant medicinal effects such as anti-inflammatory, antimicrobial, antioxidant, anticancer and defending effects against various chronic diseases (Crozier et al., 2006). While trying to obtain maximum benefits from the bioactives, there is an ongoing struggle to minimize ill effects of certain molecules such as allergens. Traditional thermal extraction processing such as soxhlet extraction, water distillation extraction, steam distillation extraction has been used to extract bioactive compounds from plants. Several drawbacks are associated with thermal extraction for example, loss of nutrients, time consuming, and requirement of bulk quantity of chemicals as well as high energy consumption

and adverse environmental impact. Thermal processing also causes changes in various physical and chemical parameters. Some research also proved that the thermal processing decreases the bioavailability of some bioactive compounds (Sun et al., 2016). To overcome these drawbacks, novel extraction techniques are being explored for extraction as well as food processing which include ultrasound-assisted, microwave-assisted and supercritical extraction systems (Dogan et al., 2019). These novel technologies have enhanced the extraction of bioactive compounds while reducing adverse effects associated with traditional technologies.

Over the past three decades, ultrasonic treatment has been deemed feasible “green” technology for extraction in chemistry, food and pharmaceutical industries. Green extraction aims to reduce solvent, energy, wastes and environmental pollution while obtaining highest yield of product (Wei et al., 2015). Ultrasonic treatment is extensively employed in food industry for various applications for example, extraction, tenderization and preservation. Full extraction with higher purity of the final product can be achieved by ultrasonication in a few minutes with high reproducibility, decreasing the usage of solvent and removing the post-processing steps involved in waste water treatment (Chemat et al., 2017). Ultrasonic treatment has a potential to fulfill FDA requirement of

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5 log reduction of microorganisms in beverages (Salleh-Mack and Roberts, 2007). However, there are some adverse effects of ultrasonic treatment on bioactive compounds. For example, Tiwari et al. (2008) reported the reduction of anthocyanin content in strawberry juice by 3.2%. An interesting group of molecules that has been studied in relation to ultrasonic treatment is allergens. Allergens are generally proteinaceous compounds that elicit exaggerated immune response in the form of food allergies. Since ultrasonic treatment can change the native confirmation of proteins (Ma et al., 2018) it can alter the resulting allergenic potential of compounds making it relatively safe for consumption in target consumer groups. Ultrasonication also has different effects on the physico-chemical properties of food products. Therefore, this review explores the other side of the coin with focus on degradation of bioactive compounds as well as allergens through ultrasonic treatment. The present review article clarifies the mechanism of ultrasonication and its effect on degradation of various bioactive compounds including vitamins, carotenoids and phenolic compounds along with any other limitations if any.

2. Working principle/mechanism

In ultrasonic technique, acoustic vibrations or mechanical waves are used on the sample with frequencies from 20 kHz to 100 MHz. Ultrasonic assisted extraction (UAE) is based on the cavitation, thermal and mechanical phenomena, and is considered to be the one of the most effective techniques for the extraction of compounds of interest (Fig. 1). This combined phenomena causes cell wall rupture, decrease in particle size as well as increment in reaction rate through mass transfer across the cell wall. Ultrasonic treatment causes mechanical impact on the cell wall. This enhances the solvent penetration into the cell. Maximum intracellular compounds thereby dissolve in solvent which is further collected and purified. Thus, ultrasonic technique can potentially accelerate the extraction of bioactive compounds (Wen et al., 2018). The extraction is carried out in gaseous or liquid solvent by the result of cavitation which builds on liquid-liquid or gas-liquid interfaces. During extraction, mechanical fragmentation of the cell wall occurs which aids in easy removal of the extract from the cell (Ilbay et al., 2013 and Dogan et al., 2019). Ultrasonication is applied for the extraction of bioactive compounds with less treatment time, reduced energy consumption, less solvent requirement and is rather simple to use as a technique (Chemat et al., 2008). This technique is one of the fastest extraction methods and very effective since the cell wall gets ruptured by the operation of ultrasonic. When the ultrasonic waves pass through the liquid, it results in compression and expansion cycles. The expansion part makes bubbles or cavities in the fluid. The processing of bubble development, growth and implosive disruption is called as cavitation (Luque-García and Luque de Castro, 2003). In the pure liquid system, the bubble retains its circular structure as the environment around the bubble is uniform. When the

circular bubble ruptures near a solid surface it becomes variable in shape and generates high-speed jets of solvent against the cell walls and enhances the solvent penetration into the cell wall. This improves the interaction between solid and liquid as shown in Fig. 2. In addition, in the solid material ultrasonic wave causes swelling, hydration and expansion of the pores in the cell wall. This increases the diffusion and hence improves mass transfer (Vinatoru, 2001). However, cavitation phenomena can also introduce new reaction mechanisms with the formation of different types of free radicals. Generally, hydroxyl radicals are formed when water is used as a solvent. Sometimes highly reactive free radicals can be liberated which can alter other compounds for example proteins but also simultaneously accelerate the extraction of bioactive compounds (Wen et al., 2018).

3. Effect of ultrasonication on bioactive compounds

Bioactive compounds are often secondary metabolites which exists in plant tissues and aid the plants against external & internal stress thereby increasing their chances of survival. These are additional nutritional compounds present in food which provide positive benefits to the human health. These compounds usually degrade during conventional thermal processing and storage. However, various studies proved that ultrasonic extraction retains most of the bioactive compounds. Bioactive compounds consist of vitamins, total phenolic compounds, carotenoids and so on. Phenols are further divided into flavonoids and non-flavonoids. Flavonoids consist of anthocyanins, flavonols, flavones etc., whereas, non-flavonoids contains tannins, stilbenes and phenolic acids (Crozier et al., 2006). Specific studies have highlighted the effect of ultrasonic technology on several key bioactives as mentioned below.

3.1. Vitamins

Vitamins are essential micronutrients help in maintaining health and growth of humans. Vitamins are a group of water and fat soluble molecules which are abundantly sourced from various natural sources. These vitamins are extensively used as functional ingredients in food, drugs and cosmetics. Vitamin C, also known as ascorbic acid, is a water soluble vitamin which has proven antioxidant activity. It is thermolabile and unstable at extreme processing parameters. The degradation of vitamin C is carried out by aerobic and/or anaerobic pathways. Tiwari et al. (2008) studied the effect of ultrasonic treatment on ascorbic acid in strawberry juice. The amplitude of the sonicator probe from 40 to 100% with fixed frequency of 20 kHz and treatment time of 2–10 min was applied to the strawberry juice. It was observed that the ascorbic acid content was reduced by 11% at the highest processing conditions. This study has mentioned the reaction mechanism of degradation of ascorbic acid. The degradation may be due to the oxidation reactions supported by the free radicals produced during sonication. The mechanism is based

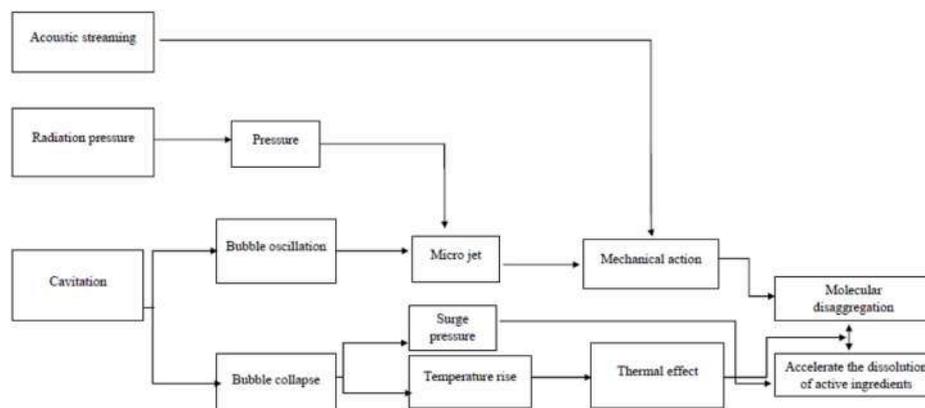


Fig. 1. Diagram of ultrasound-assisted extraction mechanism (Wen et al., 2018).

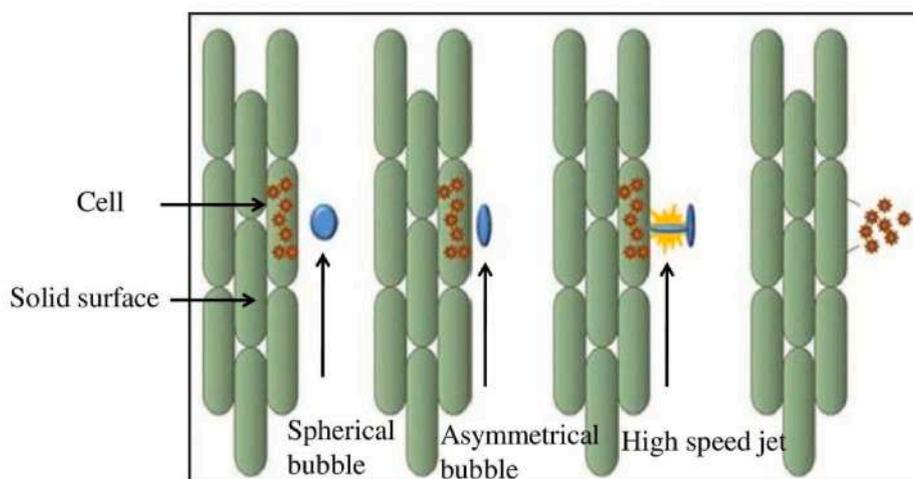


Fig. 2. Disruption of cavitation bubble and discharge of plant extract (Vardanega et al., 2014).

on the thermolysis and combustion that occurs within the cavitation bubbles, which is considered to be the primary path for the degradation. It can also be due to the interactions between free radicals and ascorbic acid causing the development of oxidation products on the bubble surface. Another study on orange juice also proved the degradation of ascorbic acid in orange juice during ultrasound processing (Valdramidiset al., 2010). A 1500W ultrasonic processor was used for the sonication of orange juice. The prepared samples were treated at fixed frequency of 20 kHz, amplitude from 24.4 to 61.01m, temperature from 5 to 30 °C and time from 0 to 10min. Treatment time has a significant effect on the decrease of ascorbic acid in orange juice. First order degradation kinetics was observed for ascorbic acid. The highest decrease in ascorbic acid content was found at 61.01m amplitude and at 30 °C temperature. Decrease in ascorbic acid led to final content being 15% of the original amounts found in fresh orange juice. In this study it was concluded that the rise in temperature and amplitude produced higher degradation of ascorbic acid.

The water-soluble vitamin B12, also known as cobalamin is also sensitive to degradation during various processing conditions such as heat, UV, oxygen and pH. The heat extraction of cyanocobalamin was compared with ultrasonic-assisted extraction and the subsequent stability of cyanocobalamin was assessed by Chandra-Hioeet al. (2020). In this study no degradation was seen during ultrasonic bath extraction treatment. This might be due to the low intensity of ultrasonication used for the extraction i.e. $<1 \text{ W/cm}^2$. Whereas, degradation of cyanocobalamin was observed in heat extraction treatment. Industrial processing has significant impact on the degradation of α tocopherol form of vitamin E. α tocopherol is very sensitive and can be readily oxidized during processing (Gawrysiak-Witulaska et al., 2009). The influence of ultrasonic processing in both bath and probe type system on the degradation kinetics of vitamin E in avocado puree was studied by Fernandes et al. (2016). Ultrasonic power-densities from 55 to 5000 W/L were used at 23 and 40 °C. Ultrasonic treatment causes the degradation of vitamin E in avocado puree and this effect is more significant in probe ultrasound with power densities from 1000 to 5000 W/L. Tocopherol was reduced by 79% at a power density of 5000 W/L during the initial steps of processing. Whereas, no major loss of tocopherol in avocado puree was found in bath ultrasound at 23 °C. The degradation of tocopherol may be due to the act of tocopherol in maintaining the levels of H_2O_2 concentration and other oxidizing agents in the cell.

3.2. Carotenoids

Carotenoids represents an essential category of natural pigments in

fresh produce. Carotenoids are classified into two groups which include carotenes and xanthophylls. α -, β -carotene, lycopene are the examples of carotenoids. Whereas, the lutein and zeaxanthin are the examples for xanthophylls (Ayelén Vélez et al., 2017). Various studies have proved the relation between high carotenoid intake and reduced chances of chronic degenerative diseases for example cancer and cardiovascular disease (Kritchevsky, 1999) thereby establishing it as an essential bioactive group to be studied. Kumcuogluet al. (2014) found a progressive increase in extraction yield of lycopene from tomato paste processing waste in both ultrasound assisted extraction and conventional organic solvent extraction treatment. In this study, it was noticed that there was initial sudden rise in the lycopene yield with increase in time in both the extraction methods. This may be because of the high lycopene concentration gradient between the solvent and the cell during the initial time of the extraction process. Further, the values of lycopene yield for 30 and 40 min for conventional organic solvent extraction matched those to the yield at 20 and 30 min for ultrasound assisted extraction method. During extraction with ultrasonic treatment, cavitation and thermal effects has a significant effect on the extraction yield. As the intensity of power increased, additional energy was moved for cavitation. This phenomenon causes the increases in lycopene yield. When the ultrasonic intensities are lower, the thermal effect is negligible because heat generated from ultrasonic may get entirely diffused. Whereas, with the increase in ultrasonic intensity, the cavitation effect reduces while there is an increase in the thermal effect. Similarly, Yilmaz et al. (2017) have done a comparative study between ultrasound-assisted extraction and conventional organic solvent extraction methods for the lycopene and β -carotene extraction from tomato-processing wastes. These authors also found the similar results for both the extraction methods. Ultrasound-assisted extraction required shorter time for extraction in comparison to conventional organic solvent extraction method. Highest lycopene and β -carotene yield was achieved at 90W ultrasonic power for 30 and 15 min, respectively. Lycopene concentration increased with the increase in time for the ultrasonic power of 50, 65, 90W. However, β -carotene concentration reduced at 90W. This may be due to the sensitivity variation of lycopene and β -carotene in thermal treatments. Lycopene was more resistant to thermal degradation in comparison with the other carotenoids for example, α -carotene and β -carotene. Li et al. (2013) found the amount of carotenoids extracted with ultrasonic treatment was equal to the amount obtained in conventional solvent extraction method. In this experiment, carotenoids were extracted from fresh carrot using ultrasonic-assisted extraction and solvent extraction. Sunflower oil was used instead of organic solvent while performing ultrasonic-assisted extraction. The maximum yield of β -carotene using ultrasonic-assisted extraction i.e.

334.75 mg/L was achieved in 20 min whereas, solvent extraction required 1 h for obtaining the similar quantity of β -carotene of 321.36 mg/L. These researchers have not obtained carotenoid degradation with ultrasonic treatment using sunflower oil. Chuyen et al. (2018) observed gradual increase in carotenoid concentration with extraction time at three different ultrasonic powers. Lutein has therapeutic property for the prevention of human eye degenerative diseases. Song et al. (2015) studied the stability of all-trans lutein under the influence of ultrasonic treatment. It was observed that the ultrasonic treatment causes isomerization of lutein to its isomers. The degradation patterns changed at different temperatures. First-order degradation kinetics of all-trans lutein was seen at 20 °C. Whereas, second-order kinetics was observed at 30–50 °C. The gradual reduction in retention rate of all-trans lutein occurred with the increment in ultrasonic frequency and power. Whereas, the retention rate of all-trans lutein enhanced with the increase in temperature.

3.3. Phenolic compounds

Phenolic compounds are the main bioactive components of various fruits and vegetables because of their influence on the sensorial properties and nutritional qualities of food positive effect on health (Dogan et al., 2019). They are further categorized into different groups such as phenolic acids, flavonoids, tannins etc. Anthocyanin is a primary group of water soluble phenolic compounds and provides color to the fruits (Gan et al., 2018). Anthocyanins have been proved to be effective in preventing diseases such as cancer, coronary heart diseases and other metabolic disorders. Anthocyanins are very unstable and degrade instantly during extraction, processing and storage (Fennema and Tannenbaum, 1996). During ultrasonic extraction, cavitation causes severe physical changes inside the bubbles. This leads to various events such as destruction of bubbles, degassing, formation of free radicals etc. This further results in changes in temperature, pressure and physical interaction between solid and liquid interfaces which induces the degradation of anthocyanins (Gomes et al., 2017). Setyaningsih et al. (2016) worked on stability of 40 phenolic compounds from the various groups such as cinnamic acids, catechins, stilbenes, flavonols, benzoic aldehydes under ultrasonic assisted extraction. The effect of treatment temperature from 10 to 70 °C for 20 min on the stability of 40 phenolic compounds was studied. Interestingly, it was observed that many of the phenolic compounds were stable at extraction temperature of 70 °C. Whereas, a few phenolic compounds started degradation at 50 °C. Therefore, to achieve more than 90% recoveries, it was suggested to perform ultrasonic extraction treatment at temperature between 10 and 50 °C for nearly all phenolic compounds except gallic acid and kaempferol. 12 and 16% degradations of gallic acid and rutin was found at starting temperature of 60 °C for UAE. However, phenolic compounds such as *p*-coumaric, *o*-coumaric and resveratrol indicated 11, 12 and 21% degradation at 70 °C under UAE.

Antioxidants delay or prevent oxidation of macromolecules such as lipids, proteins and DNA under oxidizing conditions. Various researches have confirmed that the oxidative stress is related to several diseases such as diabetes, neurological disorders, cancer as well as cardiovascular disorders (Vilkhuet et al., 2008). Antioxidant activity of bayberry juice was recorded as percent DPPH inhibition and the initial value was 193.19 mg TE/100 ml (Cao et al., 2019). Low ultrasonic intensity of 90.41 and 180.82 W/cm² did not cause significant changes in DPPH inhibition values. Whereas, significant reduction in antioxidant activity was observed after 6–10 min treatment time at intensity of 271.23, 361.64, and 452.05 W/cm². While using ultrasonic treatment, low intensity with less treatment time had no significant effect on antioxidant activity. Hence in the conclusion of this study, it was seen that with the higher ultrasonic intensity and time, the antioxidant activity gradually reduced for bayberry juice. Also, Sun et al. (2016) examined that the increase in sonication power from 200 to 500 W and processing time from 0 to 60 min, led to significant decrease in the antioxidant capacity of

pelargonidin-3-glucoside. In comparison with control sample, the maximum reduction in antioxidant activity was found to be 74.77% and 72.74%, at 500W for 60 min as analyzed by the FRAP and DPPH procedure, respectively.

In another study, UAE was compared with solvent extraction for the potential recovery of phenolic compounds from pumpkins and peaches (Altemimi et al., 2016). Optimal extraction of total phenolics from pumpkins was achieved using UAE at a temperature of 41.45 °C, power of 44.60% with a treatment time of 25.67min. Whereas, for peaches the optimum conditions for the extraction of total phenolics was at temperature of 41.53 °C, power of 43.99% and time of 27.86 min. A comparative study has been conducted on the extraction of phenolic compounds with conventional and ultrasound-assisted extraction techniques from vegetable sources (Medina-Torres et al., 2017). In this study it was demonstrated that phenomenon of acoustic cavitation is responsible for the effective recovery of phenolic compounds. Further, it was concluded that the energy requirement, treatment time and temperature in UAE is comparatively less than the conventional method of soxhlet extraction. Hence, UAE is very helpful for the extraction of thermo-sensitive compounds.

4. Effect of ultrasonication on allergens

Food allergy is an adverse immune response initiated by consumption of specific foods or food additives. Most allergens are known to be water-soluble glycoproteins having masses of 10–70 kDa that show the following functionalities (1) Ability to sensitize a genetically predisposed individual by initiating formation of IgE antibodies, (2) Binding to those generated IgE antibodies, and (3) Commencing an exaggerated immune reaction post IgE binding. Most common symptoms of food allergies are gastrointestinal, respiratory, cardiovascular, and cutaneous symptoms and eventually an anaphylactic shock. Studies have reported eight major food groups, namely eggs, milk, fish, shellfish, tree nuts, peanuts, soybeans, and wheat that account for approximately 90% of all the allergic reactions (Nayak et al., 2017).

High-intensity ultrasound equipment involves mechanical waves within a frequency range of 20–100kHz (Feng et al., 2011). The high energy waves cause physical and chemical changes through formation of bubbles in food matrix via intermittent compression and rarefaction until critically sized bubbles collapse. The increase in temperature and pressure (up to 5000K and 1000atm, respectively) as a result of the collapsed cavities forms the basis for conformation change of allergens and their resulting reactivity. High-velocity gradients and shear stress resulting from sonication lead to micro-streams that have structural effects like alteration of the native protein structure, formation of new intra-/intermolecular interactions, and breakdown of the large molecules (Soria and Villamiel, 2010). Cavitation changes the allergenic proteins and influences the ability of antibodies to interact with the modified proteins, thereby reducing the chances of IgE-mediated food allergic reactions. Additionally, high agitation caused by micro-streaming often disrupts Van der Waals interactions and hydrogen bonds in polypeptides, resulting in protein denaturation. Thus, researchers attributed lower allergenicity after ultrasonication processing to changes in solubility & hydrophobicity, molecular weight as well as conformational changes. Table 1 highlights the effect of sonication treatments on allergens from common food products such as milk, shrimp, crabs, peanuts and soy flour. Varying frequencies and treatment times for different food products led to differential reduction in allergenicity. In case of shrimp allergenicity was seen to reduce from 100% to 25.3% using ELISA assay, thereby highlighting the massive potential this technology has against allergens. Researchers have also explored ultrasonication assisted enzymolysis of proteins to obtain biofunctional hydrolysates and peptides (Liang et al., 2017). It has been well established that breakdown of high molecular weight proteins in to low molecular peptides is associated with lower allergenic profile and enhanced bioavailability (Arteaga et al., 2020). Hence many

Table 1

Ultrasonication as a strategy to reduce protein allergens in commonly consumed animal and plant protein sources.

| Allergen | Food source | Processing treatment | Effect on biomolecule | Effect on allergenicity | Reference |
|--|-------------|--|---|---|-------------------------|
| Casein | Milk | 25 kHz frequency, 900 W, 20 °C, 60 min | Colloidal casein with lower diameter | IgE binding capacity reduced from 1.09 to 0.93 | Wang et al. (2020) |
| β -lactoglobulin (BLG) and α -lactalbumin (ALA) in whey protein hydrolysate | Milk | Ultrasound-Ionic Liquid pre-treatment at 300 W ultrasonic power for 15 min | Smaller molecular weight peptides increased | Antigenicity of alcalasehydrolyzed ALA & BLG decreased by 68.54% and 66.58% | Zhang et al. (2019) |
| Tropomyosin fraction | Shrimp | High intensity ultrasound treatments (30 Hz, 800 W) for 30–180 min | Results in low molecular weight fraction | Allergenicity reduced from 100% to 25.3% (ELISA) | Li et al. (2006) |
| Tropomyosin from crab crude extract (CCE) | Crab | Ultrasonication at 200 W, 30 °C for 60 min, | IgE-binding sites on allergen destroyed | ELISA inhibition reduced approximately by half-80%–40% for chymotrypsin-Ultrasonicated samples | Yu et al. (2011) |
| Ara h 1 and Ara h 2 | Peanuts | 1–5 h at 50 Hz. | Reduced solubility of Ara h1 | Ultrasonication followed by trypsin and chymotrypsin digestion had the highest IC50 (31.95 lg/ml) | Li et al. (2013) |
| Glycinins&Conglycinin | Soy flour | Extraction with an ultrasonic dismembrator at 60 Hz, 300 W | Reduced solubility | Least amount of soy proteins detected using ELISA in sonicated samples (14 mg/ml) as compared to conventionally (28.99 mg/ml) extracted samples | Amponsah & Nayak (2016) |

hydrolysate and peptide products with higher degree of hydrolysis and resulting lower molecular weight peptides are used in sports nutrition and other functional applications.

Other novel studies have been undertaken to conjugate proteins with polysaccharide with an attempt to enhance functionality of the protein while reducing its allergenicity. One such study involves buckwheat protein isolate (BPI) conjugated with dextran through ultrasonication using 10 mm titanium tip for 80min at 70 °C and ultrasonic intensity of 544.59 W/m² (Xue et al., 2017). Thus ultrasonic treatment can be effectively used for processing allergen containing products and converting them to product safe for individuals with allergies and intolerances. This would no doubt increase consumer base and economic returns to the food manufacturers.

5. Effect of ultrasonication on physicochemical properties

5.1. Texture

Ultrasonication can have varied effects on the texture of a food product. The effect of ultrasound and cavitation on the changes in macromolecules can lead to changes in the hardness/toughness or tenderness/softening. The effect seen on texture is mainly due to mechanical breakdown of myofibrillar protein structures, rupture of collagen macromolecules and enhanced proteolysis or protein denaturation and myofiber fracture when meat is treated in ultrasound baths or probes (Alarcon-Rojo et al., 2019). Depending on whether toughness or tenderness is preferred end result, sonication can lead to desirable or undesirable end product. Hence optimization of sonication treatment for different food products, along with correlation studies on sensorial traits is necessary before application of this pre-treatment. One such study focussed on the effect of ultrasound on raw and boiled shrimps by treating them with power ultrasound (30 kHz, 800W) at 0 °C (treatment 1) and 50 °C (treatment 2) for 0, 2, 8, 10, and 30 min. Ultrasound treatment for 30 min lead to an increase in hardness in treatment 1, to a peak-1.5-fold higher than the control, compared to 27% increase in treatment 2 (Li et al., 2011). The increased hardness and chewiness of the product was not correlated with sensory traits but reduced allergenicity was seen for the treated sample. The increased hardness was attributed to osmosis and water loss from the tissue after sonication. A study focussed on using high-intensity ultrasound as pre-treatment for drying of pear slices studied the resultant texture characteristics of the dried pear slices. Ultrasound equipment at a frequency of 24 kHz and amplitudes of 25, 50, 75 and 100%, was utilized in this study. It was seen that the hardness of pear slices decreased [from untreated sample (104.72N), 50% of amplitude (93.461 N) to 100% amplitude (62.206

N)] with an increase in ultrasound intensity and all the process parameters had significant effect on the textural properties of pear slices (Dujmic et al., 2013).

Another study of beef steak analyzed the effects of low-frequency, high-power ultrasound (40 kHz, 1,500W) on meat quality. Beef steaks were sonicated for 10, 20, 30, 40, 50 or 60 min and it was observed that most sonicated beef samples showcased higher water-loss rates when compared to the control sample and 10 min-sample. Water-loss rate increased with duration of ultrasound treatment. The observed effect may be due to the disruption of the cellular structure and the resulting water loss in often associated with negative effect on sensory characteristics of steaks (Chang et al., 2015). As seen from the studies on texture of shrimp, pear and beef, the effect of ultrasonication varies depending on the type and composition of food matrix. While ultrasound has been effectively used for reducing microbial load and extending shelf life, its effect on texture cannot be ignored and needs detailed studying before adaptation of this technology. According to Fig. 3, it has been proved by Moghimi et al. (2018) that ultrasonic causes changes in structure of black cumin seeds. These authors proved that the power of ultrasonic wave has more influence on the oil extraction yield than irradiation time. Fig. 3 shows that the improved extraction efficiency of oil is due to the rupture of cell membrane and formation of several holes. The cavitation bubbles collapse on the external surface leads to deformation in the pressure zone. This phenomena causes degradation of cells' membrane and development of several holes.

5.2. Color

Color is one of the essential organoleptic parameters which determines the quality of food and helps in drawing the attention of consumers. With the increase in ultrasonic power, the color parameters are also affected. The study about the influence of ultrasonic on the extracted oil of black cumin seeds proved that the increase in ultrasonic power increases the value of color index of the extracted oil. The reason behind the increase in color index may be due to the depletion of pigments which include chlorophylls and dissociation of phospholipids during ultrasound treatment. Zhang and Wang (2017) studied the influence of ultrasound irradiation on the absolute color as well as color density of wine during storage. The color and color density of wine treated with ultrasound was higher as compared to the non-treated control sample. It was also found that more the treatment time of ultrasound, the more rise in the value of color and color density during the storage period. This study concludes that the ultrasound irradiation enhances the color of red wine. The increase in color pigments may be due to the local spontaneous increase in temperature and pressure

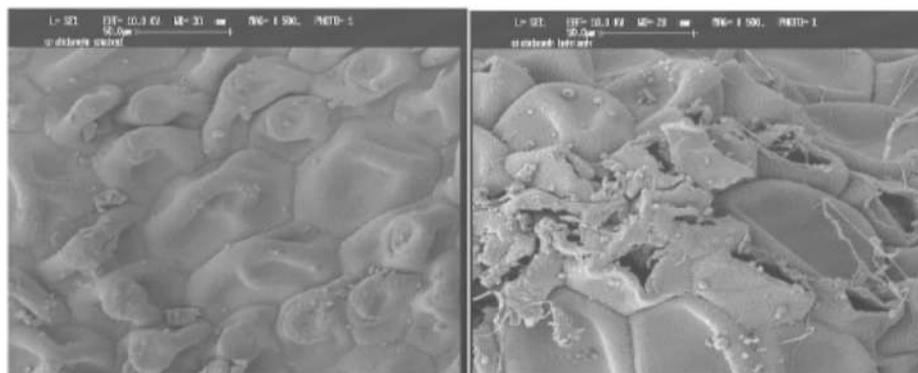


Fig. 3. SEM images of a black cumin seed structure 1) without ultrasound treatment and 2) with ultrasound treatment (Moghimi et al. (2018)).

produced by the acoustic cavitation which arbitrates free radicals and chain reactions. In another study, the effect of thermosonication on the color of clear red pitaya juice was checked (Liao et al., 2020). In this case similar observations were made that is more the treatment time and increase in temperature more the recognizable variation in color. Reduction in L^* , a^* and b^* value of thermosonicated sample with increase in input power, temperature and treatment time was observed. Degradation of betacyanin pigment which gives red-purple colour to the pitaya juice might be responsible for the observed color loss. Influence of ultrasound on the polymeric color and color density of bayberry juice was evaluated by Cao et al. (2019). Ultrasonic treatment showed no significant effect on color density of bayberry juice. Whereas, polymeric color enhanced by 6.93–31.73% with the increase in ultrasound intensity and treatment time. Compared with control sample, an increase in the value of percent polymeric color of ultrasonic treated bayberry juice by 11.99–14.19% was also observed. The development of polymeric color may be because of the production of chalcone which is a transitional product of anthocyanin degradation. This chalcone was not stable and would easily degrade to brown color which increases the polymeric color.

In addition, the results of the studies conducted by researchers on the influence of ultrasound on browning are not consistent. According to the investigation conducted by Sun et al. (2015), it was found that the ultrasound prevents the browning of fresh apple juice. Controlling enzymatic browning is found to be a major challenge for fresh apple juice processors. Fig. 4 demonstrates improved color and uniformity of ultrasonically treated fresh apple juice as compared to non-treated sample. The ultrasonically treated sample was more homogenous, less brown with no layers or bubbles. The browning index for fresh apple juice was found to be greater than the untreated samples at 15 °C. Jang and Moon (2011) reported that the combination treatment of ultrasonic and ascorbic acid was more effective in controlling the browning rate of fresh-cut apple. This is due to the inhibition of monophenolase, diphenolase and peroxidases enzymes. Whereas, individual ultrasonic treated

fresh-cut samples showed more browning due to the constrained inactivated influence on the enzymes. In another study performed on tender coconut water the combination of ultrasound with nisin treatment decreased the enzyme activity by 50%, 30% and 35% for Polyphenol oxidase, Peroxidase and Phenylalanine ammonia lyase, respectively (Rajashriet et al., 2020). This combined treatment also showed a higher browning index for tender coconut water than untreated sample. Ultrasonic treatment creates shear stress by cavitation which depolymerizes the macromolecule and produces lower molecular weight products, which may inactivate the enzymes. In this study, due to partial inactivation of browning enzymes, the residual activities of enzymes were probably responsible for the enhancement in the browning index of the ultrasonic and nisin treated tender coconut water. More studies need to be conducted at various treatment parameters to aid in complete inactivation of the implicated enzymes.

5.3. Flavor

Flavor is made up of volatile (aroma) and non-volatile (taste) components. Flavor influences the sensorial quality of food as well as overall acceptance by consumer (Zhang et al., 2020). Ultrasonic treatment has significant effect on the flavor compounds. Zou et al. (2018) reported an increase in volatile flavor components such as aldehydes, ketones and alcohols in ultrasonically treated beef samples. Various studies have proved that the ultrasonic treatment provided most flavourful extracts. Teng et al. (2019) studied the effect of UAE, ultrasound-assisted drying and microencapsulation on flavors of spices. Ultrasonic reduced the drying time which helped in maintaining the flavor of spices. UAE also reduced the extraction time and lead to higher concentration of spice flavor extract, even in comparison with advanced extraction techniques such as microwave-assisted extraction. These authors also stated that flavor can be successfully retained for a longer period with the help of ultrasound-assisted microencapsulation method. In addition, UAE coupled with vacuum distillation produces high quality of flavor compound extracts from plants. This is proved by Da Porto and Decorti (2009) in the comparative research between UAE coupled with vacuum distillation and hydrodistillation for *Menthaspicata* samples. UAE production of oxygenated compounds (5–8 times) than hydrodistillation. Extraction yield provided by UAE was in between 0.04 and 0.13% whereas 0.01–0.02% by hydrodistillation. Zhang et al. (2020) investigated the application of ultrasonic-assisted frying to improve the flavor in meatballs. The ultrasonic-assisted frying increases the speed of free fatty acid oxidation which causes the formation of volatile flavor compounds. Also, the concentration of essential amino acid improved with the application of ultrasonication method. Similar observations were made by Zou et al. (2018) for enhancing the chemical profiles of spiced beef taste and flavor. Thus, ultrasonic can be used efficiently as a potential technique to increase the flavor profile of food products.



Fig. 4. Ultrasonically (US) treated fresh apple juice at 15 °C and control check (CK) at 4 and 15 °C (Sun et al., 2015).

6. Current challenges and future perspectives

It is clear from existing literature that ultrasound technology offers several advantages such as reduction of processing time and steps involved, greater efficiency with enhanced product quality (nutritional, physicochemical & organoleptic) and improved shelf life. However it does come with some challenges. The effect of ultrasound is raw material dependent and the technology has been applied to varied plant and animal based food products such as meat, fruit and vegetables, cereals, dairy and emulsion based products (Bhargava, 2020). As seen from this review, effect of ultrasonic treatment can be positive or negative on the various biomolecules depending on the treatment parameters and the overall composition of the food under investigation. As reported, ultrasound is known to improve certain techno-functional properties while having negative effect on others. An interesting example of this would be increase in foaming capacity (techno-functional property) of food proteins that have been processed using ultrasonication. While a desirable trait for egg protein (Sheng et al., 2018) when used for its foaming applications it could reduce applicability of other proteins where foaming is undesired in the final product.

Also depending upon the unit operation this novel technique is coupled with, different end results can be expected. For example ultrasonication is often used along with filtration, freezing-thawing, dehydration, pasteurization, extraction and other existing treatments and processes. In case of ultrasonic coupled filtration, enhanced filtration is seen with reduced filtration time and prevention of membrane fouling (Kyllönen et al., 2005). Though careful optimization of ultrasound velocity needs to be undertaken to avoid any damage to the filter. Integrated use of ultrasonic treatment with other conventional or novel techniques could result in enhancement of overall quality of the processed food product. Carefully designed experimentation at pilot and industrial scale for optimization of parameters and basic research on the effect of acoustic treatment on the molecular composition of different food products needs to be undertaken. Possible adverse effect of industrial utilization of this technique needs to be studied along with energy and financial considerations.

7. Conclusion

Ultrasonic treatment used for the extraction of compounds which is free from any residual solvents, impurity or with other unwanted compounds. As conventional processing causes losses in bioactive compounds, preserving them during processing is very difficult. It could be stated that the ultrasonic treatment provides more benefits in reducing the losses and retaining the bioactive compounds compared with conventional treatments. The application of ultrasonication on expensive ingredients is an economical approach than the conventional extraction techniques, which is the need of the industry. Therefore, further studies are required on the application of ultrasonic technique for the extraction of bioactive compounds from different foods as well as on allergens. The present techniques for extraction of compounds are not consistent and used for crude extraction. Hence, more studies are required on extraction techniques for efficient production. Based on this review article, it can be possible to bring researchers from various streams such as food technology, engineering, biotechnology to provide an encouraging future for the industrial application of ultrasonic assisted extraction for bioactive compounds from various foods.

CRedit authorship contribution statement

Azza Silotry Naik: Writing – original draft, Drafting the article. **Deodatt Suryawanshi:** Writing – review & editing. **Manoj Kumar:** Writing – review & editing. **Roji Waghmare:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Original Research Article

Caffeine dependence among medical interns of a tertiary teaching hospital

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ABSTRACT

Background: Consumption of caffeine in adequate quantities has no adverse effects, but prolonged consumption makes it addictive. Medical students especially Interns due to their long working hours often indulge in excessive caffeine consumption. Objectives were to assess the knowledge of caffeine addiction among the medical interns, to assess the pattern of caffeine dependence among them and to calculate the caffeine dependence among them.

Methods: It is a cross sectional descriptive study conducted among 124 medical interns of Trichy SRM Medical College Hospital and Research Centre using a pre-tested, self-administered questionnaire.

Results: High level of knowledge was found in 47 (37.90%) participants, moderate level in 34 (27.41%) participants and low level of knowledge was found in 47 (34.67%) participants. The most preferred beverage was coffee 70 (56.45%) persons and second was tea 32 (25.80%). Majority of the study population 110 (88.7%) started consuming caffeinated products only after 5 years of age. Head ache 51 (41.12%) and exam durations 50 (40.32%) were common occasions of high caffeine intake. Among the study population, major group had either no 99 (79.83%) or just thirst 10 (8.06%) as withdrawal symptoms. Only 19.35% of them had caffeine dependence.

Conclusions: Though the dependence level is low, the magnitude of the problem is big and self-awareness of this dreadful habit is necessary. Thus, prompt recognition of symptoms of dependence, tolerance and intoxication is necessary to avoid them falling a prey to this habit in the future.

Keywords: Caffeine, Knowledge, Dependence, Tolerance, Withdrawal, Intoxication

INTRODUCTION

Caffeine is one of the well-known central nervous system (CNS) stimulants. It is popular for its cheap cost and instant action. In contrast to other such stimulants, caffeine acts by indirect effect on CNS. Caffeine is a psychoactive drug is due to its stimulant properties, which depends on its ability to reduce adenosine transmission in the brain. Caffeine acts as an antagonist to both Adenosine A1 and adenosine A2 receptors.¹ Dependence consisted of a maladaptive pattern of substance use with clinically significant impairment manifested by 3 or more

symptoms within a 12-month period. These symptoms included: 1) tolerance, 2) withdrawal, 3) substance used in larger amounts or over a longer period than intended, 4) a persistent desire or unsuccessful effort to control use, 5) a great deal of time spent obtaining, using, or recovering from the substance, 6) forgoing important activities because of the substance, 7) and substance use continued despite knowledge of having a persistent or recurrent physical or psychological problem likely to be caused or exacerbated by the substance (i.e., 'use despite harm').² Students generally prefer caffeine over other commonly available psychoactive drugs is that it

increases wakefulness and overall concentration.³ Medical interns work late due to their long shift hours and necessity to stay awake consume stimulants. Hence this study was conducted with the following objectives.

Objectives

Objectives were 1) to assess the knowledge of caffeine addiction among the medical interns 2) to assess the pattern of caffeine dependence among them 3) to calculate the caffeine dependence among them.

METHODS

Study type

It is a Cross sectional descriptive study conducted from February 2019 to March 2019

Study setting

The study was conducted in a tertiary care teaching hospital, Trichy SRM Medical College Hospital and Research Centre, Irungalur.

Sample size

Since we have included only one medical college, (Universal Sampling) All 134 CRRIs (Compulsory rotatory residential internship) doing their internship during the period from March 2018 to March 2019 were included in this study.

Inclusion criteria

Those who consumed any type of caffeinated product within the last one year which came to 124.

Exclusion criteria

Those who did not consume any type of caffeinated product within the last one year and who did not give consent.

Ethical approval

Ethical approval was got from the Institutional Ethics Committee.

Data collection and analysis

A list of all the medical interns who were doing their internships during March 2018 to March 2019 was collected from the college administration. Two medical interns collected the data for a period of one month based on their availability in the respective departments at a time convenient to them. Written and informed consent was obtained from the participants before the survey. After giving adequate information data was collected

using a self-administered questionnaire. The questionnaire was framed after studying all the variables carefully from the literature. Medical interns not belonging to this period of internship and members who did not give consent to participate in the study were excluded from the study.

The following operational definitions were modified and used due to feasibility reasons after studying the review of literature.

Knowledge level

Participants answering all 3 questions designed to assess the knowledge correctly were considered to have high knowledge level. Participants answering 1-2 questions correctly were considered to have moderate knowledge and participants answering none of the questions correctly were considered to have low level of knowledge.

Dependence

Dependence consisted of a maladaptive pattern of substance use (Caffeine) with clinically significant impairment manifested by 3 or more symptoms within a 12-month period. Not all 7 components of DSM – 3 was included (withdrawal, tolerance, intoxication were included).⁴

RESULTS

The most preferred caffeinated product was coffee 70 (56.45%). Majority of them had 60 (48.38%) had moderate and 47 (37.90%) had high level of knowledge regarding caffeine dependence.

Table 1: Demographic details of the study participants (N=134).

| Variables | Frequency | Percentage |
|-----------------------|-----------|------------|
| Age (in years) | | |
| ≤23 | 70 | 52.2 |
| >23 | 64 | 47.8 |
| Sex | | |
| Male | 75 | 56 |
| Female | 59 | 44 |

Table 2: Types of caffeine products commonly preferred among medical interns (N=124).

| Responses | Frequency (n=124) | Percentage |
|---|-------------------|------------|
| Commonly preferred caffeine product in the last year | | |
| Coffee | 70 | 56.45 |
| Tea | 32 | 25.80 |
| Colas | 10 | 8.06 |
| Dark chocolate | 10 | 8.06 |
| Nothing | 2 | 1.61 |

Table 3: Features of caffeine withdrawal symptoms among medical interns (N=124).

| Responses | Frequency (N=124) | Percentage |
|--|-------------------|------------|
| What happens if you miss routine schedule of caffeinated product? | | |
| Nothing | 96 | 77.4 |
| Just thirsty | 10 | 8.06 |
| Drowsy and sleepy | 9 | 7.25 |
| Headache and irritable | 8 | 6.45 |
| Tremors and depression | 1 | 0.80 |

Table 4: Features of development of tolerance towards caffeine products among medical interns (n=124).

| Responses | Frequency | Percentage |
|---|-----------|------------|
| Usual hours of sleep | | |
| Less than 5 hours | 5 | 4.13 |
| 5 To 8 hours | 101 | 81.45 |
| 8 Hours or more | 18 | 14.51 |
| Do you feel sleepy during day, even after taking caffeinated products? | | |
| Yes | 20 | 16.12 |
| No | 103 | 83.06 |

Table 5: Symptoms of impending caffeine intoxication among medical interns (n=124).

| Responses | Frequency | Percentage |
|--|-----------|------------|
| Impending caffeine intoxication symptoms. | | |
| Sleep disturbance only | 10 | 8.06 |
| GI disturbance | 2 | 1.6 |
| Hot flushes | 3 | 2.4 |
| Palpitation | 2 | 1.6 |
| Breathlessness | 4 | 3.2 |
| Aggression | 1 | 0.8 |
| Not experienced any of these | 101 | 81.45 |

Table 6: Dependence and knowledge level about caffeine addiction (n=124).

| Knowledge level | Frequency | Percentage |
|-------------------|-----------|------------|
| High | 47 | 37.90 |
| Moderate | 60 | 48.38 |
| Low | 17 | 13.70 |
| Dependence | | |
| Yes | 24 | 19.35 |
| No | 100 | 80.65 |

Only 13 (10.48%) members were considered to have caffeine dependence. About 96 (77.4%) had no withdrawal symptoms, 101 (81.5%) did not have any in

toxification features, 103 (83.06%) developed no tolerance towards caffeine products (Tables 1-6).

DISCUSSION

As anticipated, among various available caffeinated products, the most preferred one was coffee 70 (56.45%) persons and second highest was tea with 32 (25.80%) respondents. In a study done by Ahmad et al among medical and non-medical students of Lahore Pakistan the preferred one was soft drinks followed by tea and then coffee which varies with our results.⁵ In a similar study by Jochebed et al among undergraduate dental students, the results were similar to our results with coffee being most preferred followed by tea.⁶

Majority of the study population 110 (88.7%) started consuming caffeinated products only after 5 years of age. Of which 59 (53.63%) persons commenced only in their adolescence after 15 years of age. This is consistent with the findings of Gera et al.^{7,8} Evidently, habit of drinking coffee developed in their adolescence, where they have many challenges such as handling peer pressure and board exams. As they enter adulthood, they are more exposed to the real-world problems and these may be the probable reasons for starting to take a caffeinated product. In our study, among various occasions demanding higher extent of caffeine intake, head ache 51 (41.12%) and exam durations 50 (40.32%) tops the list. This agrees with the findings of Edward et al.⁹⁻¹²

Among 124 subjects who participated in the study, about 13 (10.48%) members were considered to have caffeine dependence. About 96 (77.4%) had no withdrawal symptoms, 101 (81.5%) did not have any in toxification features, 103 (83.06%) developed no tolerance towards caffeine products. In a study done by AtikahRamlil et al 28.3% students were caffeine dependent. This contradicts with our study.¹³ The study population and the variables used are different in our study which might be the probable reasons.

The strength of our study is that we used a validated tool and privacy was maintained as it was a self-administered questionnaire. Only one medical college was included and all items in the DSM -4 questionnaire was not used. These are be the limitations of this study.

CONCLUSION

Though the dependence level is low, the magnitude of the problem is big and self-awareness of this dreadful habit is necessary. Thus, Prompt recognition of symptoms of dependence, tolerance and intoxication is necessary to avoid them falling a prey to this habit in the future.

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Teaching learning methods effective in improving communication skills among medical students: A systematic review

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ABSTRACT

Background: Competency Based Medical Education curriculum has incorporated multiple changes from traditional curriculum one among which is teaching communication skills. This review focused on systematic analysis of research articles to identify various teaching learning methods found to be effective in improving communication skills among medical students. **Methods:** Articles on communication skills, written in English, published between the years 2000 to 2020 in Medline database were retrieved. All full text articles with intervention study design were included. A total of 1061 articles were retrieved using different search strategies. After removing duplications, title and abstract screening was done that was followed by full text review for quality assessment using Cochrane Effective Practice and Organisation of Care Review Group (EPOC) checklist. **Results:** Out of total 1061 articles retrieved, 22 were found to be focusing on communication skills training and assessment. Quality assessment were done using EPOC checklist which resulted in 18 articles for full text review. Among these 18 studies, 7 used role-plays performed either by peers/near peers or student themselves coupled with/without feedback by teachers, peers and reflection writing. Student-patient/standardized patient interaction with feedback by faculty/peers/standardized patients, recorded videoclips of roleplay/student-patient interaction, virtual simulation, structured scripts, workshop, and seminar were few other TL methods used. **Conclusion:** Role-play was the teaching learning method commonly used. Studies varied in TL methods, context of implementation, duration of intervention & follow up and assessment method. Since none of the articles reviewed were conducted in India, further studies with rigorous study design are needed to explore TL methods that would be effective in Indian context.

Key word: communication skills, interpersonal communication, medical education, medical school, medical students, teaching learning methods.

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INTRODUCTION

CBME curriculum implemented in 2019 is a major shift from traditional curriculum incorporating multiple changes from traditional curriculum.^{1,2} One among which is AETCOM module to teach attitude, ethics and communication skills to medical students

in India.³ Effective communication skills are necessary to interact with patients and their family members to aid in correct diagnosis of health issue and to communicate effectively to them about the disease course, treatment and prevention measures

and also to communicate with their colleagues and health care workers for effective team work. Better communication ability is needed to improve health awareness among patients and in the community. Higher the communication, higher would be the adherence to medication among the patients and better would be the health outcomes.^{4,5,6,7,8} Better doctor patient communication would reduce miscommunication related errors.⁴ As per the present CBME curriculum, communication skills are taught across all phases of MBBS curriculum from first to final year. Teaching communication to IMG is also included as a part of foundation course for first year MBBS even before they enter regular teaching. Though the AETCOM module includes various teaching learning (TL) methods suggested to teach communication, it also gives opportunity for faculty to use their own innovative methods in teaching the specified competencies relevant to communication skills. Under traditional curriculum, learning communication skills was a part of hidden curriculum and hence there was no uniform systematic approach in teaching the same. As per the new CBME curriculum for undergraduate medical students, communication skills are to be taught to the IMG using appropriate TL methods and the same must be assessed in each phase to ensure that the student has acquired the competencies. Hence it is essential to identify effective TL methods to improve communication skills which may then be implemented in the teaching learning process. The present study is a systematic review focusing on summarizing the findings of relevant intervention studies to explore teaching learning (TL) methods to improve communication skills among medical students.

METHODOLOGY

A systematic review of articles focusing on improving communication skills among medical students was done in the month of October 2020.

Data source and Eligibility criteria: Articles written in English, published between the years January 2000 to September 2020 available in Medline database using PubMed search engine were included. All articles fulfilling the eligibility criteria were included irrespective of country where the original research work is done. Articles published from research work done using intervention study design and only original research articles were included excluding review articles. Intervention studies that used both randomized and non-randomized control study

designs were included for review. Only free full text articles published during the specified period were retrieved to complete the review process.

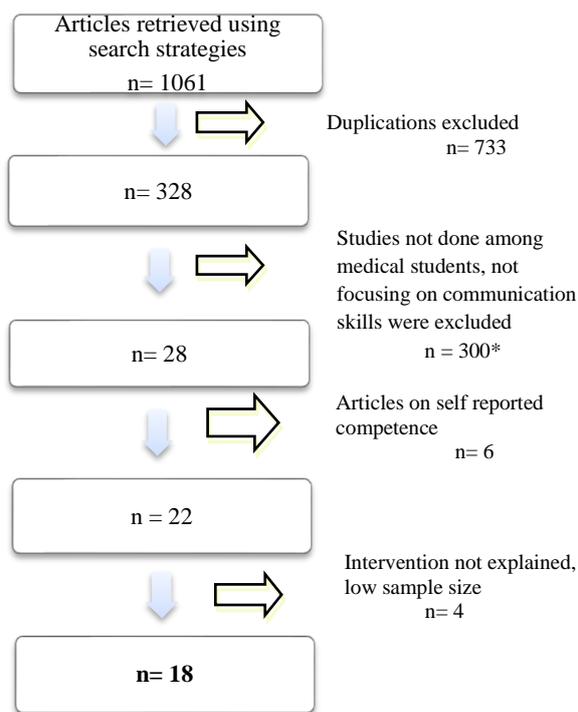
Search strategy: The search terms used were “communication”, “communication skills”, “medical students”, “medical undergraduates”, “medical education”, “medical school”, “interpersonal communication” and “interpersonal communication skills”. Box 1 describes the details of search strategy. These search strategies resulted in 1061 free full text articles.

Box 1: Search strategies used to retrieve the articles

Medical students AND communication: 127
Medical students AND communication skill: 59
Medical undergraduates AND communication skills: 29
Medical undergraduates AND communication: 43
Medical education AND communication skills: 198
Medical school AND communication skills: 163
Medical students AND interpersonal communication: 119
Medical students AND interpersonal communication skills: 58
Medical undergraduates AND interpersonal communication: 43

Review process: Article’s search and retrieval was done by two authors independently. Wherever a discrepancy was found in the selected article, it was resolved by another reviewer not related to this study and the Cronbach’s alpha for inter reviewer agreement was 0.87. In phase 1, title of the articles was screened to remove duplications which resulted in 328 articles. During phase 2 of review process, title and abstract of these articles were read to identify studies specifically done using intervention study design among medical students focusing on communication skills. Research works not done among medical students i.e., physicians, residents, specialists, nursing/dental/physiotherapy, and other allied health science students were excluded. The studies done to improve communication skill among caregivers and patients were also excluded. This resulted in 28 studies which were included for complete systematic review to explore the TL methods used in these studies and to identify effective methods in phase 3 review. Among these 28 articles, 6 studies did not assess communication skills, instead self-rated competence was studied and hence these were excluded resulting in 22 articles. In the next phase, Quality assessment of these articles was done using the Cochrane Effective Practice and Organisation of Care Review Group (EPOC) checklist and bias assessment.^{9,10} Description of sample size, randomization process, allocation

Figure 1: Flowchart explaining the process of article retrieval for systematic review



- * n= 87 (physicians/residents/specialists/primary care providers)
- n= 88 (community/family/care giver skill/patient care)
- n= 63 (Allied health/college/school students not medical students)
- n= 28 (clinical skills not communication)
- n= 17 (information management/ telehealth not communication skills)
- n= 5 (Trial protocol)
- n= 5 (Academic performance of students/language fluency not communication skills)
- n =2 (communication knowledge not skill)
- n= 2 (questionnaire development)
- n= 3 (effective communication for strategy for trial participant recruitment)

concealment, blinding of assessors/standardized patients/real patients, teaching learning methods used for intervention, pre and post intervention assessment method and proportion of loss to follow-up, precautions taken by the authors to reduce bias. The studies without clear explanation of randomization process and intervention, studies that reported inadequate sample size and loss to follow-up more than 20% were excluded¹¹⁻¹⁴ resulting in 18 articles. Figure 1 describes the complete review process.

RESULTS

A total of 18 articles were completely reviewed for the details of teaching learning methods used in these studies. Table 1 describes details of the studies included for complete review. Of these 18 studies, 2 were controlled clinical trials and rest 16 studies were randomized controlled trials. Majority of these studies were done in Germany (27.7%) followed by

USA (16.7%), Japan, Taiwan and other countries. Communication skills are not only important for an Indian Medical Graduate to interact with their patients and caregivers, but also to achieve team coordination with their colleagues and other health care professionals. Thus, we included studies that focused on doctor-patient communication and team leader communication. Of the total 18 studied reviewed, 16 were focused on improving doctor-patient communication and 2 on improving communication skills as a team leader. All these studies were done with medical students across various professional years as participants. Majority of studies were done among 5th year medical students (33.3%), followed by 4th year (22.2%) and 2nd year students (22.2%). Other participants included were students of 1st, 3rd and 6th professional year. Amongst various teaching learning methods used in these studies, roleplay by the students coupled with or without feedback was most used followed by student-real patient or simulated patient interaction. Computer based virtual simulation and simulation of nonverbal behaviour of the students and feedback for the same were also used to teach communication skills for medical students. Details of teaching learning methods used, and their effectiveness are explained in table 2. Among 18 articles reviewed, only 4 studies have compared two different TL methods. Out of these 18 studies, only two studies reported long term effectiveness of the interventions used and of which one study with videoclips and roleplay reported that few components of communication skills were retained and scores of few decreased. One study also assessed cost effectiveness of the TL method used as intervention. Of these, one study reported that roleplay by students is cost effective than using student-standardized patient interaction.

DISCUSSION

Graduate Medical Education regulation 2019 has introduced outcome-based teaching for medical students to produce competent Indian Medical Graduate. Acquiring appropriate communication skills is one such competency for an IMG. Appropriate teaching learning methods need to be used to make an IMG competent in communication skills. The present systematic review was done to provide insights into the availability of quality intervention studies and specific teaching learning methods found effective. Intervention studies that assessed improvement in communication

Table 1: details of the studies included for complete review of teaching learning methods

| Year | Country | Author | Design | TL Methods | Sample size | Assessment method | Result |
|------|-------------|---------------------------------|-------------------------|--|---|--|--|
| 2001 | London | Knowles C et al ¹⁵ | RCT | Video of a roleplay with structured feedback by physician and educational psychologist | N= 132 4 th year | OSCE | Sample group scored significantly high than control group |
| 2003 | USA | Windish DM et al ¹⁶ | RCT | Small group exercises with roleplay, reflection and feedback | N= 60 2 nd year | 30-item interpersonal checklist rating behaviors by standardized patients | Significant difference in building rapport and gathering psychosocial history. |
| 2004 | Japan | Mukohara K et al ¹⁷ | CCT | Small group Seminar | N= 105 5 th year | Videotaped OSCE | No significant difference in majority of core skills |
| 2008 | Taiwan | Ho MJ et al ¹⁸ | RCT | Videoclip and roleplay | N= 57 5 th year | OSCE | Roleplay is effective than videoclips. Both roleplay and videoclips were effective than controls |
| 2009 | Texas | Morrow JB et al ¹⁹ | RCT | Guided discovery, brief didactics, and practice role plays. | N= 17 1 st year | EHR specific communication skills checklist | EHR specific communication skills does not improve general communication skills |
| 2009 | Turkey | Ozcakar N et al ²⁰ | RCT | Verbal feedback by faculty on spot and Visual feedback by watching recorded videos of interaction with simulated patient | N= 52 2 nd year | Communication skills checklist | Feedback based on videotaped interviews is superior to feedback given based on the observation of assessors. |
| 2010 | Taiwan | Ho MJ et al ²¹ | RCT | Videoclip and roleplay | N= 57 5 th year | OSCE | Communication skills in few areas diminished significantly after a year and no significant reduction in few components |
| 2012 | Germany | Lund F ²² | RCT | Peer roleplay with feedback by teachers. | N= 84 1 st year | Communication Assessment Tool using videotaped student patient interaction | Communication score was significantly high in intervention group |
| 2013 | Germany | Werner A et al ²³ | RCT with cross over | Lecture, role-play, brainstorming session | N= 30 5 th , 6 th year | Information recall by layperson | Significantly more information items could be recalled in intervention group |
| 2015 | Germany | Bosse HM ²⁴ | RCT | Role Play by peer group and individually training with Standardized Patients including feedback by SP | N= 69 5 th year | Calgary-Cambridge-Observation-Guide Checklist in OSCE station | Roleplay was effective than SP encounter and feedback. Roleplay was cost-effective |
| 2015 | USA | Shaddeau A et al ²⁵ | RCT | Student patient interaction as small group discussion | N= 135 3 rd year | OSCE | No significant difference in communication score |
| 2015 | Germany | Castelao EF et al ²⁶ | RCT | Team leader training: Video clips followed by reflection and group discussion. | N= 180 5 th year | Validated checklist of videotaped simulation exercise | Significantly high team leader communication score in intervention group |
| 2016 | Switzerland | Perrig M et al ²⁷ | Controlled intervention | Student patient interaction with real patients with systematic feedback by teacher, peers and patients | N= 48 4 th year | OSCE | There was a significant improvement in communication skills in |

| | | | | | | | |
|------|-----------|-------------------------------|---------------------------|--|--------------------------------|---|---|
| | | | tion study | | | | intervention group but it did not had long term effect |
| 2016 | Australia | Liu C et al ²⁸ | RCT with crossover trial | Automated visual presentation of students' nonverbal behavior with feedback from standardized patient (SP) | N= 268 2 nd year | SOCA checklist | Significantly high score in intervention group |
| 2017 | Japan | Nomura O et al ²⁹ | RCT non-inferiority trial | Cross year peer tutoring (CYPT) | N= 116 4 th year | OSCE | No significant difference in OSCE scores between groups. |
| 2017 | USA | Kron FW et al ³⁰ | RCT | Computer simulation featuring virtual humans and feedback | N= 421 2 nd year | OSCE | Significantly high score in intervention group |
| 2018 | Indiana | Pettit KE ³¹ | RCT | Refresher training in scripted communication | N= 474 4 th year | Patient rated satisfaction in student patient interaction | No statistically significant difference in communication element use between the intervention and control groups. |
| 2019 | Germany | Engerer C et al ³² | RCT | Specific, structured and behavior-orientated feedback | N= 66 3 rd year | Validated checklist Com-ON-check | Five out of seven domains in communication skills improved significantly pre and post in the intervention group. |

Table 2: Teaching learning methods used and reported to be effective in various studies

| TL method | No. of TL methods used | No. of TL methods with Significant effect |
|--|------------------------|---|
| | n (%) | n (%) |
| Pre-recorder video roleplay with feedback | 2 | 2 |
| Real time Role play with feedback/reflection | 2 | 2 |
| Small group seminar | 1 | 0 |
| Real time Role play vs Videoclip | 1 | 1 |
| Videoclip vs Control | 1 | 1 |
| Guided discovery | 1 | 0 |
| Video recorded SP interaction and feedback vs on spot feedback on SP interaction | 1 | 1 |
| Role play vs control | 1 | 1 |
| Lecture/seminar/roleplay | 1 | 1 |
| Realtime roleplay vs SP interaction with feedback by patient | 1 | 1 |
| SP interaction as small group | 1 | 0 |
| Real time Student-Pt/SP interaction with 360 feedback | 1 | 1 |
| Virtual simulation | 2 | 2 |
| Cross Year Peer Tutoring vs Faculty Led Training | 1 | NA* |
| Structured script with instructions | 1 | 0 |
| Behaviour oriented feedback | 1 | 1 |
| Total | 19** | |

* Non inferiority trial

**More than one TL

method used in One study skills of medical students rather than their self-efficacy on communication were

included in this review. There were a total of 28 intervention studies retrieved initially, of which 6 studies assessed improvement in self-competence in communication skills instead of skills assessment in a patient care setting. An Indian Medical Graduate will not be confident on their communication skills unless they learn skills through scenarios in clinical setting. Hence, we included studies those assessed improvement in the medical students' communication skills during post intervention assessment compared to their baseline scores rather than assessing communication skills related knowledge and attitude.¹¹⁻³² Most of these studies are done in Germany^{22-24,26,32} and none were conducted in India. To account for ethnic and other inter country differences that might influence the need and context in which appropriate communication skills are to be used, intervention studies must be conducted among medical students in India. Two among the reviewed studies reported communication skill improvement in few domains and no significant increase in few others.^{16,32} Hence, it is difficult to interpret the overall usefulness of the TL method used in these studies. Most of the studies compared intervention using a specific TL method with conventional curriculum as control which may not provide the actual effectiveness of the TL method used unless it is compared with another TL method. Few studies have used multiple TL methods in one single intervention arm for the study purpose or in the form of curriculum implementation which makes it difficult to interpret the effect of single TL method.

Conclusion:

Role-play was the teaching learning method commonly used and found to be effective. The studies varied in TL methods used as intervention, context in which it is implemented, duration of intervention, follow up and method of assessment before and after intervention. Since none of the articles reviewed were conducted in India, further research studies with rigorous study design are needed to explore TL methods that would be beneficial for medical students in Indian context.

Scope for future research:

Intervention studies following appropriate quality guidelines among Indian medical students are need of the hour.

To assess the effectiveness of each TL method, single TL method need to be used in each intervention group rather than using multiple methods.

Researchers planning to identify the effect of a specific teaching learning method shall compare with another TL method rather than with a control group without any intervention.

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Concept of Formative assessment and Strategies for its effective implementation under Competency-Based Medical Education: A Review

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ABSTRACT

Internal assessments are routinely conducted by faculty in Medical Institution to evaluate students. In most institutions, it is done with the main objective of documenting internal assessment marks to be included in the final University Examination. Under the new curriculum for Indian Medical Graduates 2019, there has been a paradigm shift from conventional to Competency-Based Medical Education with major revisions in the assessment methods. Focus has shifted from purely summative to a combination of both formative and summative assessment. Formative assessment involves giving regular feedbacks to students which helps them to learn better and motivates faculties to improve their teaching skills. Therefore, it becomes essential for all faculties in medical institutions to know these changes through faculty development programs and understand the key strategies for its successful implementation. This article highlights the perspective of formative assessment, different methods commonly used, and the scope for future research in our settings.

Key word: Formative Assessment, Summative Assessment, Feedback, Competency-Based Medical Education.

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INTRODUCTION

Medical education in India has undergone a paradigm shift from the traditional to competency-based education which is defined as an outcome-based approach to the design, implementation, assessment, and evaluation of a medical education program using an organizing framework of competencies.¹ Apart from the change in teaching modalities, the new curriculum also demands a competency-based assessment characterized by its longitudinal nature with regular feedback and in a different context to achieve certification of competency. Thus, the system of only one summative or year-end examination is not suited for this purpose.² Most teaching faculties are familiar with the terms formative and summative

assessment. But its application is unknown or felt not important with the traditional system of medical education. The role of formative assessment (FA) in student learning is well acknowledged, but it is still not well understood across higher education.³ Evidence states that students can get trained in certain competencies within 6 to 7 months by teachers practicing FA which would otherwise take 1 year.⁴ With the implementation of Competency-Based Medical Education (CBME) it becomes essential for every medical teacher to understand how formative assessment differs from summative and effective methods for its implementation. This article throws light on some of

these aspects and provides a need for further research in our Indian setting.

Understanding Formative assessment in comparison with Summative assessment

The assessment constitutes an important component of any curriculum. It is broadly classified as a summative and formative assessment. The conventional medical education system has a lot of emphasis and weightage given to summative assessment which mostly happens at the university level and is used to decide pass or fail. Under the CBME curriculum, a major importance is given to formative assessment which is an ongoing process with an opportunity for the students to attain the competency through feedbacks from the faculty. The distinction between formative and summative assessment is primarily related to how assessment results are used, as many assessments developed for formative purposes can be used for summative purposes and vice versa.⁵ Other differences have been given by Dixson and Worrell³ (Table 1).

Table 1: Dante D. Dixson Frank C. Worrell³

| Characteristic | Formative assessment | Summative assessment |
|--------------------------|---|---|
| Purpose | To improve teaching and learning to identify difficulties faced by students | Evaluation of learning outcomes mainly for promotion |
| Formality | Usually, informal | Usually, formal |
| Timing of administration | Ongoing, before and during instruction | Cumulative, after instruction |
| Level of stakes | Low stakes | High stakes |
| Types of questions asked | What is working? What needs to be improved? | Is the student prepared for the next level of activity? |
| Examples | Observations, Homework, Self-evaluations | Model exams and University exams |

Concept and principles of Formative Assessment

Black and William⁴ defined assessment as all activities that teachers and students undertake to get information that can be used diagnostically to alter teaching and learning, which includes teacher observation, classroom discussion, and analysis of student work such as homework and tests. Assessments become formative when the information

is used to adapt teaching and learning to meet student needs through feedbacks. Sadler⁶ had identified three criteria essential for this feedback to be effective. Firstly, students must have a concept of what is expected to be learned. Secondly, they should be able to compare their present level of knowledge/performance with the expected level. Lastly, they should engage themselves in appropriate action that will lead to a decrease in this gap. Student’s autonomy with regards to learning and assessment is very important and is found to be directly correlated with increasing levels of motivation to learn, thus medical educators should actively seek out opportunities to increase student control over their educational practices.⁷

The basic element of FA is providing feedback to the students based on their performance on the assigned task. Hattie & Timperley⁸ defined feedbacks as ‘information provided by an agent (teacher, peer, self, etc.) regarding aspects of one’s performance or understanding. This information will ultimately reduce the discrepancy between the present understanding and performance to the desired level. Unfortunately, the culture for giving true feedback has not yet developed in the medical professional, unlike the others.⁹ Butler and Winne¹⁰ had stated the seven principles of feedback (Table 2) that support and develop self-regulated learning among students. Adoption of these measures will result in improved performance of not only students but also assist teachers in identifying areas of difficulty needing more instructional teaching. Moreover, meta-analysis also highlights the role of feedback given during FA as a useful tool for better learning.^{8, 11}

FA plays a key role in improving the process of learning rather than grading the students. The role of educators is primarily to motivate the intrinsic desire to learn rather than extrinsic motivation, where the concept of learning becomes the goal.⁷ Eventually it results in training students to become a life-long learner rather than exam-oriented reading. FA can be effective to serve specified purposes only when strategically implemented. Prashanthi and Ramnarayan¹² have aptly described the ten principles to effectively implement FA, known as 10 Fs- Faceless, facilitates learning, Feedback, Feedforward, Focus on learning, Flexibility, Fast, Frequent, Friendly, and Fun. Of these, faceless i.e anonymity is considered very important to identify contents difficult to learn. But this principle has a big

drawback since individual feedback is not possible. Solely relying on feedback given to students to improve their learning is not sufficient but must be accompanied with necessary action that will be taken by them to improve future learning, this principle is called the feedforward. It includes both retrospective and prospective approaches in the practice of FA.

Table 2: Seven principles of Feedback given in FA¹⁰

| S.No. | Principles of good feedbacks |
|-------|---|
| 1 | Facilitates the development of self-assessment by students in learning |
| 2 | Encourages teacher and peer dialogue around learning |
| 3 | Helps clarify what good performance is |
| 4 | Provides opportunities to close the gap (Resubmission of task/assignment) |
| 5 | Delivers high-quality information to students about their learning |
| 6 | Encourages positive motivation towards reading and self-esteem |
| 7 | Provides information to teachers to help shape their teaching |

Assessment methods

The assessment methodology employed in FA is not significantly different from those used for summative. No single method is perfect but involves different methods used multiple times to compensate for flaws in any one method.^{13, 14} Cowie and Bell¹⁵ classified FA as planned and unplanned, both have their implication and benefits. A planned form is similar to conducting a summative assessment while an unplanned way of conducting FA, is one that occurs spontaneously in the classroom. Planned FA helps to assess the performance of the whole class while unplanned FA, which is interactive, mediates the learning process. The study of literature finds interactive FA as central to teaching and learning which is more relevant in medical education.¹⁶ Several methods can be used for FA, we summarize the few standard methods for assessment during

theory (Table 3) and practical session (Table 4) commonly used.

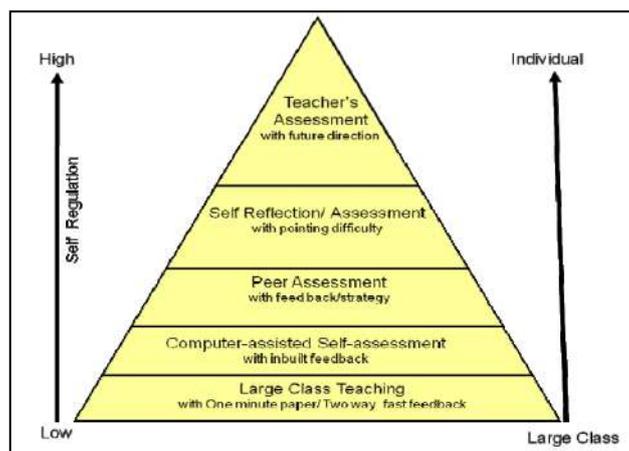
Table 3: FA Methods commonly used during theory session¹⁷⁻²¹

| Type of Assessment | Procedure | Feedback |
|-----------------------|--|--|
| Minute-paper | At the end of class, ask students to write “what is the most important point you learned today?” and “What is the least clear to you?” | Collect and review students’ responses and ensure that they have obtained the correct message. In the next class, comment on the findings or ask for peer review to discuss any discrepancies. |
| One-sentence summary | Can be used at any time during class to test knowledge about an important topic you expect them to be able to summarize. | Ensure the students have the message. |
| Directed Paraphrasing | Ask students to write a layman’s summary of any principle taught. This assesses their ability to comprehend and transfer concepts. | Peer or teacher assessed. Ensure the salient points are covered. |
| Muddiest point | Ask students to write down the concept or content of the lecture that they have not understood or found confusing. | Collect written answers or allow them to discuss with their peers. This provides feedback to both faculty and students to improve further teaching and learning respectively. |
| Knowledge probe | At the beginning of class, ask students to answer preset questions (open or multiple choice) to assess their existing knowledge. | Note any weaknesses of knowledge that need to be addressed. If open-ended could also be utilized for peer assessment. |
| Application cards | Ask students to write about a real-world application for a theory, principle, or procedure that was just covered. | Collect and pick out a broad range of examples to present to the class or allow peers to assess and discuss. |

| | | |
|--------------------------------------|---|---|
| Written exercise (MCQs, SAQs, Essay) | MCQs and SAQs are preferred to assess knowledge gained while essays help to assess in-depth understanding of the subject. | Feedback can be given immediately for MCQs and SAQs when conducted for small groups, while for essays it might take a few days depending on the teacher's correction ability. |
|--------------------------------------|---|---|

assessment by peer and teacher with one-to-one feedback will have intermediate and maximum effect respectively.

Figure 1: A model for formative assessment strategy proposed by Jeyakumar & Zed³²



A review of literature recommends the following strategies^{4,21,33-39}

Frequent short tests are better than infrequent long tests.

Assessment should be conducted within a week of teaching the topic.

The quality of questions should be kept in mind while framing the questionnaire. Tasks given to students should be relevant to real-life examples, therefore students realize the application of theoretical knowledge and importance of the topic.

Multiple methods and assessors should be used depending on the competency that is being assessed and feedback should be frequent and constructive. Feedbacks from peers also need to be obtained.

Students with poor performance should be given increased opportunities for resubmission of assignments.

Assessment based on direct observation of the trainee is better done using “qualitative approaches” such as words of judgment (e.g., satisfactory) or narrative description (trainee greeted the patient appropriately before history taking)

Feedback session can begin with the learner's self-assessment. It helps to soften the perception of harsh comments and makes corrective feedback feel more acceptable.

MCQs- Multiple choice questions, SAQs- Short answers questions

A decision on the method to be used can be done based on five criteria as stated by Van der Vleuten.²⁸ These are reliability (the degree to which the measurement of assessment is accurate and reproducible), validity (whether the assessment measures what it claims to measure), impact on future learning and practice, acceptability to learners and faculty (level of difficulty and time taken), and costs (to the individual trainee, the institution, and society at large).

Recently, many studies^{12, 29, 30} have focused on the use of FA through online mode, which is proved to be effective in given quick feedback to many students. The major advantage of this mode of assessment is that it can be done at a pace comfortable to students which can also be easily repeated according to the individual needs.

Strategies for effective implementation of FA

Black and William⁴ stated that any strategy adopted in FA should have clear goals, appropriately designed learning, and assessment tasks, including communication of criteria assessment, and providing quality feedback to students. This ultimately empowers students to assess their progress in knowledge and promote self-regulated learning,³¹ thus preparing themselves to be eager to acquire knowledge on the latest scientific development. FA is more effective and better accepted when students realize its importance in curriculum and unavoidable practice in medical education.⁴

Jayakumar and Zed³² proposed a model (Figure 1) showing the relation between the FA used and motivation towards self-regulated learning among students. Based on the model, FA methods used in lecture session have minimal effect on motivating students to improve their learning while individual

Table 4: FA Methods commonly used during practical session ²¹⁻²⁷

| Type of Assessment | Procedure | Feedback |
|--|---|--|
| Mini-Clinical Evaluation Exercise (mini-CEX) | A student performs clinical tasks (history taking or physical examination) and provides a summary of the patient encounter. Faculty members grade students on 7 parameters (interviewing skills, physical examination, professionalism, clinical judgment, counseling, organization and efficiency, and overall competence) using a 9-point rating scale for each parameter. A score of 1–3 is unsatisfactory, 4–6 is satisfactory and 7–9 is superior. | Provide structured feedback based on observed performance on interviewing skills, physical examination, professionalism, clinical judgment, counseling, organization and efficiency, and overall competence. |
| One-Minute Preceptor (OMP) | It is a 5 steps framework that helps the assessor to evaluate students' presentation skills, clinical reasoning, and knowledge. The steps involved are getting a commitment from students, probe for supporting evidence, provide general rules, reinforce what was done correctly, and lastly correct mistakes | This can be used in the OPD setting or viva session of a clinical case. It is more useful to teach about disease-specific points and differential diagnosis rather than history taking or examination skills. This framework involves giving both positive and negative feedback during the clinical training session. |
| SNAPPS | This framework differs from OMP by requiring both assessor and trainee to know the steps. It has 6 steps i.e., summarize the history and findings, narrow the differential to 2 or 3 possibilities, analyze the differential by comparing/contrasting the possibilities, probe the preceptor by asking questions, plan management for the patient's medical issues, and select a case-related issue for self-directed learning. The assessor is involved only after the 3 rd step. | This technique is more useful for advanced and self-motivated learners in the clinical setting. Feedback encourages critical thinking and identifying topics for self-directed learning. |
| Clinical Encounter Cards (CEC) | Similar to mini-CEX, scores were given on 7 parameters (history-taking, physical examination, professional behavior, technical skill, case presentation, diagnosis, and therapy) each on a 6-point scale with 1 being unsatisfactory and 6 indicates above the level of a medical graduate. | The quality of a student's performance is captured on a 4 x 6 scorecard with space for the assessor to record the feedback given to the student at the end. Useful for comparison on subsequent training sessions. |
| Blinded Patient Encounters (BPE) | Students in groups of 4–5 participate in a bedside tutorial. It starts with a period of direct observation when one of the students in the group is observed performing a focused interview or physical examination as instructed by the clinician-educator conducting the teaching session. Thereafter the student is expected to provide a diagnosis, including a differential diagnosis, based on the clinical findings. | It concludes with a feedback session in which the student receives personal private advice about his/her performance. Peer feedback is also possible in this method. |
| Direct Observation of Procedural Skills (DOPS) | Like CEC, the student is expected to perform procedures such as endotracheal intubation, nasogastric tube insertion, administration of intravenous medication, venipuncture, peripheral venous cannulation, and arterial blood sampling | Students are given specific feedback based on direct observation of each step to improve their procedural skills. |
| Standardized patients (SP) | This method uses actors who are trained to portray themselves as patients. The assessor uses a checklist to grade the students. | Feedback is given to students based on the assessor scoring and also comments from the SP. |
| Chart Stimulated Recall (CSR) | It involves dual assessment of the trainee's documentation and clinical reasoning skills. The assessor uses the case sheet documented by the trainee as a reference for structured clinical reasoning. | Assessors give feedback to students for both written and clinical decision-making skills. |

| | | |
|--|---|---|
| MultiSource Feedback (MSF) or 360° global rating | It involves the collection of performance data and feedback for an individual trainee, using structured questionnaires completed by several stakeholders (peers, senior consultants, junior specialists, nurses, and allied health service professionals) which is completed and returned to a central location for processing. Trainees should also submit a self-assessment questionnaire form. Done on routine performance, rather than performance during a specific patient encounter. | The questionnaires are collated, and individual feedback is prepared for trainees. Data are provided in a graphic form that depicts the mean ratings of the assessors and the standard mean rating. Trainees review this feedback with their supervisor and together work on developing an action plan. |
| Portfolio | Portfolios include documentation about specific areas of a trainee’s competence along with self-reflection. Includes procedure logs, peer assessments, patient surveys, literature searches, quality-improvement projects, and any other type of learning material. It also frequently includes self-assessments, learning plans, and reflective essays. | It is maximally effective when closely mentoring by teachers on its assembly and interpretation of the content, requiring considerable time and effort. |

OPD- out-patient department, SNAPPS- summarize, narrow, analyze, probe, plan, and select

Use of two-stage assignments where feedback from one stage helps to improve performance in the next stage.

Encourage bidirectional feedback i.e., allowing students to give feedback on faculty teaching. A student might be hesitant to do so, in such case ask more specific questions such as “Where would you like to see more teaching occur?”

Capacity building of all teaching staffs should be done through the faculty development program, for effective implementation of FA

Challenges in implementing FA

Several challenges have been faced even by developed countries with advanced teaching-learning environments while implementing CBME. A review article by Carraccio et al.⁴⁰ found 30 years of lag between the implementation and the actual practice of CBME. Understanding these challenges prior will help identify possible solutions. The biggest barrier to implement FA has been identified as a lack of faculty participation in conducting the assessment and giving feedback.²² Formative assessments can be negatively perceived as time-consuming by both students and teachers, especially in the clinical departments.^{39, 41} Students may lack enthusiasm initially in these assessments or with regular use, but once they recognize the concept of FA being a normal routine of an academic curriculum, it will be accepted.^{4, 42}

Most of the FA on clinical training is done among simulated patients or skill labs using mannequins. This requires training of faculty to be good observers and better assessors of student’s performances, which becomes more complex when it involves grading of

communication and emotional skills in a simulated environment rather than actual patients.³³

The infrastructural prerequisite required for implementing CBME is very demanding. Smart classrooms with voting pads and high-speed internet connection are essential for implementing unplanned FA during the lecture session. Simulation labs and patients will be needed specially to attain competency at the pre and para clinical levels. The financial support from the administration in this regard is a big challenge.³⁹

It is well known that assessment drives learning, however, it can have some unintended consequences especially with changes in the examination pattern. Training exclusively on FA can result in more focus on clinical aspects than theoretical aspects.^{28, 43} Feedback from peers promote professionalism, communication, and teamwork. This can result in students cramming for the final assessment and substituting their superficial knowledge for reflective learning.⁴⁴

It is often seen that students in medical school come from a culture that focuses on individual achievement^{45,46} and may not be prepared to accept constructive or positive feedback. They may underestimate the value of FA if it does not contribute to their overall grades.⁴⁷ The present educational culture of grading students is another barrier to the implementation of authentic formative assessment.⁴⁸ Medical students should realize that the aim is to become a holistic doctor in the real world rather than passing an exam with excellence

Scope for Future Research

Medical education research in India is the need for the hour to estimate the current level of knowledge, attitude, and perception towards conducting FA and giving feedback. We also need to understand the challenges faced by faculty and students in our setting. Only a few studies^{11,49-51} have been conducted on FA and its impact in improving students' performance. Future research to understand the benefits and barriers in implementing FA in our setting will assist in the effective implementation of CBME.

There is no agreed methodology to approach FA that demonstrates positive effects on learning that could be solely attributed to FA,⁵² therefore the other contributing factors need to be identified. New assessment tools and innovative approaches to assessing competencies such as teamwork, professionalism, empathy, etc. should be recognized to fully realize the promise of CBME.³³ There is a need to determine the feasibility of using FA tools in both theory and practical sessions in our setting, with limited resources, infrastructure, and manpower.

The basic idea behind the use of FA is to enable deep learning and feedback. Whether FA can completely replace summative assessment for health care education in the future needs to be evaluated. Research in these aspects will guide bringing about medical education reforms.

Conclusion

Research in medical education supports the shift towards CBME, where there is an alignment of training and learning with outcomes and assessment of students' performance concerning real work conditions on professional expectations.⁵³ FA has a major advantage in effectively evaluating and improving communication skills, teamwork, professionalism, ethical practice, and other procedural skills which is an essential competence that should be acquired by medical students but is not feasible to assess during the University examination due to time constraints.² Timing of assessment under CBME emphasizes more on the formative over summative assessments,⁴⁰ which has presently gained importance in our setting. This is in comparison to Western countries which focus on assessment frameworks providing a structured conceptual map of the learning outcomes of a program and allow test developers to more easily create robust assessment

instruments.⁵⁴ Purposeful assessment should be conducted with the greatest possible clarity going beyond its simple categorization as summative or formative.⁵⁵ We must repeatedly ask what the real purpose of conducting assessments is and be sure of the agenda.

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EDITORIAL

Myths and Misconceptions about Coronavirus Disease 2019 – How to Control the Infodemic?

V. Raghuram

Ever since a novel corona virus designated as severe acute respiratory syndrome coronavirus 2 originated in the Chinese city of Wuhan in December 2019 achieving pandemic proportions and subsequently spreading rapidly across major continents by international air traffic causing acute respiratory infection and death, there has been another storm brewing simultaneously. This storm is nothing but the huge amount of information being in circulation through the omnipresent social media. Most of this unverified and unreliable information reaching a large section of people is creating various myths and misconceptions regarding the cause, management and prevention of Coronavirus disease 2019 (COVID-19).

Myth is commonly considered as a folklore genre consisting of narratives/stories that play a fundamental role in human beings' everyday lives. These are often endorsed by leaders/rulers/religious preachers and explain to a great extent the functioning of a society and shape the beliefs of people. There is a potential role of cultural and religious beliefs/traditions/customs/rituals, which add a flavor to the public's mindset in a particular region/country and influence the propagation or acceptance of a myth. Myths related to various infections have been prevalent from time to time, and it takes a long battle to demystify the existing myths by providing a realistic evidence-based approach.^[1]

Currently, prevailing myths related to COVID-19 infection can be categorized into those related to the spread of infection, source of spread of infection, preventive measures, and cure.^[1]

Those myths related to spreading/transmission of COVID-19 have been bothering the public to think twice before using newspapers/vegetables, etc. and many are avoiding non-vegetarian foods with the fear of being infected. Further, many have hoarded or stockpiled antibiotics, essential oils, Vitamin C tablets, masks, sanitizers, etc. to protect themselves from running out of stock of these commodities. These myths can also lead to a false sense of security of being immune to the infection

and resultant exposure to high-risk situations. These myths can be very dangerous, as these can lead to over-complacency and lead to a reduction in actually needed practices, or following some of these myths can lead to other health hazards.^[1]

Many myths related to the spread of infection are compounded with the stigma associated with patients recovered from COVID-19 infection and the health care workers, working in COVID-19 wards/hospitals. These are leading to a social boycott of people avoiding interaction with the persons recovered from COVID-19 infection or those suspected of having COVID-19 infection.^[2]

In the times of widespread Internet accessibility to a large proportion of population, the need for regulation of content that should be made available to public is more acute than ever before. Circulation of unregulated information can facilitate perpetuation of these myths and misconceptions leading to unnecessary hospital admissions, hazardous self-medication practices, and spreading of panic among the public.

Strict legal action needs to be taken against people spreading fake news/making false claims during the pandemic. More and more stringent action and punishments must be declared by the Judiciary system to control the spread of myths/fake claims. The mainstream media should also be very cautious in presenting the different information about the COVID-19 infection. All these myths are having a widespread impact on public viewpoint and disease transmission. Therefore, possible and prompt steps should be taken by appropriate authorities to demystify the myths in due time.

Efforts to address misinformation on social media have special urgency with the emergence of COVID-19. In one effort, the World Health Organization designed and publicized

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shareable infographics to debunk coronavirus myths. Studies have shown that exposure to a corrective graphic on social media reduced misperceptions about the science of false COVID-19 prevention strategies.^[3]

While the efforts are underway to understand and control the pandemic by governments, national and international health agencies, there needs to be an effort from the public and media in quelling the infodemic which would complement these actions and help in overcoming the crisis that we find ourselves in.

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Original Research Article

A comparative study of direct health intervention and peer-led intervention on menstrual hygiene management of adolescent girls in rural India

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ABSTRACT

Background: Menstrual hygiene management (MHM) among adolescents in rural India is negatively influenced by myths and taboos which predispose them to infections which can be dispelled by health education by experts or peers. The study aimed to demonstrate the relative effectiveness of direct health education over peer led health education on MHM.

Methods: A longitudinal follow up intervention study recruiting 486 school going adolescent females divided in three groups I, II and III was conducted. Group I received peer led, while group II received direct health education and group III was control. Pre and post intervention scores of participants were compared.

Results: Direct intervention group demonstrated highest improvement in number of participants having good MHM scores ($p < 0.05$). Mean MHM scores of direct intervention group participants were the highest among all the three groups ($p < 0.05$). Knowledge score of direct intervention group was significantly higher than group I ($p = 0.001$) and group III with no difference in practice scores between group I and II ($p = 0.147$).

Conclusions: Direct health education as an intervention is more relatively effective than peer led in MHM of adolescent females.

Keywords: Adolescent health, Menstrual hygiene management, Health education, Peer led education, Direct health intervention, Myths

INTRODUCTION

According to the WHO, the adolescence period is a transition from childhood to adult hood.¹ Out of the 1.2 billion adolescent population, India is home to 253 million adolescents who constitute 20% of the world's adolescent's population.^{1,2} At any point of time, more than 1 in 10 children in India are teenagers currently experiencing puberty and more than a quarter of all

children will transition to adolescence and puberty within the next decade.³⁻⁵

As a physiological process, menstruation is unique to the reproductive cycle of females that begins at menarche and ends at menopause.^{6,7} With the onset of menstruation, a girl becomes aware of her emerging identity as a female and is greatly influenced by the feedback she receives from her family, peers and the society.⁸ The ages of onset

of menstruation is affected by heredity, racial background and nutritional status.^{9,10}

Surprisingly still today in rural India, this physiological process of menstruation is poorly understood due to myths, taboos and misconceptions attached to it. These act as a barrier for MHM thus endangering the reproductive health of the female.¹¹ Absence of adequate menstrual hygiene can predispose to urinary tract infections (UTI), scabies in the vaginal area, abdominal pain, school absenteeism and complications during future pregnancy.¹²⁻¹⁵

The myths and taboos regarding menstrual health and disease are explored by various studies one of which is related to the day when a girl attains puberty.^{16,17} If the menarche occurs on Monday, the girl will be eminently chaste, if it occurs on Tuesday, she is likely to be a widow early in her days and so on.¹⁸ The myth of impurity attached to which ascertains that the movement of the girl should be restricted. Restrictions include not being allowed to take bath, change clothes frequently, comb hair, enter religious places and dietary restrictions (taboo on the consumption of food like rice, curd, milk, lassi, potato, onion, sugarcane) during the menstrual period are also imposed.¹⁹

Various studies have demonstrated that scientific health education can bring about a positive change in knowledge and practice behavior among adolescent girls regarding MHM and can help useful to dispel age old myths, taboos and prejudices thus making the experience of menstruation more non-discriminatory and a normalized experience.^{8,20-23}

Peer-led health education which is administered by trained volunteers can be used as a method of health education to enlighten the participants. The advantage of this method is that the participants are more receptive to the advice as ice-breaking is easy, they feel more relaxed and involved in the process. It overcomes language and cultural barriers and thus helps in reinforcement. Disadvantages include selecting and training of peers, peer to participant communication process and loss of information on subsequent sessions.

Direct health education administered through the agency of an expert has also been shown to positively influence knowledge and practice behavior among the participants.^{20,24} The advantage of this method is that health education is administered by an expert and there is no loss of information on the subsequent visit. Disadvantages include the development of teacher-student relationship, didactic method of learning, language and socio-cultural barrier can impede possible information transmission.

So, the present study was aimed to compare the relative effectiveness of direct health education with peer-led health education as an intervention to improve the

knowledge and practices regarding menstrual hygiene among adolescence girls.

The study will emphasize the important role played by health education in MHM which can be used by the primary care physicians to treat common menstrual problems faced by adolescents.

METHODS

The present study was a longitudinal follow up intervention study conducted for a period of 15 months. It was a part of the project conducted from June 2015 to October 2017 in the Perambalur district of Tamil Nadu state of India. This the first part of the study can be accessed at <http://dx.doi.org/10.18203/2394-6040.ijcmph20211247>.

The study participants were high school adolescent girls of 13-15 age group selected using the random sampling technique. Review of the literature suggested that only 50% of school going adolescent females have adequate knowledge regarding menstruation and an effect size of 0.31 post-intervention. The minimum required sample computed for each group was 81, which came out to be 243 for three groups. On considering the design effect of 2, the final sample size came to be 486 study participants.

Details regarding the number of girls studying in 8th and 9th grades, location of school were collected from the district educational officer. Considering a minimum enrolment of 50 girls in class 8th and 9th, the investigators needed approximately 9 schools for the study to reach a sample size of 486. From the list of schools, 9 schools were selected by cluster random sampling. From the selected schools, all eligible consenting adolescent girls were included as study participants for the study. Schools were randomly allocated into three groups. Group I was the peer-led intervention group where health education was administered by trained students, group II was the direct intervention group where the health education was administered by the principal investigator and group III as the control group which received neither the first intervention nor the second intervention. It was made sure that the schools of various groups were located 10 kms apart in order to prevent intervention dilution. Adolescent girls of grades 8th and 9th, who have attained menarche 6 months back and who gave consent were included in the study whereas girls who were on long leave and could not be contacted in two subsequent visits were excluded from the study (Figure 1).

A self-administered, pre-tested and semi-structured questionnaire in the local language was used for data collection. The questionnaire included socio-demographic profile, menarche and menstruation details, knowledge regarding menstruation and menstrual hygiene, attitude of participants towards menstruation and menstrual hygiene, practices during menstruation and restrictions faced by participants during menstruation.

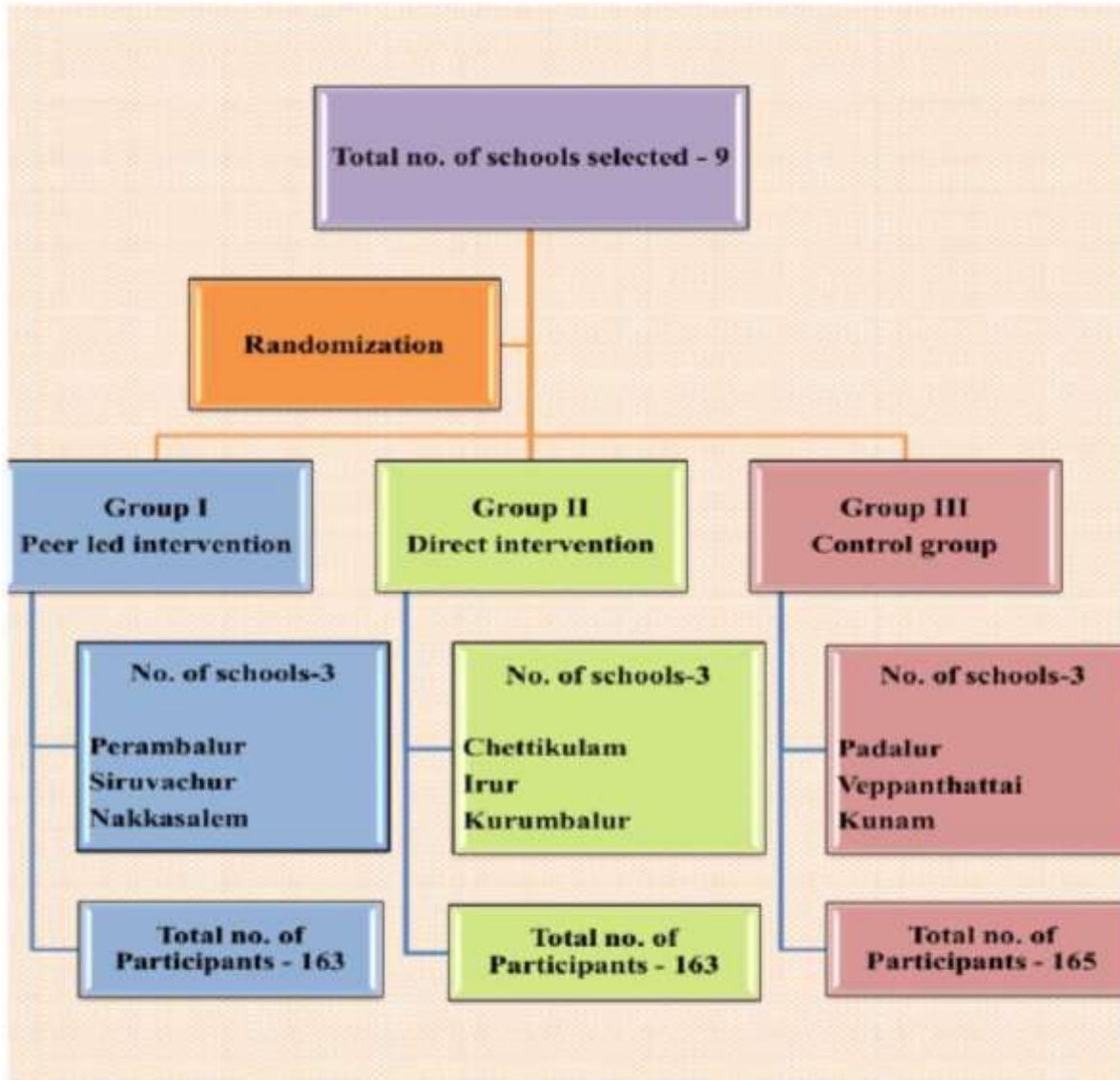


Figure 1: Selection of study schools and intervention in the three groups.

The questionnaire adopted a scoring system for ease of analysis and to demonstrate improvement. It included a knowledge score of poor:0-3, average: 4-7 and good: 8-10 and a practice score of poor: ≤ 4 , average: 5-8 and good: ≥ 9 . After explaining the purpose of the study, written informed consent of the participants was obtained and a pre-test was conducted for all the 486 participants in classroom of the schools selected. Questions were explained to the students and they were asked to answer them without discussing among themselves. It was made sure that all the questions were answered by the participants. On completion of the pre-test, participants were randomly allocated in group.¹⁻³

In the present study, health education was the form of intervention aimed at creating awareness regarding menstruation and menstrual hygiene among the study

participants. Health education material for peer led intervention and direct led intervention was developed by referring standard information education communication (IEC) materials like ANM training manual, ASHA book for adolescent health, WASH manual, UNICEF training manual.²⁵⁻²⁸ IEC material included flipbooks and pamphlets were developed in both English and Tamil (vernacular language). In addition to this, a power point presentation was developed in both the languages. All these IEC materials were corrected and finalised by experts from the specialities of obstetrics gynaecology and community medicine. Three sessions of sequential interventions for a period of 6 months were administered to groups 1 and group 2. Session 1 was through interaction and group discussion for 30 mins. Session 2 used flipbooks, pamphlets for health education for duration of 45 mins and session 3 used audio-visual aids (powerpoint presentation) for 30 mins (Figure 2).

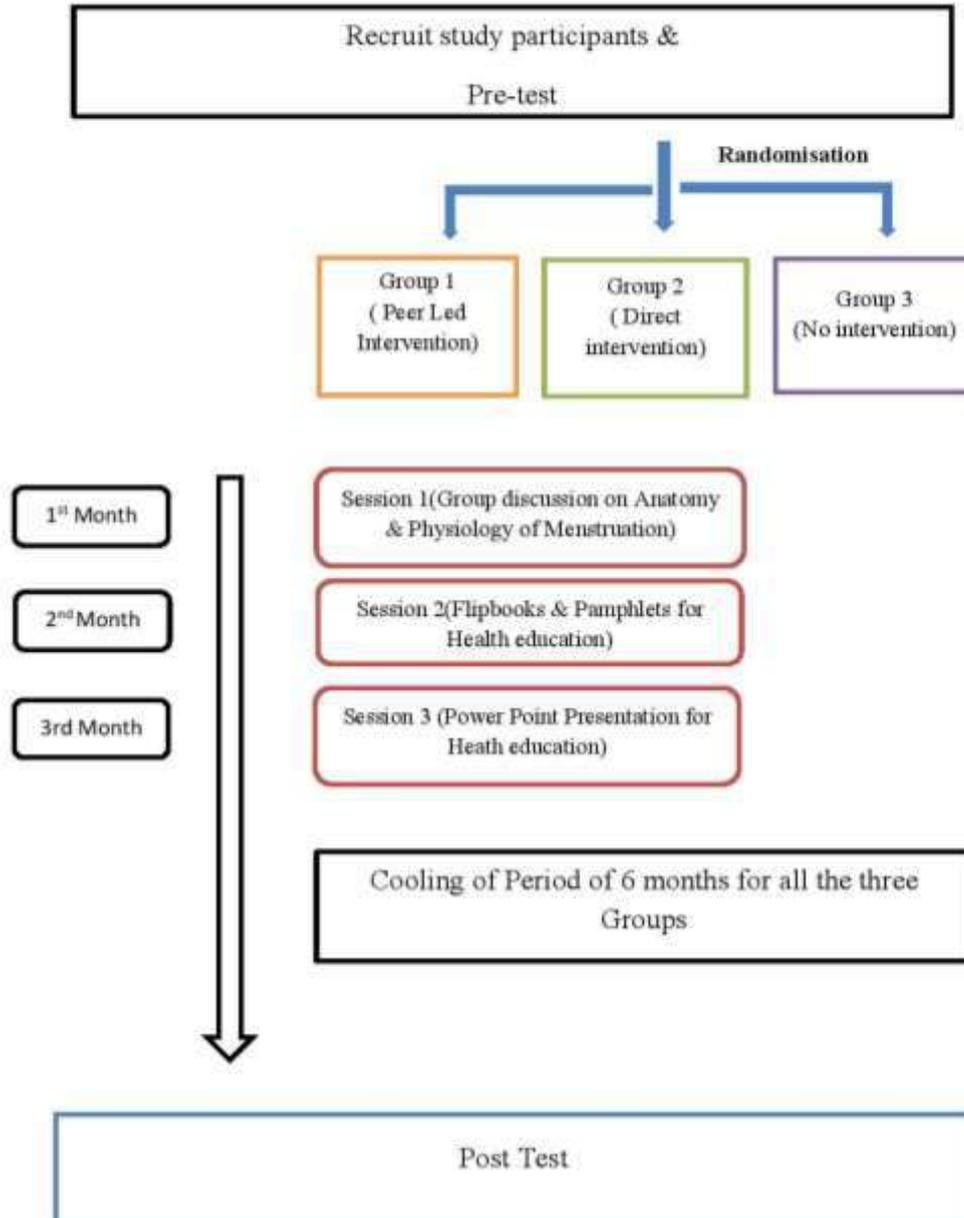


Figure 2: Steps in intervention pre and post test.

Intervention sessions were given at school class room for 1st session and in IT labs for remaining sessions. Above mentioned sessions were given for 3 months with one-month interval between sessions. The health education was given in Tamil, vernacular language to facilitate better understanding among the participants.

All the data was collected, coded, entered in microsoft excel sheet and analysed using statistical package for social sciences (SPSS) version 21. Descriptive statistics were expressed as mean and standard deviation for continuous variable and proportion for categorical

variable. Comparison of pre and post-test sample mean was carried by paired t test. Analysis of variance (ANOVA) test was done to analyse the differences in group means and multiple comparisons were done using post hoc Tukey test.

Ethical clearance for conducting the study was obtained from the institutional ethics committee of human subjects (IECHS) of Dhanalakshmi Srinivasan medical college and hospital, (Number: IRCHS/DSMCH/020 and Number: IECHS/DSMCH/017).

RESULTS

In the present study, total of 491 girls were enrolled for the study. During follow up, 489 participants were available for post-test as 2 girls were considered as loss to follow up. The numbers of participants were 163, 161, and 165 in groups I, II and III, respectively.

In the present study, the majority, 355 (73%) of participants belonged to 14 years. There was no significant difference ($p>0.05$) in age distribution between the three groups. Among the participants, 470 (96.7%) were Hindus by religion ,393 (80.8%) were from nuclear families. 65% and 70% of participant's mother and father were literate, respectively. 77.9% and 89.9% of participants mother and father were employed. Among the study participants, 221 (45.4%) live in the kutcha type of house. 289 (59.5%) of the study participants belonged to lower-middle socioeconomic class (Table 1).

Following the intervention, a post-test was conducted and the results were compared with the pre-test. Knowledge and practice scores were calculated in the post-test. Following the post-test, it was observed that the percentage of participants with good knowledge had

increased by 32.4% and 41.6% in group I and II respectively as compared to the pre intervention levels (Table 2). There was a decrease in the number of participants who had poor knowledge in all groups, comparatively more in group I and II. There was a significant difference ($p<0.05$) observed in group I and II in knowledge score. There was no significant difference observed in group III (Table 2).

The investigators observed improvement in practice scores by 33.8% and 52.1% in group I and II respectively (Table 2). There was a decrease in the number of participants who had poor practice score in all groups, comparatively more in group I and II. There was no significant difference observed in group III (Table 2).

A one-way analysis of variance (ANOVA) was calculated on follow up knowledge and practice score. The analysis showed significant difference at $p<0.05$ which indicates that there was a mean difference between the three groups in the follow up scores. The mean knowledge scores (SD) were 6.20 (2.53), 7.39 (2.11) and 5.30 (2.43) for groups I, II and III, respectively. Similarly, post-intervention, mean practice scores (SD) were 9.89 (2.19), 10.32 (2.23) and 8.73 (1.88) for groups I, II and III respectively (Table 3).

Table 1: Socio-demographic characteristic of study participants.

| Characteristics | Intervention group I; peer education; n1=163; N (%) | Intervention group II; direct education; n2=161; N (%) | Control group II; no intervention; n3=165; N (%) | Chi square value | P value |
|----------------------------|---|--|--|------------------|---------|
| Family type | | | | | |
| Nuclear | 130 (79.8) | 131(81.4) | 131(79.4) | 0.22 | 0.89 |
| Joint | 33 (20.2) | 30(18.6) | 34(20.6) | | |
| Religion | | | | | |
| Hindu | 159 (97.5) | 150 (93.2) | 161 (97.6) | 5.58 | 0.06 |
| Non-Hindu | 4 (2.5) | 11 (6.8) | 4 (2.4) | | |
| Mother's education | | | | | |
| Illiterate | 49 (30.1) | 57 (35.5) | 62 (37.6) | 2.17 | 0.33 |
| Literate | 114 (69.9) | 104 (64.5) | 103 (62.4) | | |
| Father's education | | | | | |
| Illiterate | 43 (26.4) | 51 (31.7) | 51 (30.9) | 1.27 | 0.528 |
| Literate | 120 (73.6) | 110 (68.3) | 114 (69.1) | | |
| Mother's occupation | | | | | |
| Unemployed | 42 (25.2) | 26 (16.1) | 43 (24.8) | 5.87 | 0.05 |
| Employed | 121 (74.8) | 135 (83.8) | 122 (75.1) | | |
| Father's occupation | | | | | |
| Unemployed | 23 (14.1) | 16 (10.0) | 10 (6.0) | 5.89 | 0.05 |
| Employed | 140 (85.9) | 145 (90.0) | 155 (93.9) | | |
| House type | | | | | |
| Kutcha | 70 (42.9) | 75(46.6) | 77(46.7) | 6.638 | 0.156 |
| Semi-pucca | 60 (36.8) | 63 (39.1) | 71 (43.0) | | |
| Pucca | 33 (20.2) | 23 (14.3) | 17 (10.3) | | |

Table 2: Comparison of knowledge and practice scores of participants pre (n1) and post (n2) intervention in the three groups.

| Variables | Group | Scores | Pre-intervention n1 (%) | Post-intervention n2 (%) | Mean difference (Pre, post intervention) | SD | t value | P value |
|------------------|-----------|---------------|-------------------------|--------------------------|--|------|---------|---------|
| Knowledge | Group I | Poor (0-3) | 92 (56.4) | 28 (17.2) | -1.35583 | 3.17 | -5.45 | 0.00 |
| | | Average (4-7) | 65 (39.8) | 76 (46.6) | | | | |
| | | Good (8-10) | 6 (3.6) | 59 (36.2) | | | | |
| | Group II | Poor (0-3) | 66 (41) | 9 (5.6) | -1.96894 | 2.56 | -9.74 | 0.00 |
| | | Average (4-7) | 78 (48.4) | 68 (42.2) | | | | |
| | | Good (8-10) | 17 (10.6) | 82 (52.2) | | | | |
| | Group III | Poor (0-3) | 67 (40.6) | 49 (29.7) | 0.01818 | 3.21 | 0.07 | 0.94 |
| | | Average (4-7) | 77 (46.7) | 81 (49.1) | | | | |
| | | Good (8-10) | 21 (12.7) | 35 (21.2) | | | | |
| Practice | Group I | Poor (0-3) | 23 (41) | 6 (3.7) | 1.9264 | 3.46 | 7.09 | 0.00 |
| | | Average (4-7) | 67 (41.1) | 29 (17.8) | | | | |
| | | Good (8-10) | 73 (44.7) | 128 (78.5) | | | | |
| | Group II | Poor (0-3) | 18 (11.2) | 8 (5.0) | 2.6149 | 3.49 | 9.48 | 0.00 |
| | | Average (4-7) | 87 (54.0) | 13 (8.1) | | | | |
| | | Good (8-10) | 56 (34.8) | 140 (86.9) | | | | |
| | Group III | Poor (0-3) | 11 (6.7) | 7 (4.2) | 0.2667 | 2.57 | 1.33 | 0.18 |
| | | Average (4-7) | 73 (44.2) | 50 (30.3) | | | | |
| | | Good (8-10) | 81 (49.1) | 108 (65.5) | | | | |

Table 3: Post intervention comparison of follow up knowledge and practice scores between the three groups.

| Variables | Group | Mean | SD | F | P value |
|------------------|-----------|--------------------|--------|--------|---------|
| Knowledge | Group I | 6.209 | 2.53 | 32.081 | 0.0 |
| | Group II | 7.391 [#] | 2.113 | | |
| | Group III | 5.297 | 2.4301 | | |
| Practice | Group I | 9.89 | 2.19 | 24.959 | 0.0 |
| | Group II | 10.32 [*] | 2.23 | | |
| | Group III | 8.73 | 1.88 | | |

[#]group II demonstrated increase in mean knowledge scores post intervention as compared to other groups; ^{*}group II demonstrated increase in mean practice scores post intervention as compared to other groups.

Table 4: Multiple comparisons of knowledge and practice scores between groups post intervention using post hoc Tukey test.

| Dependent variables | (I) | (J) | Mean difference (I-J) | Standard error | Significance |
|--|-----------|-----------|-----------------------|----------------|--------------|
| Post intervention knowledge score | Group I | Group II | -1.1827 [*] | 0.2629 | 0 |
| | | Group III | .9116 [*] | 0.2612 | 0.002 |
| | Group II | Group I | 1.1827 [*] | 0.2629 | 0 |
| | | Group III | 2.0943 [*] | 0.2621 | 0 |
| | Group III | Group I | -.9116 [*] | 0.2612 | 0.002 |
| | | Group III | -2.0943 [*] | 0.2621 | 0 |
| Post intervention practice score | Group I | Group II | -0.4396 | 0.2345 | 0.147 |
| | | Group III | 1.1562 [*] | 0.233 | 0 |
| | Group II | Group I | 0.4396 | 0.2345 | 0.147 |
| | | Group III | 1.5959 [*] | 0.2337 | 0 |
| | Group III | Group I | -1.1562 [*] | 0.233 | 0 |
| | | Group | -1.5959 [*] | 0.2337 | 0 |

^{*}denotes significant difference observed between the two groups.

Multiple comparisons Tukey test was used to determine whether groups differed from each other in the knowledge and the practices scores following the intervention. There was a statistically significant difference observed in knowledge score between group I versus group II ($p=0.001$), group I versus group III ($p=0.002$), group II versus group III ($p=0.001$). When comparing the mean difference, it is evident that knowledge score of group II is comparatively more than group I (Table 4).

Regarding post-intervention practice scores there was a statistically significant difference observed between the group I versus group III ($p=0.001$), group II versus group III ($p=0.001$). However, there were no significant differences observed between the group I and group II ($p=0.147$). When comparing the mean difference, it is evident that practice score of the group II is comparatively more than group I (Table 4).

DISCUSSION

To our present knowledge, this study is the first attempt to compare the relative effectiveness of direct intervention over peer-led intervention on knowledge and practices of menstrual hygiene among adolescent girls which makes comparison with the other studies a difficult proposition.

In the present study, the majority of study participants were of 14 years which is comparable to multiple studies where the age of participants was similar.^{11,29-31}

In the present study, 80% of study participants belonged to a nuclear family which is similar to study done in South India by Zaidi et al where 88% of participants belonged to nuclear family.¹¹ In the present study 65.6% and 70.3% of participants mothers and fathers were literate. This result is comparable with literacy rate of Perambalur district, which is 73.1% and 84.6% for females and males, respectively.^{32,33}

In the present study, post-intervention there was a significant increase in the percentage of participants with good knowledge and practice scores both in the peer led (group I) as well as the direct intervention (group II) compared to the control group (group III). The percentage of participants with good knowledge scores increased by 32.4% and 41.6% in group I and II respectively in the present study, which is comparable to a study conducted in Bangladesh by Haque et al which observed a 31.4% increase in follow up knowledge score post-intervention.³ Similarly, in the present study, the percentage of participants with good practice score increased by 33.8% and 52.1% in group I and II respectively post intervention which is comparable to the study conducted by Haque et al in Bangladesh which observed a 60.1% increase in good practice score following intervention.³

Post-intervention, there present study observed a significant increase in mean knowledge and mean practice scores in peer led (group I) and direct intervention (group II) as compared to the control group (group III) which is comparable with study conducted by Anitha et al in Chennai where the mean knowledge and practice score post intervention were significantly higher.³⁴

In order to compare the relative effects of intervention among the groups, Post-hoc analysis was conducted using Tukey HSD (honest significant difference). The direct intervention group (group II) showed significant increase in both the knowledge and practice scores as compared to the other two groups. There are various studies which have demonstrated the impact of educational intervention on menstrual hygiene, but comparison of direct and peer-led intervention is not to be found.^{8,20-23,35,36} In recent intervention studies conducted at Uganda, the investigators observed a significant improved in knowledge (reduce anxiety to menstruation) and practices (effective pain management) following intervention.^{35,36} A study conducted in western Nigeria by Ademola evaluated the role of school-based education involving teacher-led and peer-led intervention on reproductive health of adolescents but not specifically on menstrual hygiene.³⁷ In study conducted by Ramaiya et al in India emphasis that social and behavioural change communication (SBCC) intervention plays a positive role in influencing the knowledge, attitudes, interpersonal communication, restrictions and MHM of adolescent girls.³⁸

In present study, the direct intervention group showed more significant increase in knowledge and practices among participants owing to pictorial demonstration. Peer led intervention group also showed improvement but less than direct intervention group which can be attributed to hesitation of the volunteers to carry out the health education more effectively to the participants.

CONCLUSION

The study concludes that health education in the form of direct intervention by the trained professional has a higher impact on dispelling myths, misconceptions, taboos and adopting healthy menstrual practices among adolescence females in India. The investigators recommend that health education by a trained professional should be initialized and internalized as a programme on MHM in school curriculum.

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Original Paper

The Association Between Gaming Practices and Scholastic Performance Among Medical Students in India: Case-Control Study

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Abstract

Background: Gaming is a billion-dollar industry that is expanding at a compound annual growth rate of 9% to 14.3%, with the biggest market in Southeast Asian countries. The availability of low-cost smartphones and the ease at which the internet can be accessed have made gaming popular among youth, who enjoy it as a leisure activity. According to the World Health Organization, excessive indulgence in gaming can lead to gaming disorder. Medical students indulging in excessive gaming can succumb to gaming disorder, which can affect their scholastic performance.

Objective: This study aimed to assess the association between gaming practices and scholastic performance among medical students.

Methods: This study used a case-control design, where 448 medical undergraduate students (first year to prefinal) were preliminarily surveyed using universal sampling on their gaming practices in the last 6 months. Out of this sample, the 91 participants who admitted to gaming in the past 6 months were recruited as cases, while participants who never engaged in gaming in the last 6 months were recruited as controls. Both the cases and controls were matched for age and gender in a 1:1 ratio. The internal assessment scores (based on 2 midterms completed in the last 6 months) of cases and controls were compared. The Snedecor F test was used to determine the association between the number of hours spent gaming and internal assessment scores (%), while the Student *t* test was used to determine significant differences between the internal assessment scores of cases and controls. Odds ratios were calculated to identify the risk of poor scholastic performance among cases compared to the controls. The prevalence of gaming disorder among cases was assessed using the Gaming Addiction Scale (GAS).

Results: The frequency of gaming (in hours) was not associated with mean internal assessment scores ($P=.13$). Male cases reported significantly lower internal assessment scores compared to male controls ($P=.005$ vs $P=.01$), whereas no significant differences were observed between the internal assessment scores of female cases and controls ($P=.89$ vs $P=.59$). A negative correlation was observed between GAS scores and internal assessment scores ($r=-0.02$). The prevalence of gaming disorder using the GAS was observed to be 6.3% (28/448) in the study population and 31% (28/91) among cases. The risk of low scores (<50%) among gamers was observed to be 1.9 (95% CI 1.04-3.44, $P=.03$) times higher in the first midterm and 1.80 (95% CI 0.97-3.36, $P=.06$) times higher in the second midterm compared to nongamers.

Conclusions: The findings suggest that excessive gaming adversely affects the scholastic performance of male participants more than female participants. Awareness about gaming disorder needs to be created among students, parents, and teachers. Treatment services should be made available to medical students with gaming disorders.

KEYWORDS

gaming; gaming disorder; medical students; gaming addiction; scholastic performance; academic performance; addiction; smartphones; mobile phones; youth; medical education

Introduction

In 2019, the global gaming market was valued at US \$151.55 billion, growing at a compound annual growth rate of 9% to 14.3% and expected to reach US \$256.97 billion by 2025, with the largest market in the Asia Pacific region. Of all the available gaming platforms (PC, PlayStation, Xbox), smartphones remain the most utilized gaming platform at present, earning US \$64.4 billion in 2019 [1]. India also has a rapidly growing gaming market, with an annual growth rate of 14.3% valued at US \$890 million currently [2]. This growth is driven by the rising younger population, higher disposable incomes, the introduction of new gaming genres, and the increasing number of smartphone and tablet users [2].

Although considered a harmless leisure activity, excessive indulgence in gaming can lead to possible internet gaming disorder [3]. In the 11th Revision of the International Classification of Diseases, the World Health Organization recognized excessive gaming as a disorder “characterized by impaired control over gaming, increasing priority given to gaming over other activities to the extent that gaming takes precedence over other interests and daily activities, and continuation or escalation of gaming despite the occurrence of negative consequences” [4].

Recent studies have documented significant impairment of physical, psychological, social, and work-related problems such as insomnia, increased irritability and aggression, depressive and/or anxiety symptoms, poor academic performance, and neglect of interpersonal relationships with excessive and problematic gaming [5-7].

The medical curriculum is vast and requires extensive reading and dedication. In such circumstances, indulgence in excessive gaming among students can lead to gaming disorder, which can affect their scholastic performance. This study aimed to shed light on whether gaming practices among medical students affect their scholastic performance. Hence, this study was conducted with the following objectives:

1. To study the amount and nature of gaming practices among medical students;
2. To assess the prevalence of gaming disorder among medical students;

3. To study the association between gaming practices and scholastic performance among medical students.

Methods

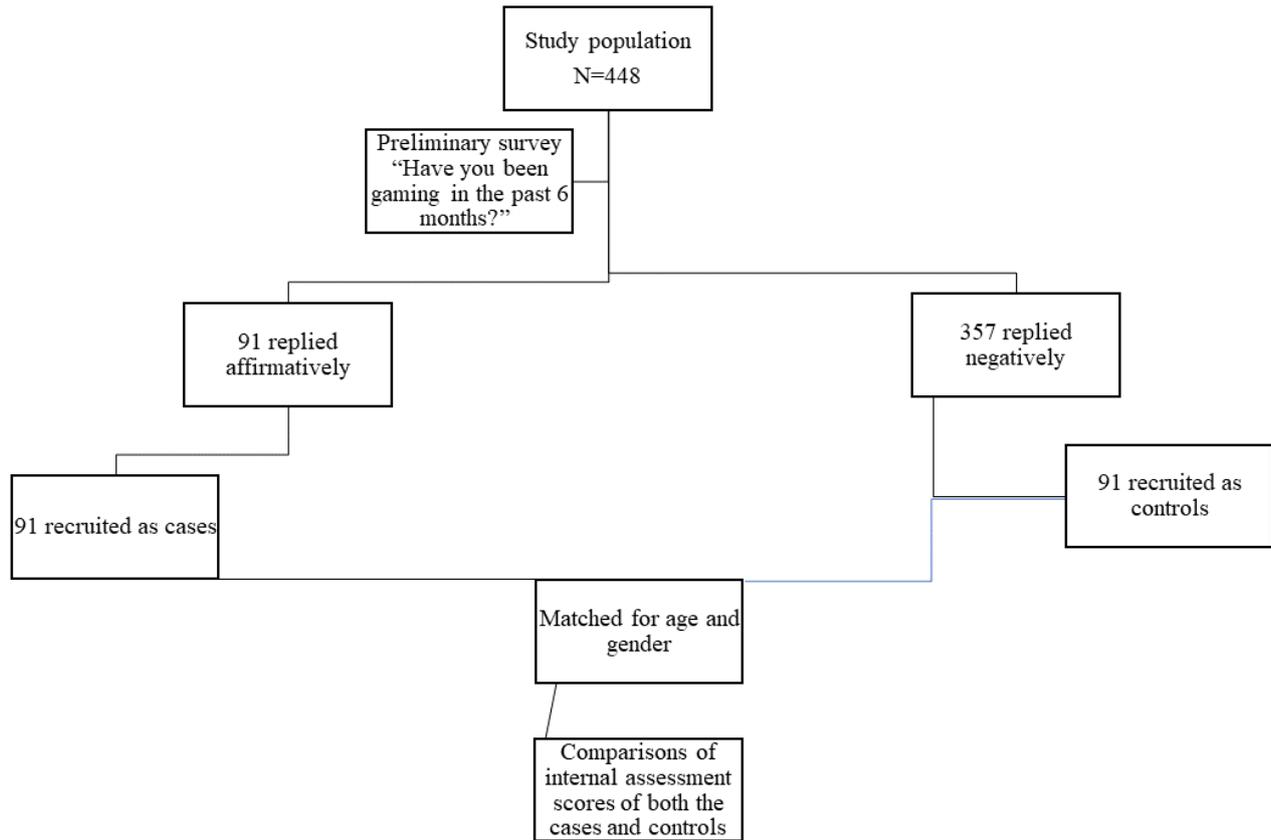
The study attempted to demonstrate the association between gaming practices and the scholastic performance of medical students.

Study Design and Ethical Clearance

The study used a case-control design and was conducted during the period of October and November 2019 in a medical college in the Trichy District of Tamil Nadu, India. Ethical clearance was obtained from the Institutional Ethical Committee of Trichy SRM Medical College (1007/TSRMMCH&RC/ME-1/2019-IEC no:039). Informed written consent was obtained from all the participants. If the enrolled participants were not interviewed on a specified date, they were interviewed subsequently at a time and place of their convenience. The purpose of the study was explained in detail and assured that the data collected would be used only for scientific purposes. Ethical principles such as respect for the person and confidentiality of their data were strictly adhered to.

Recruitment of Cases and Controls

A total of 448 undergraduate medical students in their first to prefinal year were included as participants using the universal sampling technique (Figure 1). The study preliminarily surveyed the entire study population of 448 students using personal interviews. All 448 participants were asked only 1 question: “Have you been gaming in the last 6 months?” From this preliminary sample of 448 surveyed students, 91 students replied affirmatively and were recruited as cases in the study. Following this, the investigator used purposive sampling to select 91 controls from the remaining 357 students who had never indulged in gaming in the last 6 months, and matched both cases and controls for age and gender. The controls selected were matched for age and sex using a 1:1 ratio. The frequency of gaming hours per week was assessed among the cases. The internal assessment scores of the two midterm examinations held in the last 6 months were accessed from the students’ records kept by the institution after obtaining written permission from the students and the Institutional Ethical Committee. The internal assessment scores of cases and controls were then compared and recorded in percentages.

Figure 1. Recruitment of cases and controls.

To assess the prevalence of gaming disorder, the Gaming Addiction Scale (GAS) by Lemmens et al [8] was used. The GAS is a pretested, prevalidated scale with a Cronbach alpha of .82 to .87 [8]. It has 7 items: salience, tolerance, mood modification, relapse, withdrawal, conflict, and problems. Each item has three questions with a score range of 0 to 5 with all the components scored on a Likert scale: 1=never, 2=rarely, 3=sometimes, 4=often, and 5=very often. The investigators used the monothetic format in the study, that is, a score of >3 for all items being indicative of gaming addiction. Lemmens himself hypothesized that the monothetic format would lead to a better estimate of the prevalence of addiction than the polythetic format [8]. Therefore, the investigators used the GAS according to protocol, but for the convenience of analysis, the investigators summed up the total score of all 7 items, and classified participants with a score of ≥ 63 as having a gaming disorder.

Statistical Analysis

The data entry and analysis were done using SPSS software (version 21, IBM Corp). Descriptive statistics were used for

analyzing sociodemographic details, frequency, and type of gaming. The Snedecor F test and the Student *t* test were used to determine the association between the hours spent gaming and scholastic performance, and gaming and internal assessment scores, respectively. Odds ratios were used to calculate the risk of low internal assessment scores among cases and controls. The correlation coefficient (*r*) was used to determine the correlation between the GAS scores and internal assessment scores.

Results

Of the 448 students who were preliminarily surveyed, 91 were allocated as cases and 91 as controls. Out of the 91 cases, 49 (53.8%) were female and 42 (46.2%) were male. The majority of cases (80/91, 87.9%) were aged 19 to 23 years. In terms of gaming platform, 87 (95.6%) used a mobile phone, 3 (3.4%) used a personal computer or laptop, and 1 (1.0%) used Xbox (Table 1).

Table 1. Age distribution and gaming characteristics of cases (n=91).

| Characteristic | Female, n (%) | Male, n (%) | Total, n (%) | P value |
|-------------------------------------|---------------|-------------|--------------|---------|
| Age (years) | | | | .57 |
| ≤18 | 7 (7.6) | 3 (3.3) | 10 (11.1) | |
| 19-23 | 42 (46) | 38 (41.7) | 80 (87.9) | |
| ≥24 | 0 (0) | 1 (1.1) | 1 (1.0) | |
| Gaming platform used | | | | .75 |
| Mobile phone | 48 (52.7) | 39 (42.8) | 87 (95.6) | |
| Mobile phone/PC | 1 (1.1) | 2 (2.1) | 3 (3.4) | |
| Xbox | 0 (0) | 1 (1.1) | 1 (1.0) | |
| Hours per week spent gaming | | | | .47 |
| ≤10.0 | 12 (13.1) | 16 (17.5) | 28 (30.8) | |
| 10.1-25.0 | 28 (30.7) | 22 (24.1) | 50 (55.0) | |
| 25.1-40.0 | 5 (5.4) | 2 (2.1) | 7 (7.6) | |
| 40.1-55.0 | 3 (3.2) | 0 (0) | 3 (3.2) | |
| ≥55.1 | 1 (1.1) | 2 (2.1) | 3 (3.2) | |
| Gaming Addiction Scale score | | | | .97 |
| <63.0 | 34 (37.3) | 29 (31.8) | 63 (69.2) | |
| ≥63.0 | 15 (16.4) | 13 (14.2) | 28 (30.7) | |

The frequency of playing games was assessed for a typical working day in hours and then calculated for a 7-day week. In this study, more than half of the cases (50/91, 55.0%) spent 10-25 hours per week gaming, 28 (30.8%) cases spent less than 10 hours per week, and 6 (6.4%) cases spent more than 40 hours per week (Table 1). There was no significant difference observed in the internal assessment scores of those who played games for more hours than those who played for fewer hours ($P=.13$).

Mean scores among cases were 5.2% lower compared to the controls (mean score 48.7 vs 53.9, $P=.01$) in the first internal

assessment and 4.1% lower (mean score 50.2 vs 54.3, $P=.01$) in the second internal assessment.

Male cases showed a significantly lower mean score of 9.5% on the first Internal assessment ($P=.005$) and 8.4% on the second internal assessment ($P=.01$) compared to male controls. Female cases observed 0.6% lower scores on both internal assessments than female controls ($P=.89$ and $P=.59$), as shown in Figure 2 and Table 2.

Figure 2. Comparison of mean internal assessment scores across various groups (n=91).

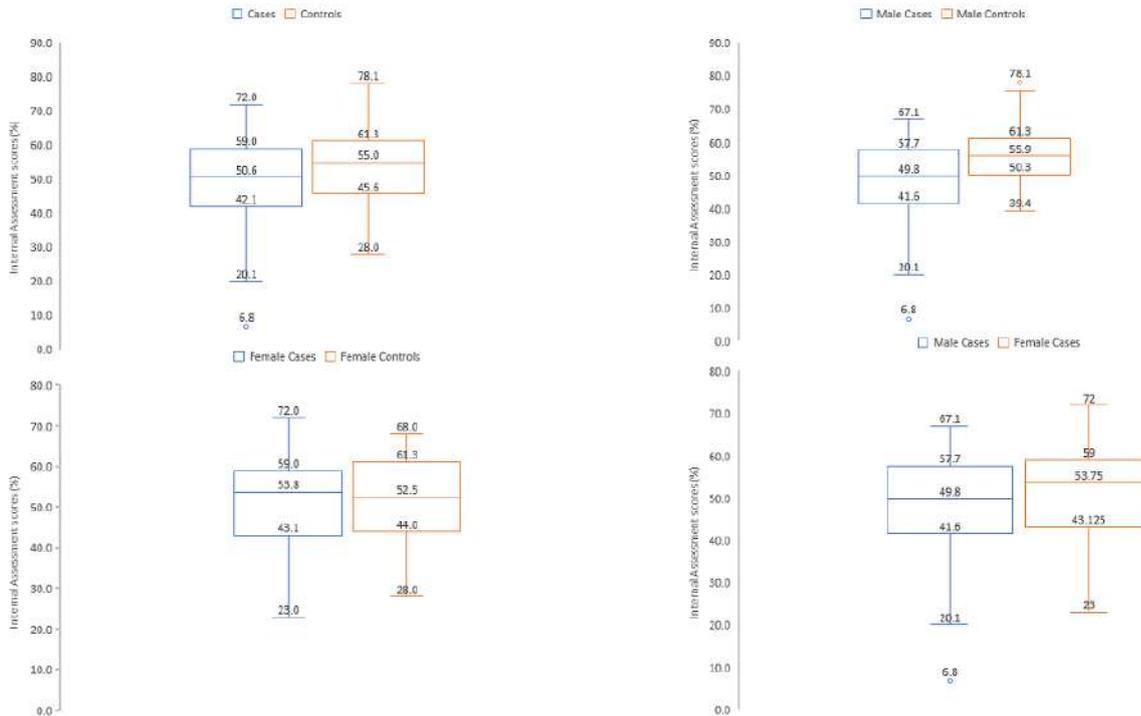


Table 2. Comparison of mean international assessment scores between various groups.

| Group | First internal assessment scores | | Second internal assessment scores | |
|-----------------|----------------------------------|-------------------|-----------------------------------|------------------|
| | Mean (SD) | P value | Mean (SD) | P value |
| Group 1 | | .01 ^a | | .01 ^a |
| Cases | 48.7 (15.0) | | 50.2 (13.9) | |
| Controls | 53.9 (12.1) | | 54.3 (11.5) | |
| Group 2 | | .05 | | .83 |
| Male cases | 45.6 (15.5) | | 50.2 (14.9) | |
| Female cases | 50.1 (14.0) | | 50.2 (13.2) | |
| Group 3 | | .005 ^a | | .01 ^a |
| Male cases | 45.9 (13.6) | | 49.3 (14.7) | |
| Male controls | 55.4 (12.1) | | 57.7 (10.6) | |
| Group 4 | | .89 | | .59 |
| Female cases | 52.2 (13.9) | | 50.8 (13.3) | |
| Female controls | 52.8 (12.2) | | 51.4 (11.5) | |

^aSignificant values.

The 7 items of the GAS were analyzed for the 91 cases. A salience score of ≥ 3 was observed in 30 (33%) participants, 24 (26.4%) had a tolerance score of ≥ 3 , 34 (37.4%) had a mood modification score of ≥ 3 , 20 (22%) had a relapse score of ≥ 3 , 26 (28.6%) a withdrawal score of ≥ 3 , 22 (24.2%) had a conflict score of ≥ 3 , and 56 (61.5%) had a problem score of ≥ 3 (Multimedia Appendix 1).

Of the 448 students who were surveyed, 28 cases had a GAS score of ≥ 63 . Thus, the prevalence of gaming disorder in this study was 6.3% among the study population and 31% (28/91) among cases.

There was a significant difference observed between mean GAS scores among male and female cases (males: mean 69.5, SD 6.4, n=13 vs females: mean 78.5, SD 9.2, n=15; $P=.008$). The GAS scores of female cases were 9 percentage points higher than male cases.

There was a negative correlation observed between the GAS and mean internal assessment scores for the cases ($r=-0.02$). Further, it was observed that the odds of scoring less than 50% were 1.9 (95% CI 1.04-3.44, $P=.03$) times more among cases than controls. A similar result was observed during the second internal assessment, where the odds of scoring less than 50%

were 1.8 (95% CI 0.97-3.36, $P=.06$) times higher among cases than controls.

Discussion

Principal Findings

To the best of our knowledge, this study is the first to use a case-control design to examine the association between gaming and scholastic performance in medical students. Since the availability of literature on internet gaming among medical students is sparse, it is difficult to draw meaningful comparisons.

This study observed that smartphones were the most commonly used gaming platform by medical students. The study observed no significant association between the frequency of gaming and internal assessment scores. Gamers (cases) showed a significantly lower score than nongamers (controls). Male gamers showed significantly lower scores compared to male nongamers, whereas the difference between scores of female gamers and nongamers was not statistically significant. The study found a negative correlation between GAS scores and internal assessment scores. Further, there was a higher risk of lower scores among those who played games compared to those who did not.

Time Spent Gaming and Internal Assessment Scores

There was no significant differences observed in the internal assessment scores and the number of hours spent gaming. This finding differs from a study by Ip et al [9], where frequent gamers (both males and females) scored less than nonfrequent gamers in examinations, with the average grades of nongamers being 9.4% higher than those of frequent gamers. A study conducted by Dumrique and Castillo [10] observed no significant relationships between the number of hours of playing and the social behavior of the respondents. The reason for this difference may be because they included assessments from the whole academic year, whereas we have included only assessments from the last 6 months. In addition, the scale of measurements differs between those studies and our study.

Internal Assessment Scores and Gaming Among Males and Females

In this study, the mean scores of the first and second midterms of those who played games were 5.2% and 4.1% lower than those who did not play games, respectively. Male nongamers had 9.5% and 8.4% higher scores than male gamers for the first and second assessments, respectively. This is somewhat similar to the finding of Ip et al [9], where the examination grades of infrequent male gamers were on average 7.2% higher compared to regular male gamers. In our study, we observed no significant difference in internal assessment scores between female gamers and female nongamers. We also observed that female gamers had higher internal assessment scores compared to male gamers despite having higher GAS scores. This indicates that although there is a greater incidence of gaming disorder among females, this is not associated with poor scholastic performance. This is similar to the findings of Ip et al [9] on gaming frequency and academic performance, where female students performed better than male students in all disciplines even though they were gaming. Contrary to our finding was Dumrique and Castillo's

[10] observation that the academic performance of students was not affected even if they played online games. This difference is because their participants had better self-control, played games preferably during the weekends, and socialized more. This finding is useful in the context of gaming as a leisure activity that is not done in excess.

Prevalence of Gaming Disorder Using Various Scales

In this study, gaming addiction, as assessed by the GAS, was found to be prevalent in 6.2% of the study population and 31% of those who played games. The prevalence of gaming disorder using different scales in various prior studies ranged from 2.0% to 22.7% [8,11-23]. This variation may be due to differences in study populations and measurement scales used.

In terms of specific studies, Lemmens et al [8] found the prevalence of the gaming addiction to be 2.3% using the monothetic format and 9.3% using the polythetic format [8]. Mentzoni et al [24], who used the GAS, observed the prevalence of problematic users (score of ≥ 4 out of 7 on the GAS) to be 4.1%. Wang et al [21] in Hong Kong identified 15.6% of study participants as having a gaming addiction. In a study conducted in Germany by Festl et al [25], 3.7% of the respondents were considered to be problematic gamers.

Correlation of the GAS With Internal Assessment Scores

We found a negative correlation between GAS scores and mean internal assessment scores—greater gaming disorder scores were associated with lower internal assessment scores, emphasizing the fact that gaming negatively affects scholastic performance. A review by Mihara and Higuchi [26] showed that many studies reported lower grades and career attainment in students indulging in excessive gaming.

Our novel study quantifies the risk of poor scholastic scores associated with excessive gaming, with gamers at higher risk than nongamers (odds ratio 1.9, 95% CI 1.04-3.44). This finding is useful in the context of restricting gaming as a leisure activity than indulging in it excessively. This observation also helps in the early identification and treatment of students who are gaming excessively to prevent poor academic performance.

Limitations

The study comes with the inherent limitations of the case-control design. The retrospective nature of the study can be used to establish an association between gaming and scholastic performance, but cannot establish causation. Additionally, it should be noted that cases and controls were matched only for age and gender since matching for other potential confounders would have led to overmatching and fewer control participants. Further, the findings of this study pertain to a single educational setting, which could limit its generalizability.

Conclusion

We conclude that gaming adversely affects scholastic performance among male students compared to female students. Awareness needs to be created among medical students about the negative effects of gaming, which can have a detrimental effect on their scholastic performance. Students, parents, teachers, and institutions should be advised on the early

detection of gaming disorder. Treatment services should be made available to those with gaming disorder in medical institutions. The study also opens new avenues for further

exploration in different educational settings using a cohort study design to examine the long-term impact of gaming on the scholastic performance of students.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Scoring of items using the Gaming Addiction Scale for cases.

[\[DOCX File , 18 KB-Multimedia Appendix 1\]](#)

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Abbreviations

GAS: Gaming Addiction Scale

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Involvement of husband in maternal and child health care in rural field practice area of a tertiary medical college in South India—A mixed method study

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ABSTRACT

Background: To commemorate the 25th anniversary of International Conference on Population and Development (ICPD) in the pursuit of Sexual and Reproductive Health Rights views of duty bearers (men) who are mostly not involved in antenatal care in a patriarchal society like India needs to be explored. **Design:** It is a mixed method study (Triangulation). **Setting and Population:** It was conducted in a rural field practice area of a private medical college in South India covering a population of 19,200. **Objectives:** 1) To determine the involvement of husband in maternal and child care. 2) To find out the perceptions of the husbands of antenatal pregnant women in maternal and child health (MCH) care. **Methods:** (Quan) A semi-structured questionnaire to find out the areas where husband is involved maximum during antenatal care (Qual). In-depth interviews was conducted to find out the factors associated with their involvement. **Results:** About 72.5% came for antenatal visits while it decreased to 27.5% during labor and further decreased to 20.3% during immunization. The reasons for decreased participation were (1) Professional Commitments, (2) Views of a Patriarchal society like India, (3) Financial Difficulties, and (4) Health Facility Related Challenges. **Conclusion:** There is a need to educate the husband regarding the importance of husband's involvement during delivery and immunization. Programs should also include men as the stakeholders for accountability and better MCH care for women.

Keywords: Husband, involvement, maternal and child health care

Introduction

The maternal and child health (MCH) has been one of the most important components of a nation's health.^[1] Maternal healthcare service comprises services provided for women during pregnancy, delivery, and postnatal.^[2] For decades, maternal mortality and infant mortality rates has been used as a measure of a country's progress toward global health goals. It marks the 25th anniversary of the Cairo of the International Conference on Population and Development (ICPD) which is a platform for all interested in the pursuit of Sexual and Reproductive Health and Rights

which includes duty bearers (men) who are mostly not involved in antenatal care in a patriarchal society like India.^[3] The MCH program in India has undergone drastic changes over the years, currently the RMNCH + A is built on the concept of continuum of care.^[1] While WHO has recommended the support and active involvement of male for better maternal and child health outcomes, this recommendation is yet to find its place in RMNCH+A.^[4]

In a patriarchal society like India, pregnancy, child birth is always considered as exclusive duties belonging to the mother/female gender. Many studies in recent times have demonstrated that involving males in antenatal care has resulted in better outcomes for mother and child.^[5] In the last two decades, many national plans in India have re-emphasized the importance of male involvement, yet without any clear policy directives and a monitoring system to measure the achievements of the program in enhancing male participation in women-related

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health programs.^[6] Tamil Nadu is making rapid progress toward reducing the maternal and infant mortality in the state.^[7] Most of this decline in mortality can be attributed to increase in accessibility to care. Involvement of male in maternal care can prove as one of the efficient ways to accelerate this progress and consolidate the gains made in previous years. So this study was undertaken to understand about the involvement of males in antenatal and child care in a rural area.

Objectives

- 1) To determine the involvement of husband in maternal and child care.
- 2) To find out the perceptions of the husbands of antenatal pregnant women in MCH care.

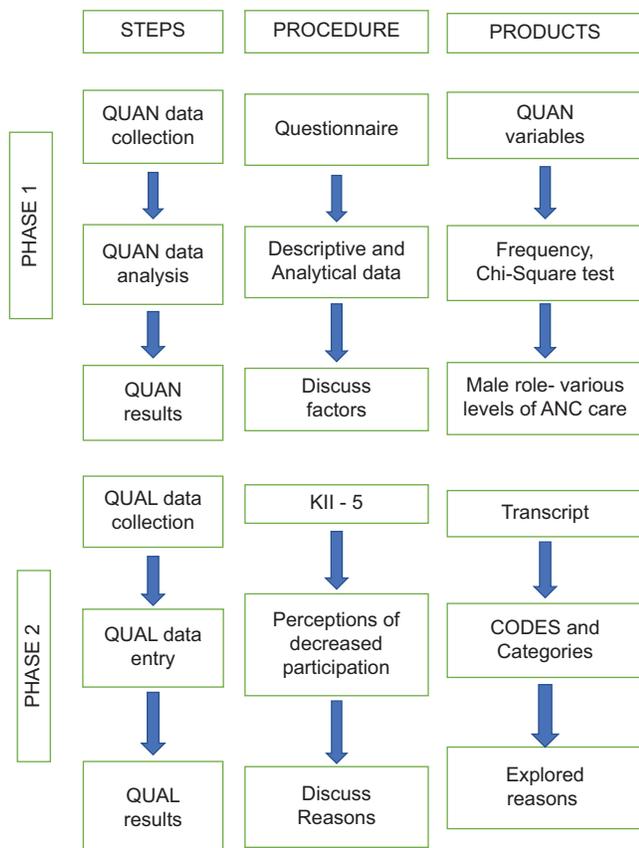
Methodology

Study design

PHASE 1 –It is a triangulation type of mixed method study^[8] where initially a questionnaire was used to find out the involvement of men in the MCH care.

PHASE 2 – Following which five Key informant interviews^[9] was conducted to explore the reasons for poor participation of men during labor and immunization.

VISUAL DIAGRAM OF THE STUDY DESIGN



Study duration

It was conducted for duration of 2 months from January to February 2019.

Study setting

The study was conducted in the rural field practice area of a private medical college. The field practice area includes one rural health and training center covering a population of 19,200.

Phase 1: Quantitative

Non-probability purposive sampling was carried out. All women having last child below 1 year attending the immunization clinics on Wednesdays in rural health center for 2 months were taken as study subjects which came to 69.

Inclusion criteria: Study participants of any gravidas who gave consent and having the last child below 1 year.

Exclusion criteria: Mother having living children above 1 year of age and who did not give consent.

Data collection procedure: The data collection was done using pre-tested semi-structured questionnaire by direct interview to the study respondents. The questions were asked in the local language (Tamil) and written informed consent was obtained prior to the interview. The questionnaire included the sociodemographic profile and the relevant questions on husband involvement. The data entry and analysis were done using SPSS software V 21. Descriptive statistics were used for summary of the findings. Chi-square test was used to find out the association between all the variables with husband involvement.

PHASE 2: Qualitative: Five KII were taken with the husbands of the women who attended the immunization clinic by contacting them with an appropriate time and place for each. They were purposively selected assuming they will shed some light into the perceptions of decreased participation. A trained interviewer interviewed the key informants using semi-structured guidelines which had open-ended questions on the decreased participation of husbands during Labor and immunization phase. The interviews were audio recorded and manual content analysis was done. Inductive and Deductive codes from the transcripts were merged to form categories.

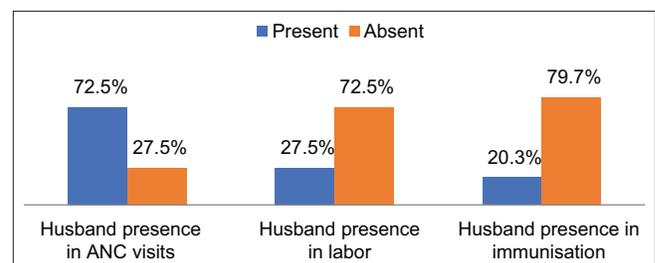


Chart 1: Husbands Involvement in Maternal and Child Health care (N = 69)

Results

(Quan) Majority belonged to Hindu religion (88.4%) and BC caste (55.1%). Almost 60% of fathers have completed only secondary education, and 50% were belonging to the occupation category of clerical/shop owner/farmer. About 50% belonged to class III socioeconomic status. About 70% have registered their pregnancy in government hospital. Around 82.6% of the delivery took place in mother's place [Table 1].

The percentage of husbands accompanying during antenatal visit were 72.5% while it decreased to 27.5% during labor and further decreased to 20.3% during immunization [Chart 1]. While coming to expenses husband contribution was comparatively good for traveling (64%) and almost half have contributed for nutrition (42%) and medicine (48%). But the expense for delivery is paid by the mother's side in 74% of the cases. Mean number of antenatal visits by the mothers were 4.8 and the mean number of visits accompanied by husband was 2.4 [Table 2]. The factors like religion, husband's education and occupation, socioeconomic

status, planning of pregnancy, registration of pregnancy and place of delivery were checked for association with spousal involvement during antenatal care, labor, and immunization but none were statistically significant.

(Qual): The mean age of the participants was 32 ± 1.48 (years \pm SD). The categories that emerged were professional commitments, patriarchal society, financial difficulties, and health facility related challenges [Table 3].

Category 1: One participant said, "I am the sole bread winner in the family. I have to run whether it is raining or a sunny day."

Category 2: "It is the duty of the mother's family." About three out of five men had misogynistic views, "Women are created for the purpose of giving birth. It is no big deal. It is the law of nature."

Category 4: "Meeting the doctor for vaccination takes lot of time as the PHC has a long queue."

Table 1: Preliminary information of study participants (n=69)

| Variables | Frequency | Percentage |
|---------------------------|-----------|------------|
| No of children | | |
| 1 | 26 | 37.7 |
| 2 and above | 43 | 62.3 |
| Registration of Pregnancy | | |
| Government | 48 | 69.6 |
| Private | 21 | 30.4 |
| Planning of Pregnancy | | |
| Planned | 55 | 79.7 |
| Unplanned | 14 | 20.3 |
| Place of Delivery | | |
| Husbands Place | 57 | 17.4 |
| Mothers place | 12 | 82.6 |

Table 2: Monetary involvement of Husband in maternal and Child health care (n=69)

| Variables | Frequency | Percentage |
|---|--------------------|------------|
| Expenses - travelling | | |
| Husband | 44 | 63.8 |
| Mothers House | 25 | 36.2 |
| Expenses - Nutrition | | |
| Husband | 29 | 42 |
| Mothers House | 40 | 58 |
| Expenses - Medicine | | |
| Husband | 33 | 47.8 |
| Mothers House | 36 | 52.2 |
| Expenses -Delivery | | |
| Husband | 18 | 26 |
| Mothers House | 51 | 74 |
| Visits of husband before and after pregnancy | Mean (S.D.) | |
| Number of visits accompanied by husband pre-pregnancy | 2.4 (1.5) | |
| Number of visits by husband post pregnancy | 5 (1.1) | |

Discussion

Sociodemographic details

Majority belonged to Hindu religion (88.4%) and 60% of fathers have completed only secondary education, 50% were belonging to the occupation category of clerical/shop owner/farmer. About 70% have registered their pregnancy in government hospital. About 82.6% of the delivery took place in mother's place. The expense for delivery is paid by the mother's side in 74% of the cases. These characteristics are specific to the context of rural population having farming as their main livelihood and choosing government setup for the maternal benefits under RMNCH + A programme in India.

The percentage of husbands accompanying during antenatal visit were 72.5% while it decreased to 27.5% during labor and further decreased to 20.3% during immunization. Mean number of antenatal visits by the mothers were 4.8 and the mean number of visits accompanied by husband was 2.4. There has been no study in India to compare directly the male involvement in various stages, however Narang *et al.*^[10] a study done in Delhi demonstrated 61% of men accompanied the women during the antenatal care which is similar to present study. However, a study conducted by Bhatta *et al.* in Nepal and Craymah *et al.* in Ghana reported 39.3% and 35% of men participated in the antenatal visits. These values are very low compared to our study as they are very low-income countries and the sample size is relatively small in our study. Mohammed S, *et al.*^[11] from Kenya in 2020 did a age specific comparison to this facet where 62.7% of the women who were in the age group of 20–29 years said that the men were present throughout their antenatal visits, women <20 years and more than 40 years said that they went to the hospital alone on all days during their antenatal period. Another study by Fatila FO, *et al.*^[12] from Kenya in 2020 reported that men involved in antenatal care was 54.5%, postnatal care 87.7%, and new born

Table 3: Perceptions of Husbands Poor Participation during Labour and Immunization

| Codes | Categories | Themes |
|---|--------------------------------------|--|
| Lack of Holidays/Paternity Leave Non - Cooperative colleagues/co-workers for duty exchange Lack of Time Management skills | Professional Commitments | Reasons of poor participation During Labour and Immunization |
| Traditional Birth Preparedness - Mothers place Lack of cooperation between in laws. Misogynistic Views Lack of Belief in Immunization. | Patriarchal Society | |
| Lack of preparedness Unplanned Pregnancy Early Marriage | Financial Difficulties | |
| Long Waiting Hours Crowded Out patient departments Vaccines - Arriving late | Health Facility - Related Challenges | |
| Results (Qual): The mean age of the participants was 32±1.48 (years±SD) The categories that emerged were Professional Commitments, Patriarchal Society, Financial Difficulties and Health Facility related challenges Category 1: One participant said, "I am the sole bread winner in the family. I have to run whether it is raining or a sunny day" Category 2: "It is the duty of the mother's family". About three out of five men had misogynistic views, "Women are created for the purpose of giving birth. It is no big deal. It is the law of nature" Category 4: "Meeting the doctor for vaccination takes lot of time as the PHC has a long queue" | | |

care 51%. This study did not explicitly mention the definitions of antenatal care, postnatal care, and newborn care which may attribute to the high percentage of postnatal care and new born care.

Male participation during labor and immunization is 47% and 44%, 10 and 20% in Bhatta *et al.* and Craymah *et al.*^[2,13] Our study reported a 27.5% attendance during labor which is very low compared to the other studies as we follow the traditional practices of conducting delivery at mother place. Fathers hardly attended the immunization rounds of their children in our study which agrees with lot of studies.^[2,6,13]

In this study factors like religion, husband's education and occupation, socioeconomic status, planning of pregnancy, registration of pregnancy and place of delivery were not significantly associated with spousal involvement during antenatal care, labor, and immunization. However, other studies have found certain factors to be significantly associated with male involvement age, religion, education, employment, income, number of children, planning of pregnancy.^[2,13,14] This may be because of the small sample size.

To explore the reasons for poor participation of men during immunization and labor, five Key informant interviews were conducted among the men who were vocal and willing to participate. The reasons were (1) Professional Commitments, (2) Views of a Patriarchal society like India, (3) Financial Difficulties, and (4) Health Facility Related Challenges. There is no qualitative study done in South India exploring these factors. Recognizing the areas of poor participation by men and exploring the thematic reasons behind them was a novel approach in this study.

Under the category of patriarchal society, one of the participant quoted, "It is the sole duty of the female, I don't understand how it is relevant to us?" (Men). This clearly states that a preconceived notion of assigned duties to the male and female gender exists among the society. This society norm is very flawed and behaviour change among the men is required for enabling better antenatal, postnatal, and child care. Another flaw was that there is no proper paternity leave in private organizations in India. These reasons should be addressed as a society and programs should be built around it. A qualitative study done in Malawi by Mkandawire *et al.*^[15] said that sociocultural beliefs, stigmatization were some of the reasons for poor participation.

The strength of this study is it is a mixed method study design,^[8] which helps us to understand the problem as a whole. Knowledge of the husband regarding antenatal care and various other postnatal variables like breastfeeding, helping in daily household chores of the child were not included. However, we should keep in mind the small sample size as it is limited to one particular area (rural) and only one teaching hospital.

Summary and Conclusion

- The husband involvement in our study is better during antenatal period but they have steadily decreased during delivery and immunization.
- The reasons for the decreased participation were (1) Professional Commitments, (2) Views of a Patriarchal society like India, (3) Financial Difficulties, and (4) Health Facility Related Challenges.
- Misogynistic views of men and society norms considering women mainly for domestic chores and child bearing and rearing requires serious behaviour change.

There is a urgent need to educate the husband regarding the importance of husband's involvement during delivery and immunization. Programs should emphasize on these factors for men to participate more in the MCH care. Further studies can be done to strengthen the participation of men in the continuum of care in India.

Ethical clearance

Clearance from the institutional research committee was obtained. Later the Institutional Ethics clearance was also obtained with (IEC No of 003) on 30.1.2020.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Original Research Article

Socio-cultural barriers for menstrual hygiene management among adolescent school girls of southern India

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ABSTRACT

Background: India is home to 20% of the world's adolescent population, with 1 in 10 children currently experiencing puberty. Menstruation, a physiological process in females is influenced not only by race, nutrition and heredity but also by the socio-cultural milieu. In Indian society, the social and cultural restrictions influence the knowledge, attitudes and the practices of adolescent girls towards menstrual hygiene. The present study was carried out to find out the level of knowledge, attitude and practice and the restrictions they face during the process of menstruation.

Methods: The study was a descriptive cross-sectional study where 489 adolescent school going females of the age group of 13-15 were recruited using simple random sampling from a cluster of schools and interviewed using a semi structured questionnaire for their knowledge, attitudes, practices and the restrictions they face during menstruation. A scoring system was adopted and categorised as poor, average and good.

Results: 423 (88.6%) participants demonstrated average to poor knowledge scores, while 279 (57.1%) participants demonstrated average to poor practice scores. There was a significant difference observed between the educational status of mother ($p=0.041$) and the knowledge scores of study participants. There was no correlation observed between the monthly per capita income of households and the knowledge ($r=0.097$) and practice scores ($r=0.0034$). 375 (76%) study participants faced multiple restrictions during menstruation like not allowed to pray or visit temples (93.6%), total seclusion (74.6%), wash clothes separately (74.6%), sleep on floor (74.6%), restriction on leisure (70.4%), eat out of separate utensils (70.4%), and restriction on consumption of food items (49.8%).

Conclusions: Knowledge and practices regarding menstrual hygiene was low among study participants and was influenced by various prevalent socio-cultural restrictions.

Keywords: Adolescence health, Barriers, Menstrual hygiene, Menstrual hygiene management, Restrictions, Socio-Cultural

INTRODUCTION

The World Health Organization (WHO) defines adolescence as a transition period from childhood to adult years.¹ India, home to 253 million adolescents constitutes 20% of the world's adolescent's population.² In addition, more than 1 in 10 children in India are teenagers currently

experiencing puberty, and more than a quarter of all children will transition to adolescence and puberty within the next decade.³⁻⁵ Menstruation, a physiological process, unique to females is part of the female reproductive cycle that begins at puberty.⁶ The first menses called "Menarche". occurs between the ages of 11 and 15 continues cyclically, except during pregnancy and

lactation throughout the reproductive years.⁷ With the onset of menstruation, the female becomes aware of her emerging identity which is not only influenced by her internal emotional and physical change but also the external environment she lives and the feedback she receives from her family, her peers and the society.⁸ The ages of onset of menstruation is influenced by heredity, race and the nutritional status.^{9,10}

Astonishingly, still today in rural India, the physiology of menstruation is poorly understood due to many myths, taboos and misconceptions which act as a barrier for Menstrual hygiene Management (MHM) thus endangering the reproductive health of the female.¹¹ Poor menstrual hygiene and inadequate self-care can lead to urinary tract infections (UTI), scabies in the vaginal area, abnormal abdominal pain, absence from school, and complications during future pregnancy.¹²⁻¹⁵

The myths and taboos regarding menstrual health and disease are explored by various studies.^{16,17} Some of the myths are related to the day when a girl attains puberty. The month, the day and the time are noted. If it happens to be on Monday, the girl will be eminently chaste. Tuesday is not favorable, as she is likely to be a widow early in her days of wedlock. If it is Wednesday, she will be wealthy and so on.¹⁸

Another myth is the notion of impurity which ascertains that the movement of the girl should be restricted. Restrictions in daily activities such as not being allowed to take bath, change clothes, comb hair, enter holy places and dietary restrictions (taboo on consumption of food like rice, curd, milk, lassi, potato, onion, sugarcane etc.) during the menstrual period are also imposed.¹⁹ The girl should have a separate place and mat for sleep, use separate vessels, mat, pillow and wash things every day morning during menstruation; sleep alone on empty floor and shouldn't throw out her dress with blood stain.²

As proximate care givers mothers, grandmothers, aunts and other relatives have a major role in influencing the attitude and practices of adolescence girls in Indian society. Absence of scientific information, incorrect practices and a negative attitude by these proximate care givers leads to intergenerational transfer of myths, taboos and misconceptions regarding menstrual hygiene. As a result, most adolescent females in rural India have incomplete and inaccurate information about menstrual physiology and hygiene, which predisposes them to infections.³

So, the present study was conducted to explore the knowledge, attitudes and practices regarding menstruation and menstrual hygiene of the adolescent females in the study area, the influencers which play a major role in their knowledge and practice behavior and the socio-cultural restrictions they face during the physiological process of menstruation.

METHODS

The present study was a part of a completed educational intervention project to improve the menstrual hygiene of adolescent girls in the Perambalur district of Tamil Nadu state of India during the period of June 2015 to October 2017. The findings from the pre-test of the study are discussed in the present paper.

The study participants were high school adolescence girls of 13-15 age group selected using random sampling technique. Review of literature suggested that 50% of school going adolescents have adequate knowledge regarding menstruation and an effect size of 0.31 post intervention. As the project involved intervention carried out on three different groups of adolescent girls the sample size computed for each group was 81, which came out to be 243 for three groups. On considering the design effect of 2, the final sample size came to be 486 study participants. The investigator recruited 490 participants in the study

Details regarding number of girls studying in 8 and 9 grades, location of school were collected from District Educational officer. Considering a minimum enrolment of 50 girls in class 8th and 9th, the investigators needed approximately 9 schools for the study to reach a sample size of 490. From the list of schools, 9 schools were chosen by cluster random sampling. From selected schools, all eligible consenting adolescent girls were included as study participants for the study. Adolescent girls of grades 8th and 9th, who have attained menarche at least 6 months back and who gave consent were included in the study.

A self-administered, pre-tested and semi-structured questionnaire in local language was used for data collection. The questionnaire included Socio demographic profile, menarche and menstruation details, knowledge regarding menstruation and menstrual hygiene, attitude of participants towards menstruation and menstrual hygiene, practices during menstruation and restrictions faced by participants during menstruation. The questionnaire adopted a scoring system for ease of analysis. It included a knowledge score of Poor: 0-3, Average: 4-7 and Good: 8-10 and a Practice score of Poor ≤ 4 , Average 5-8 and Good: ≥ 9 . After explaining the purpose of the study, written informed consent of the participants was obtained and a pre-test was conducted for all the 490 participants in class room of the schools selected. Questions were explained to the students and they were asked to answer them without discussing among themselves. It was made sure that all the questions were answered by the participants.

All the data collected were coded and entered in Microsoft excel sheet which was re-checked and analysed using Statistical Package for Social Sciences (SPSS) version 21. Descriptive statistics were expressed as Mean and Standard Deviation. To test association between

categorical variables chi-square was computed and an p value of less than 0.05 was considered significant. Correlation coefficient (r) was calculated to study the relationship between two continuous variables.

RESULTS

In the present study, totally 489 adolescent females participated in the study one participant refused to consent

Socio-demographics

Among the participants, 349 adolescent females i.e., 71.3% were in the age group of 14 years followed by 13 (22%) and 15 years (0.7%).

Among the participants 470 (96.2%) of the participants belonged to Hindu religion, 392 (80.1%) were from nuclear family. About 321 (65.6%) and 344 (70.4%) of participant's mother and father were literate respectively. Regarding occupation status of the parents, 381 (77.9%) and 440 (89.9%) of participants' mother and father were employed respectively. 222 (45.4%) of study participants lived in kutch house while 59.5% (n=291) of the study participants belonged to class IV of modified B.G. Prasad scale (Table 1).

Menarche and menstruation details

About 276 i.e., 56.5% participants attended age at Menarche at 13 years. Among the participants, 406 (83.1%) of them had regular menstrual cycles whereas, 83 (16.9%) girls had irregular cycles.

Table 1: Socio-demographic and menstrual details of the study participants (n=489).

| Variables | N | Percent | |
|--|------------------|---------|------|
| Age (years) | ≤13 | 108 | 22 |
| | 14 | 349 | 71.3 |
| | 15 | 32 | 0.7 |
| Family | Nuclear | 392 | 80.1 |
| | Joint | 97 | 19.9 |
| Religion | Hindu | 470 | 96.2 |
| | Non-Hindu | 19 | 3.8 |
| Mother's education | Illiterate | 168 | 34.4 |
| | Literate | 321 | 65.6 |
| Father's education | Illiterate | 145 | 29.6 |
| | Literate | 344 | 70.4 |
| Mother's occupation | Unemployed | 108 | 22.1 |
| | Employed | 381 | 77.9 |
| Father's Occupation | Unemployed | 49 | 10.1 |
| | Employed | 440 | 89.9 |
| House type | Kuccha | 222 | 45.4 |
| | semi pucca | 194 | 39.6 |
| | Pucca | 73 | 14.9 |
| Age at menarche | <12 years | 166 | 33.9 |
| | 13 years | 276 | 56.5 |
| | >14 years | 47 | 9.6 |
| Regularity of menstrual cycle | Regular cycles | 406 | 83.1 |
| | Irregular cycles | 83 | 16.9 |
| First Informant regarding menstruation | Friends | 68 | 13.9 |
| | Mothers | 106 | 21.6 |
| | Aunt | 121 | 24.7 |
| | Sister | 147 | 30 |
| | Others | 47 | 9.6 |

Table 2. Various knowledge, attitude, practices and the restrictions faced by the study participants (n=489).

| Variables | N | Percent | |
|--|-----|---------|------|
| Able to explain in lay terms about menstruation | Yes | 260 | 53.1 |
| | No | 229 | 46.9 |
| Know what is the cause of menstruation | Yes | 200 | 40.8 |
| | No | 289 | 59.2 |
| Know from which organ blood flows during menstruation | Yes | 113 | 23.1 |
| | No | 376 | 76.9 |
| Know whether dietary practices affect menstruation. | Yes | 268 | 54.8 |
| | No | 221 | 45.2 |
| Have you heard about menstrual hygiene | Yes | 294 | 60.1 |
| | No | 195 | 39.9 |
| Know whether poor menstrual hygiene leads to infection | Yes | 239 | 48.9 |
| | No | 250 | 51.1 |
| Knows the normal duration of menstruation period | Yes | 159 | 32.5 |
| | No | 330 | 67.5 |
| Knows the normal interval of menstrual cycle | Yes | 117 | 23.9 |
| | No | 372 | 76.1 |
| Knows the age at which Menopause occurs | Yes | 151 | 30.9 |
| | No | 338 | 69.1 |

Continued.

| Variables | | N | Percent | |
|---|---|-----------------------|---------|------|
| Knows that menstruation indicates fertility | Yes | 243 | 49.7 | |
| | No | 246 | 50.3 | |
| Attitude (n=489) | What was your emotional reaction when you got your first menses? | Afraid | 268 | 54.8 |
| | | Embarrassed | 92 | 18.8 |
| | | Guilty | 11 | 2.24 |
| | | Indeterminate | 117 | 23.9 |
| | What is your emotional reaction when you don't get your regular menses? | Relieved | 109 | 22.2 |
| | | Afraid | 181 | 37.2 |
| | | worried | 129 | 26.3 |
| | Do you think that it is better to know about menstruation before attaining menarche | Indeterminate | 70 | 14.3 |
| | | Yes | 323 | 66.1 |
| | Do you think that educating Girls regarding menstrual hygiene is necessary | No | 166 | 33.9 |
| | | Yes | 404 | 82.6 |
| | To whom are you comfortable to discuss issues regarding Menstrual hygiene | No | 85 | 17.4 |
| Friend | | 205 | 41.9 | |
| Mother | | 191 | 39.1 | |
| Sister | | 68 | 13.9 | |
| Practices (n=489) | What type of absorbent you use during your menses? | others | 25 | 5.1 |
| | | Sanitary napkin | 489 | 100 |
| | How frequently you change absorbent during day? | Cloths | 0 | 0 |
| | | <2 | 128 | 26.2 |
| | Do you change absorbent before sleep? | > 3 | 361 | 73.8 |
| | | Yes | 294 | 60.2 |
| | How do you dispose off the used absorbent? | No | 195 | 39.8 |
| | | Fair practice | 410 | 83.8 |
| | Do you practice regular cleaning of genitalia during Menstruation? | Poor practice | 79 | 16.2 |
| | | Fair practice | 251 | 51.3 |
| | What materials you use for cleaning genitalia during menstruation? | Poor practice | 238 | 48.7 |
| | | Water with soap | 250 | 51.2 |
| Water only | | 163 | 33.3 | |
| Restrictions (n=375) | Seclusion/ Stay in other room | Not washing regularly | 76 | 15.5 |
| | | Yes | 280 | 76.6 |
| | Use of Separate utensils for eating | No | 95 | 25.4 |
| | | Yes | 264 | 70.4 |
| | Restriction on cooking /going inside kitchen | No | 111 | 29.6 |
| | | Yes | 253 | 67.5 |
| | Restriction on consumption of certain foods | No | 122 | 32.5 |
| | | Yes | 187 | 49.9 |
| | Restriction on attending school | No | 188 | 50.1 |
| | | Yes | 79 | 21 |
| | Restrictions to on outdoor playing /exercise /leisure activities | No | 296 | 79 |
| | | Yes | 210 | 56 |
| Restriction on visit to relatives, friends and neighbours | No | 165 | 46 | |
| | Yes | 265 | 70.6 | |
| Advocated to use empty floor, separate place or mat for sleep | No | 110 | 29.4 | |
| | Yes | 280 | 74.6 | |
| Restrictions to visit Prayer room, Pray or Visit Temple | No | 95 | 25.4 | |
| | Yes | 351 | 93.6 | |
| | No | 24 | 6.4 | |

Table 3: Educational status of parents and their association with knowledge and practice scores.

| | | Grades | Graduation | Illiterate | Primary | Secondary | Total | P value |
|-------------------------|-------------------|---------|------------|------------|---------|-----------|-------|---------|
| Knowledge scores | Mothers education | Average | 7 | 68 | 71 | 79 | 225 | 0.041 |
| | | Good | 4 | 23 | 16 | 13 | 56 | |
| | | Poor | 3 | 77 | 48 | 80 | 208 | |
| | Fathers Education | Average | 11 | 58 | 76 | 80 | 225 | 0.19 |
| | | Good | 1 | 24 | 16 | 15 | 56 | |
| | | Poor | 13 | 63 | 58 | 74 | 208 | |
| Practice scores | Mothers education | Average | 6 | 81 | 71 | 69 | 227 | 0.284 |
| | | Good | 7 | 72 | 53 | 78 | 210 | |
| | | Poor | 1 | 15 | 11 | 25 | 52 | |
| | Fathers Education | Average | 13 | 71 | 62 | 81 | 227 | 0.452 |
| | | Good | 11 | 59 | 66 | 74 | 210 | |
| | | Poor | 1 | 15 | 22 | 14 | 52 | |

Regarding information related to menarche and menstrual cycle, 147 (30%) of the study participants replied that elder sister was the primary source of the knowledge, followed by 121 (24.7%) from aunt, 106 (21.6%) from mother, 68 (13.9%) from friends and 47 (9.6%) received information from grandmother and neighbors (Table 2).

Knowledge about menstruation and menstrual hygiene

About 260 (53.1%) of participants were able to answer that menstruation was a physiological process and 200 (40.8%) of participants were aware that it is caused by hormonal changes in the body. Among the study participants, 113 (23.1%) answered that menstrual blood flows through the uterus.

More than half, 268 (54.8%) of participants replied that diet doesn't affect menstruation. 294 (60.1%) participants said that they have heard about menstrual hygiene and 239 (48.9%) were aware that poor menstrual hygiene can lead to infection of the reproductive tract. About 159 (32.5%) and 117 (23.9%) participants respectively had appropriate knowledge regarding the normal duration of menstrual cycle and normal intervals between cycles, while 151 (30.9%) replied correctly the age at which menopause occurs. 243 (49.7%) participants were aware that menstruation indicates fertility. Cumulative knowledge scores were calculated for each participant followed by calculation of mean. Mean cumulative knowledge scores calculated was 4.22 ± 2.28 (SD). Among the study participants, only about 56 (11.4%) had good scores whereas the 423 (88.6%) were poor to average scorers.

Attitudes

Attitude of study participants towards menstruation and menstrual hygiene was assessed. When questioned regarding first reaction towards menarche 268 (54.8%) of participants responded that they were afraid when they had their first menses. About 323 (66.1%) responded that it is better to know about menstruation before attaining

menarche. Majority i.e., 404 (82.6%) of the participants responded that educating girls regarding menstruation and menstrual Hygiene is necessary before menarche.

When the participants were questioned "To whom are they comfortable to discuss menstruation and menstrual hygiene related issues?" about 205 (41.9%) of participants responded they were comfortable discussion this issue with friends followed by mother 191 (39.1%).

Practices

It was found that about all participants 489 (100%) use sanitary napkin during menstruation. 361 (73.3%) of participants replied that they change it more than 3 times in a day. They were asked whether they change pad before sleep for which 294 (60.2%) of participants responded yes. Disposal of used menstrual hygiene products was questioned. Burying, burning, disposing in waste bin after proper wrapping was considered to be fair practice and 410 (83.8%) participants were practicing it. Throwing out in open, flushing it in toilets, or throwing in toilets or corners was considered as poor practice and it was found that 79 (16.2%) participants were practicing it. 251 (51.3%) participants were cleaning their external genitalia regularly while 250 (51.2%) girls were using water and soap to clean their private parts. Those who used only water to clean were 163 (33.3%). A total of 12 points were given for practices during menstruation. Cumulative mean practice score was 8.04 ± 2.56 (SD). 210 (42.9%) had good practice scores, 279 (57.1%) participants had poor and average scores.

Restrictions during menstruation

The participants were assessed for any restrictions they faced during menstruation. 375 (76.6%) study participants faced multiple restrictions of different categories during menstruation. 280 (74.6%) of the total 375 participants, were totally secluded and not allowed to touch anyone or any objects at house. 351 (93.6%) of participants faced restriction to enter prayer room, offer

prayers or to enter temple. Restrictions on consumption of food items were present in about 187 (49.8%) participants, whereas 264 (70.4%) of participants were given separate utensils for eating. 253 (67.5%) participants were restricted from entering kitchen during menstruation. About 280 (74.6%) of them were made to sleep on floor during menstrual cycle. Almost 280 (74.6%) of the girls were made to wash their clothes separately. 79 (21%) of the study participants were not allowed to visit school. 210 (56%) participants were not allowed to play, exercise and involve in leisure activities while 265 (70.6%) participants were restricted from meeting neighbors or relatives.

There was a significant difference observed between the educational status of mother's education status and the knowledge scores of study participants (0.041) but not of the father. There was no significant difference observed between the educational status of both parents and their practice scores (Table 3). There was no correlation observed between the monthly per capita income of households and the knowledge score ($r=0.097$) (Figure 1) and practice scores of the study participants ($r=0.0034$) (Figure 2).

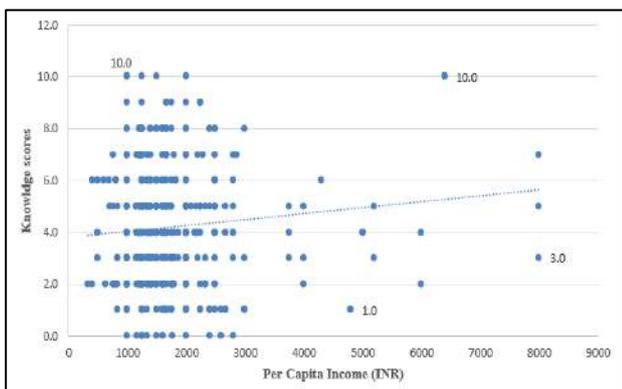


Figure 1: Correlation between monthly per capita income and Knowledge scores of study participants ($r=0.097$).

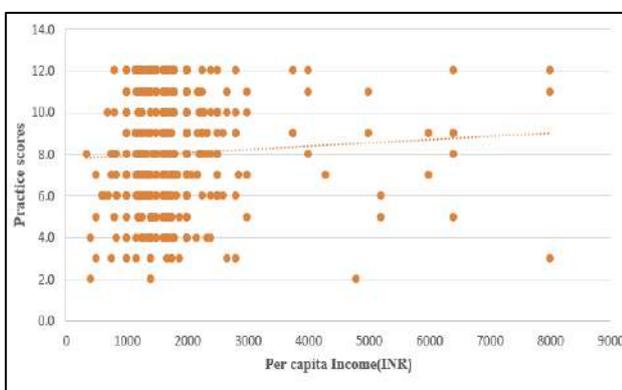


Figure 2: Correlation between monthly per capita income and practice scores of study participants ($r=0.034$).

DISCUSSION

In the present study, the mean age menarche in the 56% of study participants was 12.7 years (± 0.66) which are similar to findings of studies conducted in Nepal by Hamal et al and in Karnataka by Ramchandra et al where the mean age of menarche was and 12.94 yrs. and 12.39 years respectively.^{14,20} In the present study, 83.0% of participants had regular menstrual cycles which is comparable to study conducted in Gujarat by Rana et al and in Belgaum by Pokrel et al where 76% and 76.9% participants respectively had regular menstrual cycles.^{21,22}

In the present study, the primary source of information and practice regarding menstruation hygiene was the elder sister of the participant, which is similar to study conducted in Belgaum by Pokrel et al where elder sister was the primary source of knowledge in 25.3% of study participants.²² In contrast to this, mother was the primary source of information and practice in 41.7% of participants in the study conducted by Verma et al in Varanasi.²³

In the present study, 53% participants were aware that menstruation is a physiological process which is similar to study conducted by Pokrel et al and Allah et al where about half of the participants were aware of it.^{22,24} In the present study 41% of participants responded that menstruation occurs due to hormonal changes. In a study conducted by Kamath et al in Udupi, 45.5% of participants responded similarly.²⁵ Only 23.1% of participants in the present study responded that the blood flows through the uterus via the vagina, which is comparable to the studies conducted by Sapkota et al and Pokrel et al where only 36.0% and 29.7% of participants respectively could answer correctly.^{22,26}

In the present study, 46% of the participants replied affirmatively that food affects menstruation which is more than the study conducted by Hague et al in Bangladesh where 34% of participants replied that food affects menstruation.³ In the present study, majority (60%) of participants were aware of the term "menstrual hygiene" which is comparable to study done by Shiva et al in western Ethiopia where 75% of participants were aware of it.²⁷ This finding is in contrast to study conducted by the Tegegne et al in North east Ethiopia where only 24.5% of girls were aware of the term "menstrual hygiene".⁷ In the present study, 48.7% of participants were aware that poor menstrual hygiene predisposes to reproductive tract infection which is comparable to a study done by Sapkota et al in Nepal where 37% of girls were aware of it.²⁶ In contrast to this, the study conducted by Haque et al in Bangladesh reports a higher percentage of participants (68.3%) aware of their predisposition to reproductive tract infections in absence of proper menstrual hygiene.³

About 31% participants had correct knowledge regarding age at menopause which is in similar to 58% in a study of

Ghana.²⁸ Mean knowledge scores of study participants were found to be 4.22 (± 2.28) which is comparable with a study done by Anitha et al in Chennai where mean knowledge scores of participants were 5.27 (± 1.87). In another study conducted by Shanbhag et al in Karnataka the pretest knowledge mean score of all participants was lesser (4.04 ± 1.32).^{29,30} In the present study about 55% of participants responded that they were afraid or terrified during menarche which is similar to study in Nigeria where 53% and in Udipi where 49.6% of participants were afraid during their first menstruation experience.^{25,31}

In order to promote menstrual hygiene among young rural girls, the state government of Tamil Nadu launched distribution of free sanitary pads to adolescent girls. The above initiative reflects the reason for universal usage (100%) of sanitary pads during menstruation by the study participants. This finding is similar to studies conducted by Zaidi et al in Thiruporur and Bharathalakshmi et al in Chidambaram of Tamil Nadu where the utilization of sanitary napkins was 93.8% and 90.5% respectively.^{11,33} On contrary, in studies done by Gilany et al in Egypt and Subash et al in Nagpur lower Sanitary pad usage of 66.8% and 49.3% respectively was reported.³⁵

In present study, higher proportion of the participants changed their sanitary pads frequently (more than three times a day). This is in contrast to studies done in Nepal by Sapkota and by Patavegar in Delhi where only half of the participants changed pads frequently.^{26,36} In the present study 83.8% participants practiced hygienic methods of sanitary pads disposal whereas in studies done by Haque et al in Bangladesh and Dasgupta et al in Kolkata only 50% of participants were practicing a hygienic method of disposal.^{3,13}

About 52% of participants in the present study were practicing cleaning of genitalia regularly whereas in studies conducted by Patevagar et al in Delhi and by Subhas et al in Nagpur, 66% and 42% of participants cleaned their genitalia frequently in the present study 51% participants used water and soap to clean genitalia while in the study by Patavegar et al 47.4% of participants wash their genitalia with water and soap/antiseptic.^{35,36} The cumulative mean practice scores (SD) of the study participants in the present study was 8.04 (± 2.56 SD) which is comparable to study in Chennai which showed a mean practice score (SD) of 8.22 (± 1.18 SD), whereas the practice scores were lower (6.41 ± 1.65) in a study conducted in Karnataka.^{17,18}

Restrictions faced during menstruation

In the present study, majority (76%) of the study participants faced restrictions during menstruation. Among them, about 74 % of participants faced seclusion which is comparable to study done by Zaidi et al in Thirupurur where 66.0% of participants faced seclusion. Restriction on household activities was common among

the study participants where 67% of them faced it during menstruation. In similar study conducted by Shanbag et al in Karnataka there were restrictions on 50% of participants for doing household chores.¹⁸ Restrictions to pray and enter temple was universal (93.5%) in the study participants which is similar to studies done by Pokrel et al in Belgaum and Bhudhagaonkar et al in Maharashtra where 98.3% and 100% participants faced such restriction.^{12,37} About half of the participants said that certain types of foods were restricted which is similar to 33.5% in a study done by Srivastava et al in Madhya Pradesh.³⁸ Almost 3/4th of the participants were made to sleep separately on floor during menstruation which is similar to study done in South India where 64% of participants faced such restriction.¹¹

CONCLUSION

The knowledge and practice scores regarding menstrual hygiene was low among participants and was influenced by various prevalent socio-cultural restrictions. Parents' educational status did affect knowledge and practice of study participants but income has no effect on knowledge and practice of the study participants.

Recommendations

Training in gender education by trained professionals can bring about a change in the knowledge and practice behaviour of the participants. Further qualitative studies to explore the role played by proximate care givers in influencing knowledge and practices should be conducted and health education should be targeted in this group.

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ORIGINAL ARTICLE

A Study on Fast Food and Worm Infestation among Food Handlers in a Metropolitan City

Dr. P. Praveena,¹ Dr. L. Kannan²

ABSTRACT

Introduction: Parasitic infestation is a global public health issue, especially in developing countries like India. These diseases target a large group of population leading to malabsorption and fatal complications. Food handlers play an important role in cooking and distribution of foods to people. They are potential sources to transmit parasitic infestation to humans. The infestation in long term can predict the risk and progress of protein–energy malnutrition and iron deficiency anemia. Hence, in view of the above issues, the main objective of the study is to find out the level of addiction among food stackers of fast food and also to find out the prevalence of worm infestation among food handlers working in fast food. **Materials and Methods:** A population-based cross-sectional study was carried out from May 2018 to November 2019, to find out the prevalence of worm infestation among food handlers. Around 360 food handlers and 374 food stackers were covered in this study. Simple random sampling technique was used to select the centers. The food handlers were interviewed at one point of time. Initial face-to-face interview was performed and stool samples were collected from the food handlers and examined for intestinal infestation. The data was analyzed using SPSS version 20. **Results:** The prevalence of intestinal worm infestation was found to be 45.5% with 95% confidence interval of 39.6–49.87, the majority of them had protozoan infestation 83.8%. There was a significant contributing environmental factor with intestinal infestations among the food handlers. About 348 (93%) of food stackers regularly consumed food at fast foods on daily basis, either one meal for more than 6 months duration. **Conclusion:** This study confirms the determinant of intestinal parasitic infestation is not only limited to individual factors but also related to household and community. Control of parasitic infections is achieved by multisectoral approach- good sanitation, health education, and anti-parasitic treatment.

Key words: Food handlers, fast food addiction, parasitic infestation

INTRODUCTION

The WHO estimates 50 million people around the world suffer parasitic infections each year.^[1] The overall prevalence of intestinal parasitic infections in India varies from 12.5 to 67% depending on the organism. It is evident from the previous studies that the prevalence of intestinal parasitic infections in Chennai was around 36%. In today's fast moving world, people living in metro cities like Chennai with dense and diverse population, depend on

fast foods for their timely fill ups. Fast foods are available at every corner of road in Chennai and do not even have authentic permission from the local bodies. The food which is served along road premises is precooked reheated either on the previous day or on the same day and spicy. Consumers are not cautious on unhygienic environment. Proper hand washing is not followed by both food handlers and stackers. The infection in long term can predict the risk

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of protein–energy malnutrition and iron deficiency anemia.^[2] Hence, in view of the above issues, the objective of the study is to find out the level of addiction among food stackers and prevalence of worm infestation among food handlers.

MATERIALS AND METHODS

Study Design

This study was a population-based cross-sectional study.

Study Area

The study was conducted in southern part of Chennai urban population.

Study Population

Fast food centers were identified based on their habitation. From a total of 4846 fast food centers, 956 centers were randomly selected by lottery method. Around 374 food stackers and 360 food handlers were randomly selected from the fast food centers. All 374 food stackers were interviewed using the questionnaire. Among 360 food handlers, 354 food handlers gave their stool samples for examination. Stool examination was performed by direct saline and iodine wet mount preparation.

Study Period

The study was carried out from May 2018 to November 2019.

Sample Size

The prevalence of intestinal parasitic infections was taken as the basis for sample size calculation because of public health implications and its importance. The prevalence of intestinal parasitic infection in urban setting was reported to 37.6% by Maria *et al.*^[3] Assuming the 35% in this locality with limit of accuracy as 15% the sample size worked out to be 325.4. The anticipated non-response in providing stool samples was 2 % and accordingly the sample size arrived at 354.

Sampling Unit

Participants of all age groups were taken in as the study population who are currently employed in fast foods.

Sampling Method

A simple random sampling technique was adopted.

Development of Questionnaire

A questionnaire was prepared based on the information obtained by various studies related to the topic. Based

on the observation during pretesting, the modified questionnaire was used in the main study. Standard of living index was used instead of per-capita income for assessing socioeconomic status. Certain questions were rephrased. Informed consent was obtained from participants, prior to data collection.

The ethical approval was taken from the Institutional Ethics Committee of Sri Ramachandra Medical College (REF: IEC-NI/18/APR/64/28) dated May 21, 2018.

RESULTS

Food Stackers

Among the food stackers, a total of 374 individuals were interviewed during their consumption at fast foods. It has been found that 348 (93%) of food stackers regularly consumed food on daily basis, either one meal for more than 6 months duration and remaining 26 (7%) were consuming food regularly for <6 months duration. Age and area wise distribution of food stackers who consume food from fast food is given in Table 1.

Many of the food stackers were not aware of the health implications and their surrounding environmental conditions where food is served, they are only aware of the disposal papers kept on plate while serving food items. It has been found 37% wash their hands before eating and 63% do not wash their hands before eating.

Consumption of food regularly from same premises: It has been observed that most of the food stackers who consumes food from same premises for more than 6 months were 294 (78.6%) compared to other individuals 80 (21.4%) who consumes food on different places nearby.

Type of Food Consumed by Food Stackers

Vegetarian versus mixed diet: It has been found that 299 (79.9%) had mixed consumption of food compared to 75 (20%) who usually consume vegetarian diet. The reason coated by many of the food stackers that they usually believe taking food from plant source has got a least effect on ill health compared to non-vegetarian diet consumptions.

Table 1: Age- and area-wise distribution of food stackers who consume food from fast food

| Age | Number | Percentage | 95% CI | Chi-square | P value |
|--------------------|--------|------------|-----------|------------|---------|
| <30 years | 205 | 46.8 | 39.9–53.7 | 0.22 | <0.05 |
| More than 30 years | 169 | 44.4 | 37–51.8 | | |
| Rural | 186 | 53.8 | 46.8–60.8 | 9.64 | <0.001 |
| Urban | 188 | 37.8 | 30.9–44.7 | | |

Even though there is availability of food at home, the reason for consuming in fast food is, food is readily available at low cost amidst busy work schedule. Around 166 (44.3%) were not aware of health issues caused due to taking fast foods.

Food Handlers

Among the 360 (food handlers), 188 (50.1%) were male and 172 (49.9%) were female food handlers. The age ranged from 14 to 86 years. Of the 360 participants, 354 participants willingly gave their stool samples. It was collected and examined. The response rate was 98.3%.

Environmental Factors

Present study reveals 186 (51.6%), 91 (25.27%), 83 (23%) of food handlers were residing in kutchha, semi-pucca and pucca house respectively. Around 60% of overall prevalence of parasitic infections were contributed by kutchha and semi-pucca dwellers. About 224 (62.2%) participants were living in overcrowded houses.

Personal Hygiene

Among 360 food handlers 212 (58%) did not wash hand before serving and 234 (65%) did not wash their hand with soap after defecation. It was observed that 47.3% of the food handlers had trimmed their finger nails and 95 (26.38%) had the habit of nail biting.

Prevalence of Intestinal Parasitic Infections

Table 2 illustrates, Among 360 food handlers, 354 provided the samples for stool examination. Stool samples from 354 study participants were examined. Out of which 161 had intestinal parasite and overall prevalence of parasitic infections was found to be 45.4% with 95% confidence interval (CI) of 39.6–49.8%. Among the 161 food handlers with parasitic infestation, 83.85% had protozoal infection (*Entamoeba histolytica* and *Giardia*) which was higher than helminthic infestation, 16.15% (pin worm tape worm, hook worm, round worm), shown in Table 3

DISCUSSION

Parasitic infestation is one of the major problems that affect human health, especially in developing countries.^[1] Different studies have been conducted in the field of intestinal parasite found higher prevalence in food handlers. In this study, a prevalence 45.5% of intestinal parasites found among food handlers is different from what was reported by other authors in different parts of the world.^[4,5] Eventhough it seems to be lower compared to other studies, the prevalence of parasitic infestation is nearly half among the food handlers.

Around 348 (93%) of food stackers regularly consumed food on daily basis in fast foods, either one meal for more

Table 2: Prevalence of parasitic infections among food handlers

| Parasitic infections | (n = 354) | Percentage |
|----------------------|-----------|------------|
| Absent | 193 | 54.5 % |
| Present | 161 | 45.5 % |

Table 3: Prevalence type of parasitic infections (protozoan and helminths)

| Parasite type | Number of food handlers infected (N = 161) |
|--|--|
| Protozoa (<i>entamoeba</i> and <i>giardia</i>) | 135 (83.85%) |
| Helminths (<i>ascaris</i> , <i>tricuris hook</i> worm tapeworm pinworm) | 26 (16.15%) |

than 6 months duration which was higher addiction to fast foods compared to results of previous studies done in metro cities. This is a cause of great concern. This study is found to be similar to the prevalence of intestinal infection 46.4% reported in a study conducted in Kadar village of Tamil Nadu,^[6] while other studies in more or less similar group from different parts of India showed varied prevalence.^[7] Interestingly, when other studies done in Chennai, Andaman and Nicobar Island, Karnataka, and Darjeeling,^[8,9] showed higher prevalence of helminthic infections, the present study revealed higher prevalence of protozoal infection 83.85% (*Entamoeba histolytica* and *Giardia*) which was higher than helminthic infestation, 16.15% (pin worm tape worm, hook worm, round worm). Similar findings have been reported by Sackev and Fernandez *et al.* studies.^[2,8]

The prevalence of single parasitic infection was around 38% and less compared to multiple parasites infestations. A study done in Darjeeling^[11] among children showed lower prevalence of single parasitic infection reported 28.2% and higher of multiple infection 23.3% hence it may be concluded that the intensity of multiple infections was comparatively high in the present study. This difference in the prevalence may be attributed to the different geographical area of the latter study. The type of parasites mostly commonly seen is *Entamoeba histolytica*; *Giardia lamblia* which resembles available studies.^[12,13,16]

INTESTINAL PARASITES AND ASSOCIATED FACTORS

Water and Parasitic Infections

In the present study, it has been reported that improper water supply, source of water and cooking practices contributed to parasitic infection. On comparing this with Sugunan *et al*, Ali *et al* studies^[9,14]. The higher prevalence of waterborne protozoal infection was attributed to their use of untreated water

Type of Home and Overcrowding

In the current study around 186 (51.6 %), 91(25.27%), 83 (23%) of food handlers were residing in kutchha, semi-pucca and pucca house respectively. Food handlers living in kutchha and semi pucca houses constituted about 60% of overall parasitic prevalence compared to pucca dwellers. Similar significant association between type of house, low socio economic status and prevalence parasitic infestation was illustrated in a study at Egypt by Curtale^[15]

This study revealed that around 62.2 % of food handlers were living in over crowded environment, among them more than half of the food handlers were found to have intestinal parasitic infestations due to increased intrafamilial transmission in overcrowded houses These observations were nearly similar to study done in Argentina^[16] It stated that there was positive correlation between the presence of internal parasitic infection and overcrowding. Majority of the study participants belong to joint family, importance of personal hygiene among food handlers, was not adequate. There are high risk of transmitting the infection to other members of the family, especially among children.

Personal Hygiene of Food Handlers

Among 360 food handlers 212 (58%) did not wash hand before serving and 234 (65%) did not wash their hand with soap after defecation. It was observed that 47.3% of the food handlers had trimmed their finger nails and 95 (26.38%) had the habit of nail biting. Similar to reports of Sackev and Fernandez *et al.* studies.^[2,8] Therefore personal hygiene among the food handlers was found to be very poor.

The environmental factors of the food handlers were found to be favorable for the transmission of parasitic infections which resembles Carter *et al.*^[4] contributions.

CONCLUSION

The study confirms the determinants of intestinal parasitic infestations is not only limited to individual factors but also related to household and community. Multisectoral approach to control of parasitic infections must be adopted as advocated by the WHO. Control of parasitic infections is achieved by good sanitation, health education, and anti-parasitic treatment. Low socioeconomic factors, levels of education, surrounding environmental factors, solid waste, and nuisance have statistically significant association with intestinal infestation. Behavioral changes of food handlers were very important. Hence, strict law / policy should be implemented among food handlers as public health importance. Frequent practice of thorough hand washing with soap and water to be encouraged among both food handlers and food stackers.

FUNDING

No funding.

CONFLICTS OF INTEREST

None declared.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of SRMC &RI (SRU), Chennai (REF: IEC-NI/18/APR/64/28), dated May 21, 2018.

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Pregaming on Alcohol Products among Male College Students in Puducherry-Mixed-Methods Study

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Abstract

Background: There are some risky practices such as preloading or pregaming which exist among college students. When students pregame, compared with drinking episodes when they do not, they consume a greater number of drinks and have higher blood alcohol concentrations. **Objectives:** (1) To explore the perceptions about pregaming among male college students in Puducherry. (2) To study the prevalence of pregaming among current alcohol users. **Materials and Methods:** A sequential exploratory mixed-method study (Qualitative-Focus Group Discussion [FGD] to explore pregaming followed by Quantitative-self-administered questionnaire [survey]) was conducted among 450 male engineering college students by simple random sampling. **Results:** The prevalence of pregaming among current alcohol users was 66.7%. Among all occasions, the students were involved in pregaming mostly on birthdays 92.5% and marriages 92.5% followed by college cultural events 90%. All of the students 100% wanted to pregame for anticipated alcohol cost problems, 100% pregame for fun and 87.5% easy conversations with the opposite sex and majority 66.6% had the intention to quit pregaming among current users. **Conclusion:** The prevalence of pregaming is high among current users however, the majority of them had the intention to quit this behavior. Counselors and health care professionals working in alcohol de-addiction centers should specifically question pregaming and its associated symptoms. Tailor-made interventions should be promoted to target the concept of pregaming-related consequences of alcohol addiction.

Keywords: Alcohol-use, college students, pregaming

INTRODUCTION

Alcohol consumption has been a part of human culture since the beginning of documented history. The effects of alcohol consumption on health are detrimental, with an estimated 3.8% of all global deaths and 4.6% of global disability-adjusted life-years attributable to alcohol. The prevalence of alcohol use remains low in India as compared to other countries. However, recent data from India (National Family Health Survey III and IV) suggest increasing consumption of alcohol and the harmful effects across Indian society. These effects have been more pronounced among youth.^[1]

There are some risky practices, which exist among college students that lead to binge drinking. Those risky practices are called “pre-loading,” “front-loading,” or “pre-gaming.” Though there is no exact definition of pregaming in the literature, the common entity that exists in the definition is consuming alcohol in large amounts before attending a social event, where additional alcohol may or might not be

available and/or consumed. When students pregame, compared with drinking episodes when they do not, they consume a greater number of drinks and have higher blood alcohol concentrations. Individuals pregame for various reasons like waiting for an event to start, a desire for rapid intoxication, anticipated alcohol access issues (e.g., due to cost reasons or limited availability), and/or safety reasons (e.g., greater awareness of the contents of one’s drink).^[2,3] Pregaming has been linked to many general and specific alcohol-related consequences such as neglecting responsibilities, feeling sick, and passing out. They also experience blackouts and temporary loss of memory while drinking.^[4]

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Although there has been extensive research on pre-gaming in western countries, no study on pre-gaming among students in India has been done so far. This study will help the health professionals working with these student populations not only to assess the overall quantity and frequency of alcohol consumption but also their pre-gaming conditions. Hence, the present study was undertaken with the following objective to explore the perceptions about pre-gaming among male college students in Puducherry and to study the prevalence of pre-gaming among current alcohol users and the factors influencing their intention to quit this behavior.

METHODS

Study setting

Puducherry is famous for the partying culture that attracts tourists from all over Tamil Nadu. The economy of the state highly depends on the market of alcohol.^[5] Many colleges are located near the Pondicherry border, having numerous liquor shops on the highway. Hence the study was conducted in a private engineering college in Kalitheerthalkuppam Puducherry where access to alcohol products is easier. A total of 1653 undergraduate students were studying in the college, of which 1042 were male and 662 were female.

Study participants

Inclusion criteria

Only male college students aged 18–21 years who gave consent were included, as male students are more prone to consume alcohol in our culture and also the minimum legal age for drinking in Puducherry is from 18.^[6]

Exclusion criteria

Female students and male students less than 18 years.

Study type

It is a sequential Exploratory Mixed-Method study^[7] design where equal weightage was given to both study designs. **Qualitative:** Focus Group Discussion (FDG) was done using grounded theory to explore the topic of pre-gaming and to frame the questionnaire. **Quantitative:** A descriptive cross-sectional study, where self-administered pre-tested questionnaire was used.

Sample size

Based on a previous study done in America, by Haas *et al.*,^[8] the prevalence of pre-gaming was found to be 68%. Considering an alpha error of 5%, 7% relative precision and a 5% nonresponse rate the sample size was calculated to be 445. About 490 students were included in the study.

Qualitative study tool

Two FGDs were conducted, separately among ten 1st year and ten final-year students. Students who participated were purposively selected as they were vocal and willing to participate.

Written informed consent was obtained from each participant and the FGDs were conducted by a trained facilitator.^[9] The

discussions were audio-recorded and noted simultaneously by a note-taker who was also trained in qualitative research methods and manual content analysis of the data was done by two investigators. Codes were derived from the transcripts to form a logic to produce a universal claim from observed instances. Later, similar codes were merged together to establish the categories. Any incongruity between the two investigators was resolved by mutual discussion. The findings were used in developing a tailored questionnaire that was administered to the students.

We achieved data saturation after the second FGD as pre-gaming codes were getting repeated.

Quantitative study tool

The questionnaire was framed after reviewing literature and including the components of the FGD and pilot tested to 40 male students (10% of the sample size approximately) in a neighboring engineering college followed by a group discussion where consensus regarding terminologies was met by a group of experts.

The following operational definitions were used for the present study.

Pregaming is defined as consuming any form of alcohol (>3 pegs) at a time before attending a social event, where additional alcohol may or may not be available and/or consumed. Every user of alcohol is defined as a student who had used any form of alcohol at least once in their lifetime. Current user of alcohol is defined as a student who had used any form of alcohol at least once in the past 30 days.

Intention to quit pre-gaming

Any student who has contemplated quitting pre-gaming of alcohol products for the past 30 days.

A list of courses in the engineering college was obtained from the college authorities. Out of five branches, two branches (Mechanical and IT) were selected by lottery method. As there were only male students in the Mechanical branch, we completely enumerated the male students from another branch as well. Of the 490 students in those branches, 19 students were not present at the time of the survey and 21 students did not fill the forms, which came down to 450 students. The data were entered and analyzed in SPSS 24 software (SPSS Inc., Chicago, Illinois, USA) package. Descriptive statistics such as frequency, means, and percentages were used to describe the data.

Ethics

Ethical clearance was obtained from the Institutional Ethics committee. Written informed consent was obtained from the participants before the survey. Assurance was given about the anonymous nature of the study.

RESULTS

The mean age of 450 male students who participated in the study was 19.23 ± 1.34 years (Mean \pm SD). Out of 450 students, 183 (40.7%) of them belong to the 1st year. About 425 (95.4%) of the students were day scholars. Most of the participant's father

53.1% and mother 57.8% were educated up to the secondary level. Based on Modified B. G. Prasad's classification (2016), 43.2% and 41.8% of students belonged to class I and class II socio-economic status, respectively. The prevalence of ever and current alcohol usage among students were 85 (18.4%) and 60 (13.3%) respectively.^[10] The prevalence of pregaming among current users was 40 (66.7%) [Table 1] shows that among 85 ever users, the common form of alcoholic beverage used were breezer 51 (60.0%), followed by beer 48 (56.5%). The most common form of alcohol among current users was beer 29 (48.3%), followed by breezer 28 (46.7%). Among all occasions, the students were involved in pregaming mostly on birthdays 37 (92.5%) and marriages 37 (92.5%) followed by college cultural 36 (90.0%), night outs (27.5%), tours (20%), house parties (17.5%) and pubs (5%). Table 2 shows that all the students wanted to pregame for anticipated alcohol cost problems and fun 35 (87.5%) of them pregamed for easy conversations with the opposite sex. The majority 40 (66.6%) had the intention to quit pregaming among current users. Advice by close ones 16 (40%) and self-realisation 10 (25%) were the major factors influencing their intention to quit their behaviour [Table 3].

Category-1: Birthdays

"Birthdays are the time to have a ball! Irrespective of whose" (Male, 20 years). Sometimes when they get 'high' they also go out of their rooms and indulge in fights (assaults and verbal abuses) with their neighbors.

Category-2: Marriages

"We get so many varieties of drinks for free and there is no compulsion to go home" (Male, 19 years). The irony is that sometimes they do not even wake up to enjoy the marriage rituals.

Category-3: Bachelor parties are perceived as the last gateway to freedom. "We have substances like magic mushrooms" (Male, 19 years). These are usually available in hill stations like Kodaikanal. Usually, we consume it along with alcohol while pregaming."

Category-4: Tours and excursions

"The backseat is always loud and fun" (Male, 20 years) When the vehicle is constantly moving, the feeling of getting high escalates very fast with the rise in the number of drinks. The main risk involved during this process is some of them demand to drive the car which can cause accidents and injuries.

Category-5: Night outs and pubs

"When parents are away the house turns into a party room." (Male, 21 years) "Getting drunk fully and entering a pub is a fashion these days." The pub's parties involve money, as the drinks served, are very costly and due to less pocket money, they get drunk fully outside and go so that they can bear the relatively cheaper entry ticket.

Category-6: After exam parties

"After exams students unwind from stress by binge drinking." (Male, 21 years). The most common complications stated was blackouts, vomiting, gastritis, physical assaults, and high-risk

sexual behavior. Many of them purposefully pregamed to behave in an uncontrolled manner to vent out their feelings.

DISCUSSION

The prevalence of pregaming among current users is 66.6% in our study. The students involved in pregaming mostly on birthdays (92.5%) and college cultural (90.0%) followed by marriages (92.5%). The occasions in which student's pregame in the west are waiting for a football match to start, sorority parties, before attending a musical concert. And in private residences before going to parties and bars.^[2,3] Though the occasions in which students pregame differs from the west, this confirms the existence of pregaming culture in India as well. The occasions differ as the system of education, the exposure and cultural differences exist between the countries. This is very context-specific as the students living in the developed countries have the habit of partying more. Reasons for pregaming included anticipated cost issues as they get very little pocket money, to have an easy conversation with the opposite sex, intention to get high and social anxiety which were consistent with the findings from the west.^[2,3]

Table 1: Alcohol products used by college students (multiple responses)

| Alcohol products used by college students | Ever user (n=85), n (%) | Current user (n=60), n (%) |
|---|-------------------------|----------------------------|
| Beer | 48 (56.5) | 29 (48.3) |
| Breezer | 51 (60.0) | 28 (46.7) |
| <i>Kallu, padhani</i> ^[10] /any other local form | 10 (11.8) | 5 (8.3) |
| Spirits (vodka, rum, gin) | 10 (11.8) | 4 (6.7) |
| Wine | 22 (25.9) | 15 (25) |
| Brandy/whisky | 6 (7.1) | 3 (5.0) |

Words in italics are in Tamil language^[10]

Table 2 Reasons for pregaming among current users (n=40) (multiple response)

| Reasons | Frequency, n (%) |
|--|------------------|
| Intention to get high | 30 (75) |
| Desired alcohol expectancies | 20 (50) |
| Fun seeking behavior | 40 (100) |
| Anticipated alcohol cost issues | 40 (100) |
| Social anxiety | 30 (75) |
| Easy conversations with the opposite sex | 35 (87.5) |

Table 3: Factors influencing intention to quit pregaming among current alcohol users (multiple responses)

| Reason for intention to quit (n=40) | Frequency, n (%) |
|---|------------------|
| Advice by closed ones | 16 (40) |
| Pressure by girl friend | 2 (5) |
| Self-realization | 10 (25) |
| Negative social implications of alcohol use | 11 (27.5) |
| Economic factors | 1 (2.5) |

The students who consume alcohol both (ever and current) were (18.8%) and (13.3%) respectively. Mini SS *et al.* from Kerala, Mahanta *et al.* from Assam reported the alcohol prevalence to be 36% and thirty-eight percentage respectively.^[11,12] These studies also included the home-based alcohol drinks which they consume as a part of their culture. This may be the reason for the escalated prevalence.

There is no research done on pregaming yet in India. Officially Indians are still among the world's lowest consumers of alcohol as per government statistics. The percentage of the drinking population aged under 21 years has increased from 2% to >14% in the past 15 years, according to studies in the southern state of Kerala by Alcohol and Drugs Information Centre India.^[13] There are very few studies that clearly state the prevalence of heavy drinking. Only when there are researches that explore heavy drinking will they discover a phenomenon called pregaming, as the no of people who drink heavily are more likely to pregame.^[14] Secondly the definition of pregaming is not so forthright and common. The occasions in which the student's pregame drastically vary from the west and hence was less thought of as a problem in India. Most importantly the price factor also plays an important role. The country-specific price ratio between procuring drinks on-premise compared with off-premise, the greater the percentage of predrinkers.^[13,14] These are the probable reasons why pregaming is under-researched in India.

Pregaming may increase the overall level of alcohol consumption among young people. They are found to have higher blood alcohol concentrations on predrinking days compared with non-predrinking days.^[13,14] Another potential problem with predrinking is that it may facilitate the use of recreational drugs like cannabis, cocaine, or magic mushrooms. The complications related to pregaming reported in our study in the FGDs were blackouts, vomiting, high-risk sexual behavior, and physical assaults. They reported that pregaming is not only risky in the short-and immediate-term, but such behavior can influence one's permissive attitudes toward drinking and future heavier drinking patterns.

The strength of the present study is that it is a mixed-method study which provides a more complete understanding of the research problem. Students from only one college were included, hence we cannot generalize this finding. The pregaming component required more depth as the number of pegs consumed per pregaming episode was not quantified. The complications related to pregaming could have been explored and quantified in detail.

CONCLUSION

The prevalence of pregaming is high among current users. The

majority of them wanted to quit this behavior. Counselors and professionals working in alcohol de-addiction centers with this population should explicitly question pregaming and its associated symptoms. Tailor-made interventions should be promoted to target the concept of pregaming-related consequences of alcohol addiction.

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Conflicts of interest

There are no conflicts of interest.

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Original Research Article

A cross-sectional study on awareness and knowledge of menstrual hygiene practices among college going girls in Coimbatore district, Tamil Nadu

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ABSTRACT

Background: Women in India are restricted in their daily activities and mobility due to taboos, cultural barriers associated with menstruation and menstrual practices. Poor menstrual hygiene can make them susceptible to various reproductive tract infections. In this study we assessed the awareness, knowledge of menstruation and practice of menstrual hygiene among adolescents.

Methods: A college based cross-sectional study was conducted in the field practice area of an urban health training centre (UHTC) of a tertiary care hospital in the district of Coimbatore. Universal sampling method was used for selection of participants. A structured proforma was used for data collection. Microsoft excel 2007 was used for data entry and data analysis was done using SPSS version 27.

Results: It was found that 80.4% had formal education on menstrual hygiene. 94.5% of them had the habit of changing pads/cloths before they go to bed. 94.1% had the habit of washing with water every time and 76.49% of those who had the habit of washing used soap/antiseptic while washing.

Conclusions: Knowledge about menstruation and menstrual hygiene should be provided in schools and colleges. Ensuring availability of sanitary products, water, privacy and appropriate waste disposal in all public services and institutions can address the challenges.

Keywords: Menstruation, Menstrual hygiene, Sanitary material, Waste disposal

INTRODUCTION

According to WHO, the term 'adolescents' refers to people belonging to age group between 10 and 19 years.¹ Adolescence is a period of transition from childhood to adulthood. During this period, pubertal development and

sexual maturation take place.² The first physical change that occurs in about 60% of girls is thelarche.^{3,4} Menstruation is cyclical shedding of the endometrial layer under the influence of the hypothalamo-pituitary axis.^{5,6} The first menstruation is called as menarche. Menstruation and menstrual practices are overshadowed

by socio-cultural taboos and is linked with several false practises leading to adverse health outcomes.⁷ Unhygienic menstrual practices can lead to untoward consequences like pelvic inflammatory disease and infertility.⁶ Exclusion and shame lead to misconceptions and unhygienic practices during menstruation thereby resulting missing school and self-medication practices. Girls also tend to refrain from social interaction. One of the main issues regarding menstruation and menstrual hygiene for young girls is privacy. Anand et al states that many studies conclude that both reproductive tract infections (RTI) and vaginal discharge are related with non-use of hygienic methods during menstruation.⁸ For better menstrual hygiene especially among college going students, there is need for improvement in knowledge and practices as well as better access to materials like sanitary pads, clean toilets with running water and privacy. Social restrictions for women during menstruation have an impact on their emotional state, mindset and lifestyle. Large number of girls in less economically developed countries drop out of school at menarche.⁹ There is also considerable doubt whether used sanitary materials come under biomedical or plastic waste, thereby making its safe disposal a problem. Proper menstrual hygiene can promote healthy sexual maturation and prevention of diseases. Knowledge about menstruation is also important to distinguish between normal and abnormal uterine bleeding and also for the purpose of knowing fertile periods and contraception.¹⁰ The role of male in reproductive health has been acknowledged as an important contributor.¹¹ Hence this study was planned to assess the awareness and knowledge of menstrual hygiene practice among college going girls in the district of Coimbatore.

METHODS

This cross-sectional study was conducted in February 2018 among 710 private college girls in the field practice area of Urban Health Training Centre in the district of Coimbatore, Tamil Nadu after Institutional ethical committee approval. Prior permission was obtained from College authorities. A validated self-administered semi-structured proforma containing questions related to menstruation and menstrual hygiene was used for this study. All the college girls who were present in class on the day of data collection and who were willing to participate in the study were included. All the participants were explained about the nature of the study, their rights and details about confidentiality and how this study will help in future contribution to the field. Written informed consent was obtained from those who were willing to participate. The proforma which contained questions related to their awareness on menstrual hygiene and their menstrual hygiene practices apart from the basic socio demographic questions was administered. The participants were asked not to mention their name and identity on the proforma. The data collected was entered in Microsoft Excel and was analysed using SPSS

software version 27. The results were expressed as frequencies and percentages.

RESULTS

710 college girls between the age group of 18-22 years responded. Among the study population, 1.1% attained menarche by 10 years of age, 4.4% attained menarche by 11 years of age. 21.1% attained menarche by 12 years of age, 31.3% attained menarche by 13 years of age. 24.5% attained menarche by 14 years of age, 15.5% attained menarche by 15 years of age and 2.1% attained menarche by 16 years and above (Table 1). 87% of the study population had regular menstrual cycles. 72.7% of the study population had 3-5 days of menstruation while another 23.1% had flow for 6-8 days (Figure 1). 65% of the study population had cycles between 28 and 32 days. 21.86% of the study population had cycle less than 28 days (Figure 2). 97.7% of the study population used disposable sanitary napkins. Only 2.3% of them used cloth. Of those who used cloths, 40 % washed the cloths and reused them (Figure 3). 80.4% of the study population had formal education on menstrual hygiene. 94.5% of them had the habit of changing pads/cloths before they go to bed. 94.1% had the habit of washing with water at every change and 76.49% of those who had the habit of washing used soap/antiseptic while washing (Table 2).

Table 1: Distribution of study population according to age which they attained menarche.

| Age (year) | Frequency | Percentage (%) |
|--------------|------------|----------------|
| 10 | 8 | 1.1 |
| 11 | 31 | 4.4 |
| 12 | 150 | 21.1 |
| 13 | 220 | 31.3 |
| 14 | 174 | 24.5 |
| 15 | 110 | 15.5 |
| 16 and above | 15 | 2.1 |
| Total | 710 | 100 |

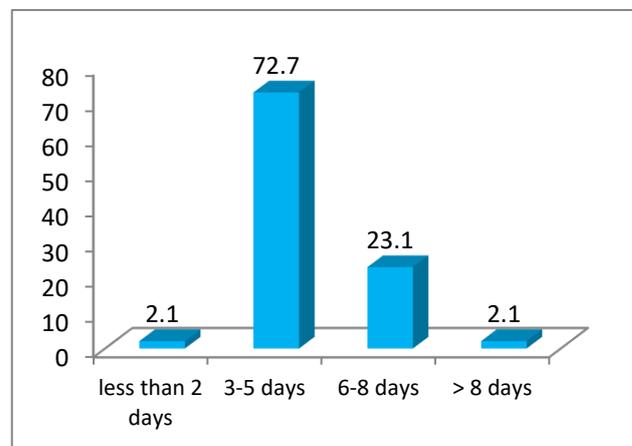


Figure 1: Distribution of study population according to duration of menstruation.

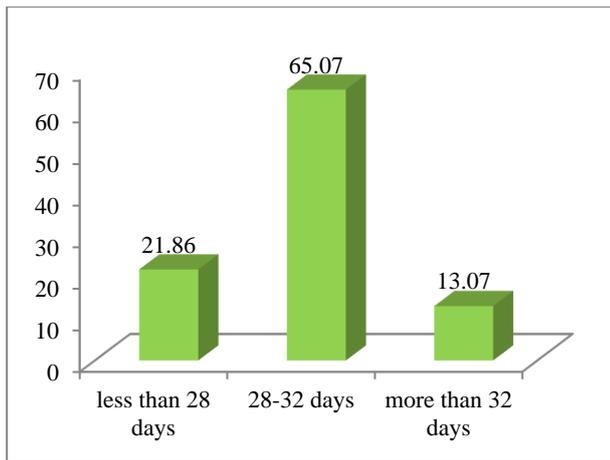


Figure 2: Distribution of study population according to length of each cycle.

65% had cycles between 28 and 32 days. 21.86% had cycle less than 28 days.

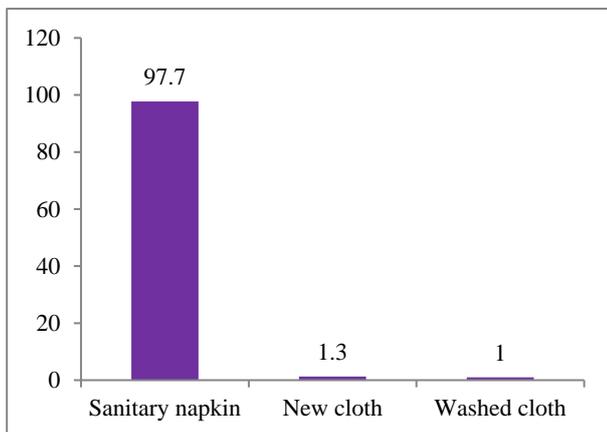


Figure 3: Distribution of study population according to type of absorbent used.

Table 2: Distribution of respondents according to their hygienic practices during menstruation.

| Practice | Number | Frequency |
|--|--------|-----------|
| Having previous education on menstrual hygiene | 571 | 80.4 |
| Change pads/cloth before going to bed | 971 | 94.5 |
| Washing with water every time | 668 | 94.1 |
| Using soap/antiseptic while washing | 511 | 72 |

DISCUSSION

This study was planned to know the awareness/knowledge of menstruation and menstrual hygiene practices among girl students in private college in the field practise area of UHTC of a tertiary care hospital in the district of Coimbatore.

In this study, mean age of attaining menarche were 13. Many studies done among different populations in various places in India showed similar mean age.^{7,12-14} 87% of the participants had regular menstrual cycle which is high when compared to study done by Singh et al.¹⁵ Menstrual cycles are often irregular during adolescence.^{16,17} Immaturity of the hypothalamic-pituitary-ovarian axis during the early years after menarche often results in anovulation and cycles may be somewhat long; however, 90% of cycles will be within the range of 21-45 days, although short cycles of less than 20 days and long cycles of more than 45 days may occur.¹⁸ By the third year after menarche, 60-80% of menstrual cycles are 21-34 days long, as is typical of adults.¹⁷⁻¹⁹

About 97.7% of the participants used disposable sanitary napkins and 2.3% of them used clothes which is in accordance with study done by Juyal and Adhikari et al.^{12,20} Socio economic reasons are the main cause for use of cloth followed by lack of knowledge on menstrual hygiene.^{7,8,12,13} In our study, 80.4% of the participants had a formal education on menstrual hygiene which is almost as same as the study done by Sharma et al.²¹

However due to the media awareness/school and college health awareness program there is improved knowledge and more use of sanitary napkins. Moreover, issue of free sanitary napkins since 2011 in Tamil Nadu has also decreased the use of cloths.²²

About 94.1% of participants had the habit of washing their private parts every time they changed pads which is in accordance with a study done by Juyal et al whereas in a study done by Sharma et al only 42% of the study population had the habit of washing their genitalia every time which is very less compared to our study.^{15,21} In a study done on menstrual hygiene by El Gilanya in Egypt documented that personal hygiene is highly affected due to lack of privacy.⁶ Lack of privacy and proper toilet facilities were also attributed in other studies.^{7,8,13} Since our study was done in a private college, the privacy and proper sanitation would have resulted in good results.

There were several limitations associated with this study. A self-administered questionnaire was used rather than conducting interviews and so the reliability of the answers could not be verified.

CONCLUSION

Millions of women are now suffering from reproductive tract infections and its complications. A major risk factor for reproductive tract infections is lack of menstrual hygiene. Women should be educated about the facts of menstruation and its physiological implications before bringing any change in menstrual practices. This can be achieved through educational TV programmes and knowledgeable parents. The mean age at menarche was 13 years which means that formal education on

menstruation and menstrual hygiene should start from school so that they will be well informed on this normal phenomenon.

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A Case of Accessory Anterior Hepatic Fissure: Cadaveric Study with Retrospective Radiological Investigation into its Vascularity

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Abstract

The hepatic diaphragmatic surface is usually smooth and featureless. Although the existing literature mentions grooves or fissures on the diaphragmatic surface, there is no consensus regarding its causative factors. During routine dissection, the authors found a liver with a broad accessory fissure on the anterior surface of the anatomical right lobe. An enlarged hepatic area was observed on the left side of this accessory fissure. The undersurface of the right hemidiaphragm had a diaphragmatic band that extended into the hepatic fissure. The morphometry of the accessory fissure was studied. Retrospective magnetic resonance imaging showed that the middle hepatic vein was to the immediate right of the accessory fissure. Histological examination was done to confirm the presence of muscle tissue in the hypertrophied diaphragmatic band. As the need for surgeries and transplantations of the liver rises, there is a need to understand the hepatic macroscopic anatomy and its radiological correlation.

Keywords: Diaphragmatic bands, diaphragmatic groove, magnetic resonance imaging

INTRODUCTION

The hepatic diaphragmatic surface is usually smooth and featureless but may present one or more grooves named variously as diaphragmatic or hepatic grooves, sulci, or accessory hepatic fissures.^[1] The hepatic fissure is an abnormal, permanent vertical indentation that partially subdivides the liver parenchyma and the existing literature puts the incidence at 5%–40%.^[2-4] These may be confused with hepatic abscesses, malignancies, or macronodular liver during surgery or radiological imaging. The radiological imaging shows that the accessory fissures usually occur in the junctions between the branching of the right or left portal veins that correspond to the position of the right and middle hepatic veins and their tributaries in 67% of cases.^[5] Although various hypotheses have been proposed for the occurrence of hepatic fissures, no causative factors have been defined.^[5] The anatomical study of hepatic variations is needed due to the rise in hepatic imaging and surgical interventions. This work involved morphometric and retrospective radiological

analysis of a cadaveric liver with an accessory anterior hepatic fissure and histological examination of the tissue from the diaphragm.

CASE REPORT

A liver with an accessory fissure on the anterior aspect of the anatomical right lobe was found during routine dissection in All India Institute of Medical Sciences, Bhubaneswar, on a donated body of an 88-year-old Indian male [Figure 1]. The medical reports of the body donor showed no history of any hepatic or pulmonary disease. The liver had no other variations.

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A hypertrophied muscle band on the inferior aspect of the right hemidiaphragm interlocked with the said accessory fissure *in situ*.

The accessory fissure was wide, smooth-walled, and indented the hepatic inferior border 78.95 mm to the right of the fissure for the ligamentum teres hepatis [Figure 1]. The enlarged hepatic area between the accessory fissure and the falciform ligament could qualify as an accessory lobe,^[3] and corresponded to surgical segment IV. The fissure was 67.37 mm long and the average depth was 1.8 mm and the average width was 12.98 mm measured by a digital vernier caliper. The liver weighed 1.020 kg.

The magnetic resonance imaging (MRI) traced the three main hepatic veins: the right, middle, and left to the inferior vena cava and delineated their relation to the accessory fissure. The veins from the accessory lobe (corresponding to surgical segment IV) were tributaries of the left hepatic vein. The deep projection of the accessory fissure contained no major hepatic veins. The middle hepatic vein was located immediately to the right of the accessory fissure [Figure 2].

Routine H and E staining confirmed that the diaphragmatic slip/band was skeletal muscle. The ImageJ software showed that the diaphragmatic slip was 2.8 mm thick in contrast to a tissue sample from another area of the right hemidiaphragm, which was 1.1 mm thick [Figure 3].

DISCUSSION

Similar to our finding, previous studies have also reported a single, wide, curved fissure on the anterior aspect of the hepatic right lobe [Table 1]^[3,5-10] with an incidence of 5%–40%.^[2,4] The broad range of incidence in existing literature may be due to these fissures being asymptomatic and incidental findings during surgery,^[2] autopsy,^[5] radiological imaging,^[3] or cadaveric dissection.^[6-10] Their occurrence showed racial variation^[2] and was more common in females after 15 years.^[11] The incidence rose with age and affected 71% of individuals between 81 and 84 years.^[3] Previous studies show that the accessory fissure of the liver was common in the anterosuperior surface at the boundary of the two hepatic lobes;^[3] Maachi *et al.* in 2005 state that accessory fissures were on the hepatic right side in 88% of cases as seen in this study.

When the main accessory sulci (MAS) were single, its mean length was 8.8 ± 2.1 cm; the mean depth was 1.7 ± 0.7 cm, and the average width was 0.8 ± 0.7 cm.^[11] The fissure in the present study was 67.37 mm (6.73 cm) long; its average depth and width were 1.8 mm (0.18 cm), 12.98 mm (1.3 cm), respectively.

Previous studies have used radiological imaging, corrosion cast of the vascular tree of the liver, or cadaveric dissection to study these fissures.^[5,11] The morphometric analysis of a cadaveric liver specimen followed by an MRI scan was done in this case report.

Studies show that localized band-like thickening of the diaphragmatic muscle (diaphragmatic slip/band) protruded

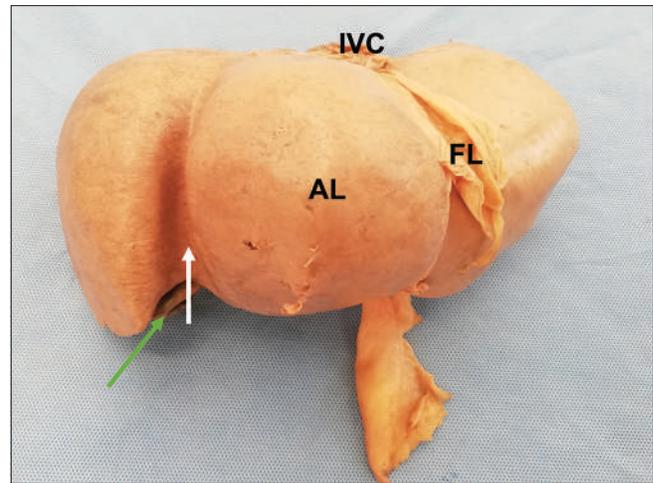


Figure 1: Photograph showing diaphragmatic surface of the liver; White arrow – Accessory fissure; Green arrow – Gall bladder; AL: Accessory lobe (hypertrophic segment IV); FL: Falciform ligament; IVC: Inferior vena cava

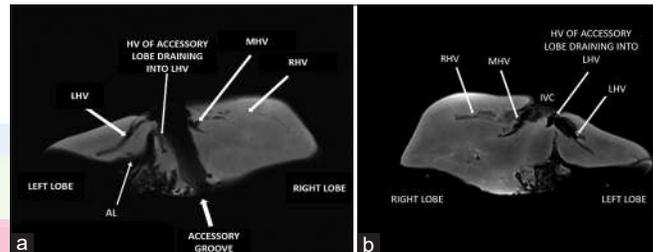


Figure 2: Magnetic resonance imaging (a - Posterior view; b- anterior view) showing the accessory fissure or groove; middle hepatic vein was immediately right to accessory groove; hepatic vein of accessory lobe draining into the left hepatic vein; right hepatic vein

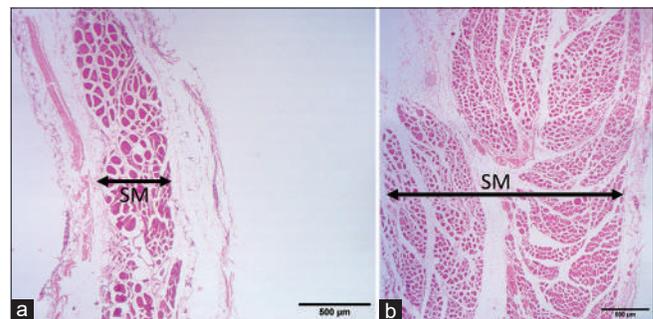


Figure 3: H and E stained section of normal diaphragm (a) and band part of the diaphragm (b). Band part is more than twice the thickness of normal part which was measured by the ImageJ software. SM: Skeletal muscle

from its undersurface and indented the liver and these bands become prominent with age.^[12] The present study reports similar findings, and routine histological staining has confirmed the diaphragmatic band as muscle tissue.

An imaging study by Maachi *et al.* in 2003 reported a correspondence between the location of the accessory fissure on the hepatic surface and the occurrence of either the right or middle hepatic veins or their tributaries in the deep projection

Table 1: Review of literature

| Author | Mode of study | Number of specimens | Accessory fissure (%) | Diaphragmatic slips/band (%) |
|---|----------------------------|---------------------|-----------------------|------------------------------|
| Sreekanth (2016) ^[6] | Cadaveric | 45 | 31.1 | 4.4 |
| Ono <i>et al.</i> (2000) ^[7] | Cadaveric | 420 | 11.9 | 0.2 |
| Auh <i>et al.</i> (1984) ^[3] | Radiological and cadaveric | 120 | - | 25 |
| Chaudhari <i>et al.</i> (2017) ^[9] | Cadaveric | 80 | 35 | 7.5 |
| Macchi <i>et al.</i> (2003) ^[5] | Autopsy | 48 | - | 40 |
| Reddy <i>et al.</i> (2016) ^[8] | Cadaveric | 52 | 53.8 | 26.9 |
| Mehar <i>et al.</i> (2017) ^[10] | Cadaveric | 33 | 27.2 | 12.1 |

of these fissures in 67% of cases. Our study showed no such correspondence like the remaining 33% of cases of the study by Maachi *et al.* 2003.^[5] The lack of correspondence between fissure and the main hepatic veins or their tributaries maybe because of the territorial boundaries between hepatic segments and portal venous sectors (delineated by the occurrence of the major trunks of hepatic veins) were not flat but undulating planes.^[5]

Many factors have been proposed in the past to explain the occurrence of accessory fissures which can be broadly classified as acquired (extrinsic) and embryological factors.

The acquired factors often cited in the past include respiratory diseases causing chronic cough (cough furrows) and long-term use of tight corsets for cosmetic or medical reasons (corset liver).^[5,13]

External hepatic compression can produce accessory fissures that show an age-related rise in incidence.^[3,9] It may be due to the pressure applied by the whole diaphragm,^[5] partial diaphragmatic eventration,^[3] or diaphragmatic bands^[14,15] that cause localized invagination of the liver tissue (with the incidence of 45.9% in existing literature).^[3,5,9,12] In this case report, we have found a hypertrophied diaphragmatic band extending into the floor of the said hepatic fissure.

The radiological and corrosion cast studies of hepatic vasculature have shown that there was a correspondence of the MAS with the right portal fissure and the right hepatic vein in 71% of cases.^[11] According to Maachi *et al.* in 2005, the upper part of the right portal fissure represents an avascular watershed plane which is a potential weak zone in the superficial hepatic parenchyma that is likely to be deformed by external pressure.^[11]

The pressure exerted by the diaphragm as a whole may lead to nonuniform growth of hepatic parenchyma at specific watershed regions in the young subjects,^[11] which corresponds to weak hepatic zones described earlier.^[5,11] In adults, diseases may cause a chronic increase in the diaphragmatic activity, which in turn exerts pressure mainly on the preformed weak zones in the hepatic parenchyma to produce accessory fissures.^[5] Clinically, accessory fissures can be taken as markers of weak zones and areas of low vascularization that can be used as planes for surgical resection of the liver.^[5]

These fissures are more common in fetal age and may represent delayed intersegmental fusion.^[16] The multiple signaling pathways such as beta catenins, Wnt genes, E-cadherins, fibroblast growth factors, tumor necrosis factors, and epithelial–mesodermal transformation play a role in prenatal hepatic development. Thus, defective molecular regulation may contribute to the formation of accessory fissures.^[11,17]

The hepatic developmental anomalies are rare and attributed to excessive development (accessory lobes) or defective development.^[14] The enlarged hepatic area between the falciform ligament and the accessory hepatic fissure (corresponding to the surgical segment 1V) could be due to excessive hepatic development although the exact cause is not known.^[9,16]

CONCLUSION

The accessory fissures are normal anatomic variants but are likely confused with hepatic or other abdominal pathologies during imaging and laparoscopy. As the incidence of hepatic resections and transplantations rises, the knowledge of detailed hepatic anatomy and its variations are useful to surgeons, radiologists, and fellow anatomists.

Acknowledgment

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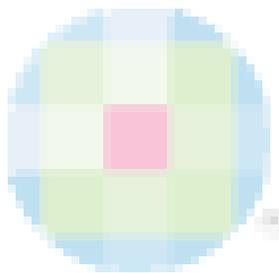
Conflicts of interest

There are no conflicts of interest.

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Comparative evaluation on the effects of balanced crystalloid solution versus ringer lactate on acid base and electrolyte status in patients undergoing elective neurosurgeries

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Abstract

Objectives: To compare the effects of balanced crystalloid solution versus lactated ringer's solution on acid base and electrolyte status in patients undergoing elective neurosurgeries **Materials and methods:** The study was conducted at Ananthapuri Hospitals and Research institute, Thiruvananthapuram A total of 80 patients were enrolled in the study. Randomisation was done using the sealed envelope technique. **Result:** Balanced Crystalloid Solutions provide stable acid base, sodium and potassium levels throughout the perioperative period. **Conclusion:** Being isotonic, Balanced Crystalloid Solutions can be recommended for Neurosurgeries.

Keywords:electrolyte,status

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Introduction

Peri-operative fluid administration is an important aspect of surgical care but is often poorly understood. Foremost intravenous fluids (IVF) gained therapeutic importance in the treatment of cholera in the 1830s[1]. Since 1880 IVF began to be administered peri-operatively to compensate for the "injurious" effects of anesthesia. Clinical improvements were consequently noted, though the adverse effects of saline were observed. The most widely used intravenous crystalloid solutions differ considerably from human plasma in composition, tonicity, or both. The most frequently prescribed crystalloid solutions are normal saline and Ringer's lactate solutions. Possible negative effects of these solutions on acid base status and plasma tonicity prompted the development of so-called 'balanced' solution[2].

A balanced electrolyte solution has the physiological electrolyte pattern and infusion of such a balanced solution is devoid of the risk of iatrogenic disruptions except for potential volume overload[2]. As one might expect excessive use of saline has been observed to result in hyperchloremic acidosis which has been identified as a potential side effect of saline based solutions. There is debate about the morbidity associated with this condition, although some consider the associated morbidity is probably low. It has been suggested that the use of balanced solutions may avoid this effect[3].

Materials and methods

The study was conducted at Ananthapuri Hospitals and Research institute, Thiruvananthapuram. Proper institutional Ethics Committee approval was obtained and a written informed consent was acquired from all patients who participated in the study. The study is a prospective observational study for a period of one year (1st June 2014 to 31st May 2015). A total of 80 patients were enrolled in the study. Randomisation was done using the sealed envelope technique. In order to get statistically significant results; a sample size of forty was allocated to each group.

Inclusion criteria

- ASA physical status I and II.
- Elective neurosurgery of more than two hours duration
- Capable of giving Informed Consent
- Age between 25-65 years.

Exclusion criteria

- Patients with extensive trauma.
- Patients with diabetic ketoacidosis
- Patients with renal failure
- Patients with pre-existing hypotension
- Procedures requiring massive blood transfusion

Sample size (4) was calculated according to the formula:

$$Z_{\alpha} = 1.96 \text{ for } \alpha = 0.05$$

$$Z_{\beta} = 0.84 \text{ for } \beta = 0.20$$

$$\mu T = \pi c \text{ (difference in mean)}$$

$$\sigma = \text{pooled standard deviation}$$

$$\text{Standard deviation of post op base excess } (\sigma) = 2.754$$

$$\text{Difference in post op base excess } (\Delta) = 1.8$$

$$N = \frac{2(1.96 + 0.84)^2 \times 2.754^2}{1.8^2}$$

Final sample size for the study is 37 rounded to 40 in each group

Patients were randomly allocated using the sealed envelope technique into two groups. Sealed opaque envelopes (equal to the number of patients) each containing a card was made. On half the number of cards it was written "Lactated Ringer's Solution" and on the other half it was written "Balanced Crystalloid Solution" indicating the intravenous fluids to be used for the surgery. The envelopes were shuffled and one picked randomly when each patient was taken up.

40 patients received Lactated Ringer's solution and 40 received Balanced Crystalloid solution (Sterofundin). Urine output was maintained at more than 0.5ml/kg/ hr as far as possible.

Baseline arterial blood gas analysis was done to measure pH, pCO₂, bicarbonate and base excess levels. Baseline serum sodium, potassium and chloride levels were also noted. Anesthetic management was standardized to propofol 2.5mg/kg, Fentanyl 2microgram/kg and vecuronium 0.1mg/kg for induction and vecuronium infusion and sevoflurane for maintenance of the patients were monitored via arterial and central venous catheters.

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Arterial blood gas analysis and serum sodium potassium and chlorides levels were measured intra-operatively after three hours and post-operatively after one hour and every three hours for the next six hour.

Mechanical ventilation was performed to maintain the arterial oxygen tension at 200-300 mmHg and the arterial carbon dioxide pressure at 35-40 mmHg. Intra-operative monitoring included end tidal carbon-di-oxide, Cardiac electrical activity, Central venous pressure, arterial blood pressure and pulse-oximetry.

Statistical analysis

Descriptive statistics such as percentage, mean and standard deviation were used to describe the variables used in the study. Inferential statistics such as independent test and chi square test was used to test the homogeneity of study variables between study drugs. Independent t test was used to compare outcome variables such as pH, PCO₂ etc at different interval of time between the study groups. SPSS 17.0 version was used to analyse data.

Result

Table 1: Distribution according to American Society of anesthesiologist physical status classifications (ASAPS)

| ASA | Lactated Ringer Solution | | Balanced Crystalloid Solution | | Z ² | P |
|----------|--------------------------|---------|-------------------------------|---------|----------------|-------|
| | Count | Percent | Count | Percent | | |
| Grade I | 19 | 47.5 | 23 | 57.5 | 0.8 | 0.370 |
| Grade II | 21 | 52.5 | 17 | 42.5 | | |

In table 1 the Chi square statistics t Z1 – U^{R-P} – P-005) showed that the groups were compared based on the ASA grading of patients.

Table 2: Comparison of MAP (mmHg) between groups at different time interval

| MAP (mmHg) | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | t | p |
|------------|--------------------------|-----|----|-------------------------------|-----|----|------|-------|
| | Mean | SD | N | Mean | SD | N | | |
| 30 Min | 65.0 | 2.1 | 40 | 65.4 | 2.0 | 40 | 0.76 | 0.449 |
| 1 hr | 65.0 | 2.1 | 40 | 65.5 | 2.1 | 40 | 0.91 | 0.367 |
| 2 hr | 64.7 | 2.1 | 40 | 65.2 | 2.0 | 40 | 0.96 | 0.339 |
| 3 hr | 65.0 | 2.1 | 40 | 65.4 | 2.1 | 40 | 0.73 | 0.466 |

Table 2 shows that the groups were compared based on their MAP in the intra-operative period. The MAP in both groups was stable throughout the surgery.

Table 3: Comparison of CVP (mmHg) between groups at different time interval

| CVP (mmHg) | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | t | p |
|------------|--------------------------|-----|----|-------------------------------|-----|----|------|-------|
| | Mean | SD | N | Mean | SD | N | | |
| 30 Min | 7.8 | 1.4 | 40 | 8.0 | 1.4 | 40 | 0.72 | 0.471 |
| 1 hr | 7.8 | 1.4 | 40 | 7.9 | 1.4 | 40 | 0.56 | 0.579 |
| 2 hr | 7.8 | 1.4 | 40 | 8.1 | 1.4 | 40 | 0.82 | 0.414 |
| 3 hr | 8.0 | 1.4 | 40 | 8.3 | 1.4 | 40 | 0.88 | 0.380 |

In table 3 the groups were compared based on their CVP in the intra-operative period.

Table 4: Comparison of pH between groups at different time interval

| pH | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | t | p |
|----------------------------|--------------------------|------|----|-------------------------------|------|----|------|-------|
| | Mean | SD | N | Mean | SD | N | | |
| Pre Op | 7.42 | 0.06 | 40 | 7.43 | 0.04 | 40 | 1.41 | 0.162 |
| Intra Op after 3 Hrs | 7.41 | 0.07 | 40 | 7.41 | 0.06 | 40 | 0.39 | 0.698 |
| Post op 2 nd Hr | 7.43 | 0.09 | 40 | 7.44 | 0.09 | 40 | 0.6 | 0.547 |
| Post op 5 th Hr | 7.42 | 0.09 | 40 | 7.42 | 0.08 | 40 | 0.38 | 0.704 |
| Post op 8 th Hr | 7.45 | 0.07 | 40 | 7.44 | 0.08 | 40 | 0.5 | 0.621 |

Table 4 shows that the mean pH does not differ between two groups at all time intervals (0.05)

Table 5: Comparison of PaCO₂ between groups at different time interval

| PaCO ₂ (mmHg) | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | t | P |
|--------------------------|--------------------------|------|----|-------------------------------|------|-------|------|-------|
| | Mean | SD | N | Mean | SD | N | | |
| op | 36.14 | 7.26 | 40 | 37.01 | 6.04 | 36.14 | 0.58 | 0.039 |
| after 3 Hr | 35.24 | 6.88 | 40 | 38.46 | 6.86 | 35.24 | 2.1 | 0.994 |
| 2 nd Hr | 31.26 | 8.34 | 40 | 31.25 | 7.48 | 31.26 | 0.01 | 0.159 |
| 5 th Hr | 33.16 | 7.84 | 40 | 35.57 | 7.29 | 33.16 | 1.42 | 0.391 |
| 8 th Hr | 33.03 | 6.85 | 40 | 34.28 | 6.12 | 33.03 | 0.86 | 0.393 |

Table 5 shows that PaCO₂ levels were measured intra operatively after three hours and respectively after one hour and every three hour from the next six hours

Table 6: Comparison HCO₃- between groups at different time interval

| HCO ₃ - | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | I | P |
|-------------------------|--------------------------|------|----|-------------------------------|------|----|-----|-------|
| | Mean | SD | N | Mean | SD | N | | |
| Pre Op | 23.41 | 3.34 | 40 | 24.59 | 2.13 | 40 | 188 | 0.064 |
| Intra Op after 3 Hrs | 22.66 | 2.83 | 40 | 23.95 | 2.93 | 40 | 24 | 0.049 |
| Post 2 nd Hr | 21.37 | 3.28 | 40 | 22.71 | 3.60 | 40 | 174 | 0.086 |

| | | | | | | | | |
|-------------------------|-------|------|----|-------|------|----|-----|-------|
| Post 5 th Hr | 22.56 | 3.70 | 40 | 23.17 | 3.99 | 40 | 071 | 0.480 |
| Post 8 th Hr | 23.38 | 3.16 | 40 | 24.12 | 3.04 | 40 | 107 | 0.289 |

Table 6 shows that in Lactated Ringer Solution group, mean HCO₃⁻ value is less when compared to Balanced Crystalloid Solution in all ABG samples but the difference is statistically significant (p value = 0.049) in intra operative samples.

The HCO₃⁻ level decreases in intra operative period and postoperative 2nd hour samples. The decrease was maximum in

postoperative 2nd hour samples in both groups. Then HCO₃⁻ levels gradually increases to reach the preoperative level during 8th hour of post-operative period. In Balanced crystalloid solution group HCO₃⁻ values were within the normal range.

Table 7: Comparison of Base Excess between groups at different time interval

| Base Excess (mEq/L) | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | | |
|-------------------------|--------------------------|------|----|-------------------------------|------|----|------|-------|
| | Mean | SD | N | Mean | SD | N | I | P |
| Pre Op | -1.16 | 3.50 | 40 | 0.28 | 3.13 | 40 | 1.93 | 0.057 |
| Intra Op after 3Hrs | -2.2 | 3.3 | 40 | -0.8 | 3.5 | 40 | 1.95 | 0.055 |
| Post 2 nd Hr | -3.5 | 3.9 | 40 | -2.2 | 3.9 | 40 | 1.43 | 0.156 |
| Post 5 th Hr | -2.5 | 4.2 | 40 | -1.7 | 4.4 | 40 | 0.77 | 0.442 |
| Post 8 th Hr | -1.2 | 3.3 | 40 | -0.6 | 3.5 | 40 | 0.8 | 0.424 |

Table 7 shows that base excess decreases during intra operative and postoperative period samples in both the two groups. The decrease is more for the Lactated Ringer solution group but the difference is not statistically significant. The decrease in base excess was maximum in the 2nd hour of postoperative period in both the groups.

Table 8: Comparison of Na⁺ between at different time interval

| Na ⁺ (mEq/L) | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | | |
|-------------------------|--------------------------|-----|----|-------------------------------|-----|----|------|-------|
| | Mean | SD | N | Mean | SD | N | I | P |
| Pre Op | 138.3 | 5.4 | 40 | 138.0 | 5.1 | 40 | 0.28 | 0.784 |
| Intra Op after 3 Hrs | 138.3 | 4.7 | 40 | 138.1 | 6.7 | 40 | 0.17 | 0.862 |
| Post 2 nd Hr | 132.7 | 6.7 | 40 | 132.2 | 9.0 | 40 | 0.28 | 0.779 |
| Post 5 th Hr | 129.9 | 5.9 | 40 | 131.9 | 7.3 | 10 | 1.33 | 0.189 |
| Post 8 th Hr | 130.4 | 4.3 | 40 | 132.9 | 8.1 | 40 | 1.73 | 0.087 |

Table 8 shows that serum sodium level decreases in the postoperative period in both the groups. The mean serum sodium does not differ between the two groups.

Table 9: Comparison of K⁺ between groups at different time interval

| K ⁺ (mEq/L) | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | | |
|-------------------------|--------------------------|-----|----|-------------------------------|-----|----|------|-------|
| | Mean | SD | N | Mean | SD | N | I | P |
| Pre Op | 3.6 | 0.6 | 40 | 3.5 | 0.5 | 40 | 0.79 | 0.434 |
| Intra Op after 3 Hrs | 3.6 | 0.5 | 40 | 3.6 | 0.5 | 40 | 0.31 | 0.761 |
| Post 2 nd Hr | 3.4 | 0.9 | 40 | 3.3 | 0.6 | 40 | 0.98 | 0.330 |
| Post 5 th Hr | 3.3 | 0.6 | 40 | 3.3 | 0.7 | 40 | 0.11 | 0.915 |
| Post 8 th Hr | 3.5 | 0.5 | 40 | 3.4 | 0.6 | 40 | 0.23 | 0.820 |

Table 9 shows that the difference in mean serum potassium between the two groups in all time intervals was not statistically significant. Cl⁻(mEq/L)

Table 10: Comparison of Cl⁻ between groups at difference time interval

| Cl ⁻ | Lactated Ringer Solution | | | Balanced Crystalloid Solution | | | | |
|-------------------------|--------------------------|-----|-------|-------------------------------|-----|------|------|-------|
| | Mean | SD | N | Mean | SD | N | I | P |
| Pre Op | 100.2 | 4.0 | 100.4 | 5.4 | 40 | 0.21 | 0.98 | 0.832 |
| Intra Op after 3 Hrs | 99.9 | 3.9 | 40 | 100.4 | 6.0 | 40 | 0.46 | 0.649 |
| Post 2 nd Hr | 97.3 | 7.4 | 40 | 95.3 | 9.4 | 40 | 1.97 | 0.289 |
| Post 5 th Hr | 92.6 | 7.2 | 40 | 95.2 | 7.4 | 40 | 16 | 0.115 |
| Post 8 th Hr | 93.4 | 6.2 | 40 | 95.6 | 7.3 | 40 | 1.47 | 0.146 |

In table 10 the serum chloride decreases in the postoperative periods in both the groups. The different in mean serum chloride between the two groups is not statistically significant.

Discussion

The present study was designed to compare the effect of Lactated Ringer's Solution and Balanced Crystalloid Solution (sterofundin) on intra operative and post operative acid base status and serum electrolyte changes mainly sodium, potassium and chloride levels.

This was done by analysis of a sample of arterial blood for taking the values of blood for HCO₃⁻, base excess, Na⁺ K⁺ and Cl⁻ values. The blood gas analysis is done at preoperative period, intra operative period after 3 hours and post operative 2nd, 5th and 8th hour samples.

80 patients were included in the study and 40 received Lactated Ringer's solution, the rest 40 received balanced crystalloid solution (Sterofundin). The anesthetic technique was same in all the cases.

The results of our study shows that sterofundin may be superior to

Lactated Ringer's solution in terms of maintaining pH balance providing a more stable acid base profile and reducing risk of hypernatremia which is in concurrence with the study done by Padmapriya et al.[5]. Whereas there is no statistically significant difference in pH profile and serum sodium status between Balanced Crystalloid solution and Lactated Ringer's Solution group were noted in our study.

The difference in mean PaCO₂ during intra operative period after 3 times is statistically significant (0.039) but the value appear to the in conge in both the two groups. The mean PaCO₂ at postoperative periods at 2nd hour, 5th hour and 8th hour is less in both the groups, but they are not statistically significant. This may be due to the spontaneous respiratory effort by the patient. Pain and anxiety night

be the reason for hyper ventilation[6]. The MAP and CVP in both groups were stable throughout the surgery.

Unlike the findings of the study done by Potura E.Lindner G et al.[7], where patient had hyperkalemia with administration of acetate buffered balanced crystalloid, our study showed serum potassium values within the normal range in both Balance crystalloid and Lactated Ringer's group. They also noted less hyperchloremia and metabolic acidosis which is in line with the findings of our study.

In our study we had stable acid base profile and lower serum chloride levels for sterofundin group which is similar to the findings of the study done by Disma N.Mameli L.et al.[8,9], who concluded that sterofundin is safer than normal saline in protecting young children undergoing major surgery against the risk of increasing plasma chlorides and subsequent metabolic acidosis.

Likewise our study also reverted lower serum chloride levels in postoperative period for the both Balanced Crystalloid and lactated Ringer group which are significant with the study of EM. Stand T et al.[10], which shows lower serum chloride levels in HES balanced group. The serum potassium levels were not significantly changed in both the groups and acid base profile of balanced crystalloid solution were improved. Similar changes were observed in the study of Hadmioglu N.Saadway et al.[11], where plasma-lyte was used as balanced crystalloid solution.

The inference about Lactated Ringer's Solution in our study proved to be consistent with the findings of Frumento et al.[12], who revealed less hyperkalemia and acidosis in Lactated Ringer group. Both the solutions avoided the development of hyperchloremic metabolic acidosis.

Though various studies were done[13,14,15] comparing lactated Ringer's and Balanced Crystalloid solution because of the potential clinical differences between solutions and the lack of definitive data, a comparative study is planned, on patients insisting the importance of choosing the right fluid for the right patient.

Conclusion

There is a statistically significant difference in the serum bicarbonate level in the intra operative period between Lactated Ringer's solution and Balanced Crystalloid Solution (Sterofundin). Balanced Crystalloid Solution provides a more stable acid base profile, but there is not much difference in pH profile between both the groups probably because of metabolic compensation.

Our study showed stable sodium levels during intra operative and postoperative periods for both Lactated Ringer's Solution and Balanced Crystalloid Solution (Sterofundin) but no statistical significance difference in serum potassium levels in intra operative & postoperative period in both the groups. Also the stable serum chloride levels in postoperative period for both the Balanced Crystalloid and lactated Ringer group reveals the fact that there is no significant hyperchloremic acidosis with both Lactated Ringer's Solution and Balanced Crystalloid Solution (Sterofundin).

Recommendations

- Balanced Crystalloid Solutions provide stable acid base, so it can be recommended for prolonged surgeries and trauma.
- In contrast to other crystalloid solutions, Balanced Crystalloid solutions provide stable sodium and potassium levels throughout the perioperative period.
- Being isotonic, Balanced Crystalloid Solutions can be recommended for Neurosurgeries.
- Since few studies reported Hyperchloremic acidosis while using normal saline, we recommended further studies to compare Normal Saline with Balanced Crystalloid Solutions.
- We recommended Balanced Crystalloid Solutions in patients with hepatic disorders as the metabolizable anion used in Balanced Crystalloid Solution is mainly Acetate.

Conflict of Interest: Nil Source of support: Nil

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Assessment of mental health status among adolescents in Puducherry, India – A mixed method study

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ABSTRACT

Context: Adolescence is a crucial period during which biological and psychosocial changes occur in an individual. The prevalence of mental disorders among Indian adolescents was 7.3%. Early recognition and intervention will help to have favorable outcomes. **Aims:** To determine and compare the prevalence and risk factors associated with mental health illness among urban and rural adolescents in Puducherry. **Methods and Material:** An explanatory mixed-method design wherein the quantitative phase (an analytical cross-sectional study) was followed by qualitative phase (focus group discussion). Adolescents aged 13–17 years attending Government schools in urban and rural Puducherry were selected by stratified random sampling. Mental health status was screened using a validated Youth Report Measures for Children and Adolescents – SDQ and students with higher score were considered to be at risk of mental health illness. **Results:** Among 329 adolescent, 25.5% are found to be at risk of mental health illness. The mean total score and sub-domain scores of hyperactivity and emotional symptoms were found to be significantly higher in urban when compared to rural. Among those at risk of mental health illness, significant difference between urban and rural area was seen with respect to variables like family monthly income and parent's occupation. Behaviour change and deterioration in academic performance were the most common presentation as perceived by the teachers. **Conclusions:** One fourth of the adolescents were found to be at risk of mental health illness, so periodic screening could be done at schools, for early identification and proper treatment of mental disorders.

Keywords: Adolescent, mental health status, strengths and difficulty questionnaire

Introduction

Adolescence is a very crucial period during which biological and psychosocial changes occur. The appearance of certain health problems of an adolescent has an impact on the mental, social, and physical well-being while growing up. About one sixth of the global population and one fifth of Indian population are constituted by adolescents.^[1]

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Globally 10–20% of children and adolescents suffer mental health illness of which 50% begin by the age of 14 years.^[2] Children with mental health illness face challenges in their routine life like stigma, isolation, and loss of access to education and health care facilities.

The prevalence of mental disorders in the age group 13–17 years is 7.3%, being nearly equal in both genders. Prevalence of mental disorders was nearly twice (13.5%) in urban metros as compared to rural (6.9%) areas.^[3] Early recognition and intervention would help to have favourable outcomes. Nearly 9.8 million of young Indians aged between 13 and 17 years were in need of active interventions. Thus, prevention and management of mental distress among adolescents and young adults should begin from an early age.

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The key strategy to reach adolescents would be schools and school teachers as they are the focal point to identify their behavioural changes at the earliest.^[4] In addition to this, the preventive services can be initiated from teachers as their care and guidance would be extremely helpful in the adolescent's mental well-being.^[5]

In India's flagship programme for adolescents (school health programme), the mental health screening through RBSK (Rashtriya Bal Swasthya Karyakram) targets only on behaviour disorders (autism and attention deficit hyperactivity disorders) and learning disorders. There is no specific approach to screen for other mental health illness.^[5] Hence, the family physicians at the primary health care level should be trained to diagnose early such mental health illness among adolescents for the early intervention and management.

There have been very few studies on mental health illness among adolescents in South India. Hence, this study was conducted with the aim to determine the prevalence of mental health illness and factors associated with it among the school-going adolescents and to explore the perceptions of mental health illness among the school teachers in Puducherry.

Subjects and Methods

Study setting

This study was conducted in a rural and an urban government school selected from the urban and rural service area of the tertiary care hospital in Puducherry. The government schools selected were approached to obtain their permission and the list of students in the age group of 13–17 years.^[3]

Study design

Sequential explanatory mixed-method design was adopted for this study. The quantitative component comprised a cross-sectional design among the school going adolescents and the qualitative component included focus group discussion (FGD) among the school teachers.

Study period

6 months (July 2019–December 2019)

Study population and data collection

Quantitative data

The list of students was obtained and eligible participants were selected by stratified random sampling from each class. The estimated sample size was 160 in each group.^[3] Data were collected from 329 study participants using Strengths and difficulty questionnaire (SDQ – youth report measures for children and adolescents).^[6] It is a 25-item behavioural screening questionnaire with 5 scales (of 5 items each) such as emotional symptoms, conduct problems, hyperactivity, peer relationship problem, and prosocial behaviour to produce a total score of 0–40. All participants were also asked about sociodemographic

characteristics. The data were collected after obtaining permission from Institute Ethics Committee (No. 200/IEC-25/F-7/2019).

Qualitative data

FGDs were conducted in Tamil with eight school teachers. Informed consent was obtained from the participants for their participation and also for the audio-recording of the interviews. The FGD was conducted over approximately 1 hr with two moderators who were trained in qualitative research methodology. An FGD guide was prepared in line with the objective of the study based on the available literature.

Data entry and analysis

Quantitative data

Data entry was done in MS Excel 2007. The data analysis was done using SPSS Version 16. Results were represented in the form of descriptive and inferential statistics. The continuous variables were represented in the form of mean and standard deviation. The categorical variables were summarized in percentages and proportions. The prevalence of mental health illness was calculated as proportion of adolescents who scored high-risk (20–40) total difficulties scores. The findings between the groups were compared using Chi-square test and Fisher's exact test. Logistic regression model was applied to find the significant factors associated with depression. A *P* value of < 0.05 was considered as significant.

Qualitative data

Manual content analysis of the transcripts was done. The analyses of interview transcripts were begun after the interview. The transcript was read at least twice and inductive and deductive codes were derived from the transcript. Later similar codes were merged together to form subthemes.

Results

Quantitative results

The data were collected from 166 urban school going adolescents and 163 rural adolescents. The mean age of the study participants was 13.9 + 1.2 years, which was similar in both groups. More than half of the study participants were males but females (52%) were more among rural school going adolescents. More than 95% of the study participants were from Hindu religion [Table 1].

Among 329 adolescent, the overall prevalence of mental health illness among adolescents was found to be 25.5% with 27.7% in urban and 23.3% in rural [Figure 1]. The mean total score, hyperactivity scale, and emotional symptoms scale were found to be significantly higher in urban adolescent when compared using Mann–Whitney U test [Table 2].

Among those at high risk of mental health illness, significant difference between urban and rural area was seen with respect to variables like family income and parent's occupation [Table 3].

Table 1: Socio-demographic profile of study participants (n=329)

| Variables | Urban n (%) | Rural n (%) | P |
|-----------------------|-------------|-------------|-------|
| Age | | | |
| 13 years | 96 (57.8) | 73 (44.8) | 0.000 |
| 14 years | 27 (16.3) | 53 (32.5) | |
| 15 years | 17 (10.2) | 25 (15.3) | |
| 16 years | 16 (9.6) | 3 (1.8) | |
| 17 years | 10 (6.0) | 9 (5.5) | |
| Gender | | | |
| Male | 96 (57.8) | 79 (48.5) | 0.089 |
| Female | 70 (42.2) | 84 (51.5) | |
| Parents occupation | | | |
| Unskilled | 46 (27.7) | 111 (68.1) | 0.000 |
| Skilled | 120 (72.3) | 52 (31.9) | |
| Parents education | | | |
| Illiterate | 82 (49.4) | 70 (42.9) | 0.240 |
| Literate | 84 (50.6) | 93 (57.1) | |
| Family monthly income | | | |
| <10,000 | 94 (56.6) | 156 (95.7) | 0.000 |
| >10,000 | 72 (43.4) | 7 (4.3) | |
| Religion | | | |
| Hindu | 163 (98.2) | 156 (95.7) | 0.216 |
| Others | 3 (1.8) | 7 (4.3) | |

Table 2: Comparison of mean rank on all scale of SDQ in the study group based on the place of residence (Mann-Whitney U test)

| Domains | Mean rank | | P |
|---------------------------|-----------|--------|-------|
| | Urban | Rural | |
| Emotional Symptoms Scale | 176.76 | 153.02 | 0.022 |
| Conduct Problem Scale | 156.81 | 173.34 | 0.106 |
| Hyperactivity Scale | 192.55 | 136.94 | 0.000 |
| Peer Problem Scale | 155.78 | 174.39 | 0.073 |
| Total Difficulties Score | 176.55 | 153.24 | 0.026 |
| Prosocial Behaviour Score | 170.20 | 159.24 | 0.283 |

As depicted in Table 4, family monthly income was significantly associated with high risk of developing mental health illness among urban and rural adolescents using logistic regression.

Qualitative results

We conducted focus group discussion with eight teachers at a meeting room in the Government school in our urban field practice area. Of the eight teachers selected purposively for the FGD, five were females and three were males. The participant's age ranged from 29 years to 43 years. About six subthemes were grouped into two thematic fields which were derived from the content analysis of the interviews [Table 5].

Theme 1: Common presentations of mental health illness

According to the respondents, the most common presentation was the behaviour change of the students followed by few signs like drowsiness and lack of concentration. Most of them made

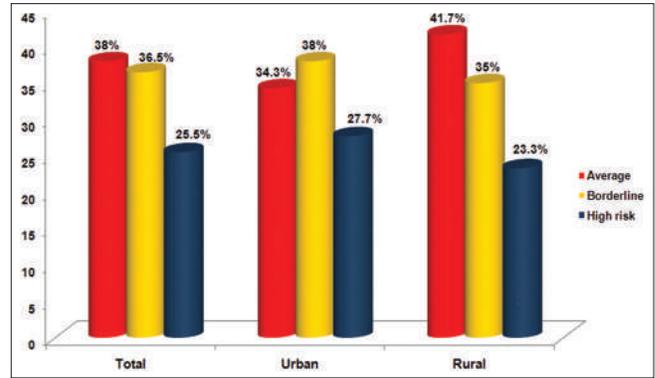


Figure 1: Distribution of mental health illness among adolescents based on SDQ scores (N = 329). (SDQ scores – Average: 0–15; Borderline: 16–19; High risk: 20–40)

a note on student's rude behaviour within their peer group and toward teachers. Here are the words of respondents who had experienced student's behaviour change.

“They won't bring notebooks. When we question them, they would get into arguments with teachers. Few students would file complaint against teachers.”

– Female respondent of 29 years

“A student would hide in a particular teacher's class and sleep. He wouldn't show his face also to us. Few students even bunk the class.”

– Female respondent of 42 years

“Few students would mark their classmates who laugh at them and then beat them when teachers leave the class.”

– Male respondent of 33 years

Deterioration in the academic performance of the student was mentioned by few respondents. A few teachers reported that these students were reluctant when they were asked to perform in class hours. As said by them,

“Boys are very shy to open up in front of fellow classmates and don't want to be questioned. Few students are giving importance to physical appearance. They are very reluctant to do things in front of their classmates like reading in front of them.”

– Female respondent of 43 years

“There's a boy, who would perform well, became drowsy in class hours and his academic performance started to deteriorate.”

– Male respondent of 40 years

“With the change of circumstances, their scholastic performance drops. Even a good performer deteriorates within a quarter.”

– Female respondent of 38 years

Table 3: Comparison of risk factors for mental health illness among high-risk adolescents based on the place of residence (n=84)

| Risk factors | Urban (%) (n=46) | Rural (%) (n=38) | P | Odds ratio |
|-----------------------|------------------|------------------|-------|---------------------|
| Gender | | | | |
| Male | 54 | 55 | 0.933 | 1.038 (0.438-2.461) |
| Female | 46 | 45 | | |
| Parents occupation | | | | |
| Unskilled | 41 | 74 | 0.003 | 0.251 (0.099-0.637) |
| Skilled | 59 | 26 | | |
| Parents education | | | | |
| Illiterate | 52 | 50 | 0.843 | 1.091 (0.462-2.577) |
| Literate | 48 | 50 | | |
| Family monthly income | | | | |
| <10000 | 61 | 95 | 0.000 | 0.086 (0.018-0.404) |
| >10000 | 39 | 5 | | |
| Religion | | | | |
| Hindu | 96 | 100 | 0.193 | 1.864 (1.524-2.279) |
| Others | 4 | 0 | | |

Table 4: Predictors of risk for mental health illness among high-risk adolescents by binary logistic regression analysis (n=84)

| Risk factors | B | S.E. | Wald | P | Adjusted odds ratio (95% CI) |
|-----------------------|-------|-------|-------|-------|------------------------------|
| Parents occupation | 0.897 | 0.515 | 3.028 | 0.082 | 2.452 (0.893-6.734) |
| Family monthly income | 2.088 | 0.813 | 6.600 | 0.010 | 8.070 (1.641-39.692) |

B: estimated logit coefficient; SE: standard error of the coefficient; Wald: test statistic using Chi-square test; CI: confidence interval

Table 5: Themes derived from FGD among school teachers

| Themes | Subthemes |
|--------------------------------|--|
| Common presentations | Signs and symptoms Behaviour change |
| Probable reasons and solutions | Academic performance Social media influence Peer group pressure Family oriented |

Theme 2: Probable reasons and solutions

According to the respondents, the most perceived cause for their mental health illness was family-oriented problems and its impact on them. Few teachers had also mentioned that the social media influence and peer-group pressure as probable reasons. They had stated that,

“Students with family issues would stay silent and we can easily pick their abnormality. When these students are probed, they disclose that they are victims of some domestic violence or being brought up by a single parent”

– Male respondent of 37 years

“Mostly seen in students who are being brought up by a single parent”

– Female respondent of 38 years

“These students do not have care or support of their family”

– Female respondent of 42 years

“Most of the students attending government schools are having one or the other family issues. They’re the ones who are misbehaving.”

– Female respondent of 29 years

“Their behaviour changes might also be because they are addicted to social media or being influenced by their friends”

– Female respondent of 41 years

When discussed about their perception on managing such students, most of them suggested about counselling these students and their parents. But they deferred on medical management as it was not required immediately. Few of their statements,

“That should be rectified from the root. So, we also counsel them regarding the same.”

– Female respondent of 29 years

“Problem oriented solution is best. So, parents should be counselled first.”

– Female respondent of 41 years

“If the peer group pressure is the reason for a student’s behaviour change, then we should help them to come out of that surrounding. We would inform their parents that your child’s behaviour is getting worse because of this friendship, so better help them to come out of it.”

– Male respondent of 40 years

“We haven’t seen children with such extreme behaviour problems that required to be referred to a psychologist or psychiatrist. As this is not a clinical or psychological problem, it’s a socioeconomic problem. So the change should begin within the family.”

– Male respondent of 37 years

Only one of the respondents was aware of the national program on adolescent health. And none of them were aware of the adolescent clinic days organized in the primary health care. As said by the respondent,

“They (the teachers) are not aware of such a program. Our education department had organized a session on this national program and one teacher per school had attended the same. I had attended the session but I couldn’t share the materials with my colleagues.”

– Male respondent of 37 years

Discussion

This study showed that one in every four adolescents is at risk of mental health illness. Puwar T *et al.*^[7] and Arman *et al.*^[8] also stated similar findings (27% and 26%, respectively) in their study done in Gujarat, India, and in Iran, respectively. Kollabathula M *et al.*^[9] found that the prevalence was about 16% among the adolescent girls in Andhra Pradesh. The difference in prevalence was probably because the study was conducted only among adolescent girls in contrast to the present study which included both genders. Bhola P *et al.*^[10] found 10.1% of pre-university students in Bangalore were at risk of mental health illness. The smaller proportion was because the researchers had considered individuals at high risk when the SDQ total difficulties score was above the 90th percentile of the data. Thus the risk of adolescents for mental health illness does not vary much across various regions of the country.

It was also found that family income and parent's occupation were significantly associated with risk of mental health illness among urban and rural adolescents. Similar association of parent's occupation was stated by Puwar T *et al.*^[7] in Gujarat. Personal factors like history of physical or verbal abuse, difficulties with studies, lack of safety, and non-traditional lifestyle practices were independently associated with mental health illness in a study conducted in Goa.^[11] Various studies had shown other risk factors like type of family, difficulties in reading at home, type of school, suicidal attempts, visual disturbances, parent's education, and financial difficulties were also associated with mental health illness among adolescents.^[7,9-13] Hence, the planning of intervention should be multi-dimensional involving the adolescents, their family background, and teachers in addition to the health care settings. Other than the routine adolescent health services available at the primary health care level, prompt mental health screening, and specialist care should be provided periodically for the opportune management.

The present study found that the difference was significant in emotional symptoms scale and hyperactivity scale when compared based on the area of residence. Reddy KR *et al.*^[14] also reported that emotional symptoms scale difference was most pronounced among females than male adolescents in a study conducted in Bangalore among adolescents. Hence, the prediction of mental health illness risk might be more frequent with emotional symptoms scale among various groups.

There were very few studies conducted to explore the perceptions of teachers about adolescent's mental health illness. From this study, it was found that behaviour change was the most common presentation as perceived by the teachers. Deterioration in academic performance was also stated by few participants in this study. Similarly Michaud PA *et al.*^[15] also stated deviant behaviour and change in school performance or behaviour as one of the signs of overt mood changes among adolescents. They had also mentioned that family conflicts were one among the various reasons for the adolescent's mental health illness similar to this

study. It was also found that adolescents influenced by social media and peer group pressure had presented abnormal from this study. However, most of the teachers were unaware of the national program for the adolescents and adolescent friendly clinics.

Strengths

This study adopted sequential explanatory mixed-method study wherein an explanation to the quantitative findings was obtained through the qualitative research. It fetched two types of information together which gave us greater understanding and insight into the adolescent mental health illness that may not have been acquired by analysing and evaluating data independently.

Limitations

The clinical assessment by a trained health care professional was not a part of the study. Also, this study had failed to address suicidal attempts, sexual and reproductive behaviour, and substance abuse.

Conclusion

The current study had shown that one in every four school going adolescents were at risk of developing mental health illness. Intervention should be made at all levels including school teachers and family members and make them aware of importance of mental health status. Life skill education would help adolescent to deal with the mental health issues. And it also emphasized the need for improved policy for training teachers and effective networking between the teachers and health professionals. The present study highlights the importance of future intervention studies involving the primary care physicians, specialists, school teachers, parents and the adolescents, with periodic follow-up.

Acknowledgement

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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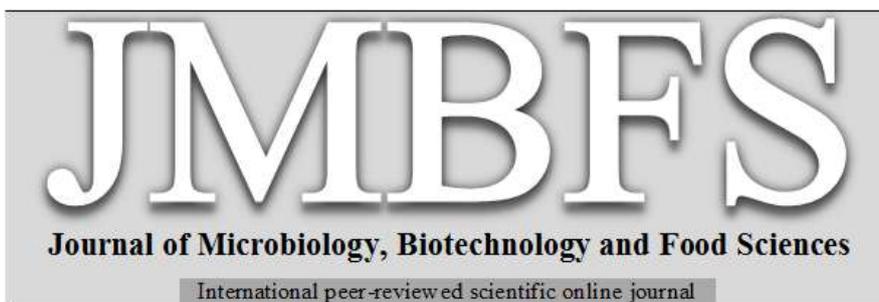
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Conflicts of interest

There are no conflicts of interest.

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EVALUATION OF PHYSIO-CHEMICAL CHARACTERISTICS AND ACRYLAMIDE CONTENT IN CRISP FRIED DOUGH WAFERS MADE FROM NIXTAMALIZED PEARL MILLET

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ABSTRACT

Pearl millet (*Pennisetum glaucum*), a drought tolerant crop belonging to the family Poaceae is an important type of millet. It is a sturdy plant that can sustain adverse growing conditions. It is rich in vitamins, minerals, oil and phytochemicals. The phytates or phytic acid present acts as antinutritional factor which bind to the proteins and minerals thereby reducing the digestibility and bioavailability of the proteins, carbohydrates and minerals. In the following study, nixtamalization, a process widely used for the treatment of maize, is being used on the millet to lower the antinutritional properties of the millet. The grain was cooked at 95°C with varying lime concentration (0.5%, 1.0%, 1.5%, 2.0%) while keeping the cooking time (30 min) and steeping time (2 hours) constant. Product (crisp fried dough wafers) was made from the milled nixtamalized flour and different physio-chemical parameters and acrylamide content of the crisp fried dough wafer was analysed. The product made from flour treated with 1.5% lime showed the best results and overall acceptability. This research could be beneficial for increasing the utilization of pearl millet in different parts of the world especially in under developed countries as a health food. Also, the phytochemicals of pearl millet have various health benefits and this study can be used to increase their concentration and thereby promote the use of pearl millet for enrichment of various food products.

Keywords: Nixtamalization, Pearl millet, Tannin, Anti-nutritional factors, Acrylamide, FTIR

INTRODUCTION

Nixtamalization or alkaline cooking is a method where alkaline solutions are used for the treatment of whole grains (Gaytán-Martínez et al. 2017). In Central America and some parts of Mexico this method is widely used for the treatment of corn kernels (Boniface and Gladys, 2011; Rendón-Villalobos et al. 2009). Lime, wood-ash and lye are the common agents used for nixtamalization process. During the process there are physical, chemical and structural changes observed in the whole grains (Owusu-Kwarteng and Akabanda, 2013). The calcium content and the bioavailability of niacin increases with nixtamalization. The treatment helps in the reduction of aflatoxin and improves the protein quality in the final product (Owusu-Kwarteng and Akabanda, 2013; Rajeswari et al. 2015). The process helps in improving the flavour and aroma of the grain (Sefa-Dedeh et al. 2004). The treatment helps in increasing the protein digestibility of the grain, makes the grains softer thereby making it easier to ground into flour (Boniface and Gladys, 2011).

Pearl millet (*Pennisetum glaucum*) also known as Bajra is one of the most important type of millet. It is a drought tolerant crop belonging to the family Poaceae (Abdel-Hafez et al. 2017; Adebisi et al. 2016). It is widely cultivated in Indian and South African subcontinents (Sandhu and Siroha, 2017). It can grow well in difficult conditions like low fertile soil, high temperature condition and soil with high salinity. Due to its high tolerance to adverse growing conditions pearl millet can grow in areas where other cereals like wheat, corn, sorghum fail to grow (Shaikh et al. 2017). Compared to other cereal crops it has a higher oil content (Jain and Bal, 1997). It is a rich source of protein, dietary fibre, starch, phytochemicals (tannins, phytic acid, ferulic acid and other phenolic compounds), vitamins like vitamin E, vitamin K and B complex vitamin (Thiamine, Riboflavin, Niacin, Vitamin B6, Folate and Pantothenic acid) (Lestienne et al. 2005; Jain and Bal, 1997). It is a well known source of calcium, iron and zinc and essential amino acids (Lestienne et al. 2007). Pearl millet consists of antinutritional factors in the form of condensed tannins, phytates, etc which bind to the proteins and minerals thereby reducing the digestibility and bioavailability of the proteins, carbohydrates and minerals (Gaytán-Martínez et al., 2017; Lestienne et al., 2007).

International Agency for Research on Cancer (IARC) has classified acrylamide as a probable carcinogen. Acrylamide is chiefly found in fried product such as potato French fries (Abdul Hamid et al., 2018). The formation of acrylamide is associated with Maillard reaction (Salazar et al., 2014). The presence of carbonyl compounds or similar groups which can react with asparagine amino acid and form

Schiff base are responsible for acrylamide formation (Salazar et al., 2014; Hidalgo et al., 2009). Various chemical agents such as asparaginase, acids, divalent actions, phospholipids and some amino acids that are used as food additives are found to be reducing the formation of acrylamide in thermally processed foods (Salazar et al., 2014; Kalita and Jayanty, 2013).

The purpose of this study were to evaluate the effect of nixtamalization on fried food prepared from nixtamalized pearl millet flour and to analyse the changes associated with the food product in terms of its proximate composition, antioxidant scavenging activity, total polyphenol content, tannin content, colour analysis, TPA and detection of acrylamide in the crisp fried dough wafers.

MATERIAL AND METHODS

Materials

Raw pearl millet was procured from local supermarket. Calcium oxide of analytical grade was purchased from a local dealer. Gallic acid, ascorbic acid, vanillin, Folin Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sisco Research Laboratory Pvt. Ltd. (SRL). Catechin was purchased from Sigma Aldrich. Reagents like methanol and HCl was purchased from Merck and Co. All the reagents and solvents used were of analytical grade.

Methods

Nixtamalization of the grain

Pearl millet was manually cleaned to remove damaged seeds or any debris. The grains were stored in a clean and dry environment until further use. These grains were cooked in lime solution of concentration 0.5%, 1.0%, 1.5% and 2.0% for a period of 30 min and steeped for 2 hours. The ratio of grain to water used for cooking of all the samples were 1:3 w/v. The cooking temperature were kept constant for all the samples i.e. 95°C. After steeping, the nixtamalized grains were washed thoroughly to remove excess of lime and extraneous pericarp material. Distilled water has been used for cooking and washing the grains throughout the experiment. The grains were dried in a tray drier at a temperature of 65 ± 2°C for 3 hours. Following this, the grains were pulverized using a disc attrition mill and sieved using 0.5mm mesh screen. All the flours were stored in an air tight container

and were kept away from direct sunlight at 37°C temperature until samples were prepared.

Preparation of crisp fried dough wafers

For preparation of crisp fried dough wafers, nixtamalized pearl millet flour was rehydrated with enough water and mixed with a small amount of clarified butter and salt to make a dough of proper consistency respectively. The dough is shaped into thin disc of 1mm in thickness and having a diameter of 4cm. These discs were deep fried for 1 min on each side in rice bran oil at a temperature of 180°C. After frying the crisp fried dough wafers were cooled on an absorbent paper towel and were tested for proximate composition, antioxidant scavenging activity, total phenolic content, tannin estimation, colour, texture profile analysis and presence of acrylamide. They were stored in clean air tight container until further use.

Proximate analysis

All the samples were analysed for moisture content, ash content, calcium content, protein content and total fat content. Total ash content was determined by method 08-03.01 (AACC, 2000), calcium content was estimated using 983.35 method of (AOAC, 1997), protein content was determined using the method of (Kisan et al., 1973), and total fat was estimated by (AACC, 2000). All analysis were performed in triplicate.

Extract preparation

Methanolic extracts of the fried samples were prepared using the method reported by (Gaytán-Martínez et al., 2017) with some slight modification. About 1g of fried sample were mixed with 10ml of methanol. The mixture was kept away from direct sunlight and stirred in a magnetic stirrer at an rpm of 450 for 30 min at 25°C. The extracted sample along with the residue was then vortexed and then centrifuged at 3000 RPM for 6 min. The supernatant was collected and stored at -20°C until further use. These extracts were further used for determination of antioxidant activity, total phenolic content and tannin content. All the results were an average of triplicate readings.

Antioxidant scavenging activity using DPPH+

The free radical scavenging activity was measured using a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method reported by with some modification. A total of 100µL of methanolic extract were mixed with 900 µL of freshly prepared 60 µM DPPH solution. The reaction mixtures were vortexed and placed in dark for 30 min at room temperature. The optical density was measured at 515nm using a UV-Vis spectrophotometer (UV-1700 Pharma Spec, Shimadzu). All the results were an average of triplicate readings. The inhibition percentage was calculated against a reagent blank and the results were expressed.

Estimation of phenolics by Folin-Ciocalteu assay

The total phenolic content of all the methanolic extracts were determined by Folin-Ciocalteu method as mentioned by (Samshuddin et al., 2015) with some slight modification. The reaction was initiated by oxidizing 100 µL of sample extract with 200µL of freshly prepared Folin Ciocalteu reagent (10%) and 800 µL of sodium carbonate (700 mM). This mixture was kept in dark at room temperature for 30 min. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV-1700 Pharma Spec, Shimadzu). The results were expressed as µg of gallic acid equivalents per gram of sample (µgGAE/g). All the results were an average of triplicate readings.

Estimation of tannins

Tannin content were determined using a method reported by (Gaytán-Martínez et al., 2017). Methanolic extract (200µL) were mixed with 800 µL vanillin reagent (0.5% vanillin, 4% HCl in methanol) and kept for 20 min. The absorbance of the mixtures were measured at 492nm using a UV-Vis spectrophotometer (UV-1700 Pharma Spec, Shimadzu). The tannins were expressed as µg (+)- catechin equivalent per gram of sample (µg CAE/g). All the results were an average of triplicate readings.

Colour analysis of crisp fried dough wafers

The colour of all the crisp fried dough wafers were analyzed using a Hunter Lab Colorimeter (ColorFlex EZ, Hunter Lab, Reston USA). L*, a* and b* were the three colour coordinates that were examined, where L* represents the lightness or darkness, a* represents redness or greenness and b* represents yellow or blue. All the results were an average of the triplicate readings.

Texture Profile Analysis (TPA)

Texture profile of all the fried samples were analysed using CT3 texture analyzer (probe: needle probe [TA9, 20mm L]; pre-test speed: 1.00 mm/s, test speed: 0.50 mm/s, post-test speed: 0.50 mm/s; load cell: 10 kg). The resistance of the material to the applied forces is measured by a calibrated load cell and the results were expressed in either grams or Newton (Rana et al., 2018; Ghosh et al., 2017; Yi et al., 2016). The software used for all the results was Texture Probe CT Software.

Fourier Transmission – Infrared (FTIR) Spectroscopy of crisp fried dough wafers

Attenuated total reflection (ATR) infrared spectra of the samples were obtained using a FTIR Spectrophotometer (FTIR-8400S, Shimadzu). Background spectra of the instrument were collected before mounting the samples (0.1g of each milled sample) on the instrument. All the spectra were recorded with characteristic peak in wave numbers from 500 to 4000 1/cm. All spectra measurement were carried out room temperature.

Statistical analysis

All data were expressed as means ± standard errors of triplicate measurements and analyzed by SPSS for Windows (ver. 16.0). One-way analyses of variance (ANOVA) were carried out to test significant differences (p≤ 0.05). Mean and standard deviation were computed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Proximate analysis

Proximate composition of crisp fried dough wafers made from nixtamalized pearl millet flour is shown in **Table 1**. The ash content for the samples was found to be increasing from 0.5% lime concentration to 2.0% lime concentration. The ash content for the treated sample ranged in 1.86% – 2.86% while the ash content for the untreated sample was 1.56%. It can be said that there was an increase in ash content of the sample. The moisture content of the sample is found to be in the range of 2.95-3.15%. During nixtamalization calcium oxide gets incorporated into the grain thereby increasing the ash content of the flour and ultimately the product made from the flour (Salazar et al., 2014; Villada et al., 2017). The calcium content for the samples were also found to be in an increasing order from 20.24 mg/g for 0.5% lime concentration to 28.09 mg/g for 2.0% lime concentration. The calcium content for untreated samples were a low value of 16.08 mg/g. The increase in the calcium content for the treated sample were a result of Ca⁺ ions being absorbed into the grain during the lime cooking and steeping (Owusu-Kwarteng and Akabanda, 2013).

The influence of nixtamalization on the protein content was seen with a decrease in the protein content due to starch gelatinization (Obadina et al., 2016). The protein content of the untreated grain was estimated to be 9.73 g/100g and the protein in the treated samples ranged from 7.29 - 9.1 g/100g. The decrease in the protein content is a result of heat treatment of the grain during alkaline cooking which resulted in the changes in the protein structure thereby improving protein digestibility and making it easy for absorption (Gomez et al., 1989). The decrease in the protein content can also be due to high temperature at the time of frying. Further studies need to be done upon the starch structure during nixtamalization.

The fat content of the crisp fried dough wafers is responsible for the mouthfeel of the product and also has an effect on the sensorial characteristic of the product. An increase in the total fat content of the product were observed. The untreated sample shows a lower total fat content of 30.75 g/100g. The total fat content of the treated sample ranged from 31.59 to 36.81 g/100g. Thus there was an increase in the total fat content of the crisp fried dough wafer and this can be due to rapid loss of moisture during cooking. Xu and Kerr, 2012; Salazar et al., 2014 reported similar findings on maize.

Table 1 Proximate composition of crisp fried dough wafers made with nixtamalized pearl millet

| Concentration of Lime (%) | Moisture content (%) | Ash Content (%) | Calcium Content (mg/g) | Protein Content (g/100g) | Total Fat Content (g/100g) |
|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|----------------------------|
| 0% | 3.18 ± 0.04 ^a | 1.56 ± 0.06 ^a | 16.08 ± 0.05 ^a | 9.73 ± 0.05 | 30.7 ± 0.05 ^a |
| 0.50% | 3.02 ± 0.03 ^b | 1.86 ± 0.07 ^b | 20.24 ± 0.09 ^b | 7.32 ± 0.04 ^b | 31.59 ± 0.06 ^b |
| 1.00% | 3.07 ± 0.05 ^c | 2.1 ± 0.02 ^c | 21.72 ± 0.07 ^c | 9.1 ± 0.08 ^c | 33.46 ± 0.06 ^c |
| 1.50% | 2.95 ± 0.02 ^d | 2.64 ± 0.03 ^d | 24.13 ± 0.06 ^d | 8.11 ± 0.08 ^d | 35.36 ± 0.07 ^d |
| 2.00% | 3.04 ± 0.09 ^c | 2.86 ± 0.08 ^e | 28.09 ± 0.04 ^e | 7.29 ± 0.09 ^e | 36.81 ± 0.07 ^e |

*Different small letters following the values in same column indicate differences for each concentration of lime (P < 0.05).

Influence on Antioxidant Scavenging Activity of DPPH⁺ Radical

The antioxidant activities of the methanolic extracts of the product was assayed against a basic free radical known as 2,2-diphenyl-1-picrylhydrazyl (DPPH⁺). The antioxidant scavenging activity of DPPH was evaluated using ascorbic acid as the standard antioxidant. In accordance with **Table 2**, there is an increase in the antioxidant scavenging activity of the nixtamalized samples. The increase in the activity can be seen from 0.5% lime treated sample exhibiting the antioxidant activity of 59.79% whereas that of 2.0% lime treated sample is 61.99%. The highest antioxidant activity is seen from 1.5% of lime treated sample which is 62.89%. The phenolic and tannin content in the grains are responsible for the antioxidant activity exhibited of the grain (**Gaytán-Martínez et al., 2017**). These phenolic compounds are usually found in a bound form in the grains where the form esterified bonds with the cell wall components of the whole cereal grains. Many physical and chemical processes such as alkaline hydrolysis, extrusion cooking, etc has been effective in liberating these bonds and turning the phenolics into a free form which in turn makes them available for the antioxidant activity (**Acosta-Estrada et al., 2014**). The release of these antioxidant is greatly influenced by higher level of alkaline agent and temperature used during cooking (**Oufnac et al., 2007**).

Effect on Total Phenolic Content

According to **Table 2**, significant variation in the total phenolic content can be seen. Decrease in the total phenolic content of the nixtamalized crisp fried dough wafers was observed. The total phenolic content of the sample made from non-nixtamalized pearl millet was found to be 42.51 µg GAE/g whereas the total phenolic content for the treated sample ranged from 6.64 to 40.04µg GAE/g. At lime concentration of 0.5% the total phenolic content was 40.04µg GAE/g. The

decrease in the content was observed with an increase in the lime concentration. This effect is because of the lime concentration and the steeping time (**Gaytán-Martínez et al., 2017**). Pearl millet consists of phenolic compounds situated in the pericarp, which gets removed from the grain at the time of cooking and steeping. This leads to the reduction of total phenols in the treated grains (**Adetunji et al., 2015**). **Rajeswari et al. (2015)** observed reduction in free, bound and total phenolic content in foxtail millet after treatment with alkaline solution. It was found in this study that these reductions increases with alkaline concentration. In the alkaline environment some molecular structural changes of the phenolic compounds are also observed leading to disruption of these compounds

Effect on Tannin Content

Tannin content of the nixtamalized crisp fried dough wafers decreases as the lime concentration is increased (**Table 2**). The tannins in the untreated sample is higher 74 µg CAE/g than that in the nixtamalized samples. The tannins in the nixtamalized sample range from 5.08 µg CAE/g to 3.004 µg CAE/g. The process was successful in lowering the tannin content of the final finished product. Tannins in the cereal are polyphenolic in nature and they exhibit antinutritional properties, but many tannins are anti-nutritional factors. They are known to form complex bonds with divalent cations and proteins thereby making them insoluble for digestion. Hence they reduce the availability of divalent cations and proteins in the body by making them escape the intestinal absorption and are excreted (**Lestienne et al., 2005**). This results in deficiencies. In nixtamalization, the use of high temperature at the time of cooking leads to the removal of pericarp and saturates the tannins content of the treated cereal (**Gaytán-Martínez et al., 2017**). **Ocheme, Oludamilola and Gladys (2010)** have reported similar studies in which they have observed a significant reduction in tannin content with lime concentration.

Table 2 Effect of nixtamalization on antioxidant scavenging activity of DPPH⁺ radical, total phenolic content, tannin content of crisp fried dough wafers

| Parameter | Concentration of Lime (%) | | | | |
|-------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | 0 % | 0.50% | 1.00% | 1.50% | 2.00% |
| Antioxidant Scavenging Activity (%) | 51.04 ± 0.05 ^a | 59.79 ± 0.06 ^b | 60.3 ± 0.09 ^c | 62.89 ± 0.08 ^d | 61.99 ± 0.08 ^c |
| Total Phenolic Content (µg GAE/g) | 42.51 ± 0.08 ^a | 40.04 ± 0.06 ^b | 28.63 ± 0.06 ^c | 26.22 ± 0.09 ^d | 6.64 ± 0.08 ^e |
| Tannin Content (µg CAE/g) | 7.44 ± 0.06 ^a | 5.08 ± 0.07 ^b | 4.27 ± 0.09 ^c | 3.72 ± 0.07 ^d | 3.05 ± 0.04 ^e |

*Different small letters following the values in same row indicate differences for each attribute (P < 0.05).

Influence on Colour and Texture

The effect of colour was determined by monitoring the changes in L*, a* and b* values of the crisp fried dough wafers. Colour is considered as one of the most important parameter which helps in determining the acceptability of fried products. The L* value represents darkness when low and lightness when high. As observed in **Table 3**, L* value of the product was lower which gave the product a darker hue. The a* and b* values of the product was lower than the untreated sample signifying a change in the colour. There are various factors that have an effect on the colour of the final product. These factors include cooking time, temperature, mineral content, etc. **Lovera et al., (2014)** reported similar finding that increase in the calcium content led to a darker finished product.

The texture of the food is one of the important parameter that affects the overall acceptability of the food product. It is characterised by hardness, adhesiveness, fracturability, cohesiveness, springiness, chewiness and gumminess. Hardness of the product can be defined as resistance of a material to deformation, indentation or penetration by means such as abrasion, drilling, impact, scratching or wear. It

can be affected by various factors such as cooking time, cooking temperature, moisture absorption to name a few. Increase in the lime concentration of the crisp fried dough wafers, increases the hardness of the product. Properties such as fracturability and springiness also increases with the increase in the lime concentration. The increase in the fracturability and springiness is due to the increase in the firmness or rigidity of the product. Similar findings were reported by **Rana et al., (2018)** for jackfruit. The increase in the rigidity of the product can also be due to the increase in the calcium content of the nixtamalized grains (**Lovera et al., 2014**). Colour and texture were greatly improved with nixtamalization, perhaps due to the removal of extraneous pericarp materials during washing after lime cooking and soaking. Hydration of the millet grains during the alkaline cooking process leads to calcium ions incorporation into the millet grain. In these phases of cooking and steeping, hydration and partial gelling of the grain starches occur simultaneously, along with the diffusion of calcium ions, which determine the physicochemical and textural properties of the final product (**Owusu-Kwarteng and Akabanda, 2013**).

Table 3 Effect of nixtamalization on the L*, a*, b* values and texture of crisp fried dough wafers

| Properties | Concentration of Lime % | | | | |
|--------------------|---------------------------|---------------------------|---------------------------|----------------|----------------|
| | 0% | 0.50% | 1.00% | 1.50% | 2.00% |
| L* | 46.03 ± 2.43 ^a | 34.51 ± 2.24 ^b | 40.54 ± 2.48 ^c | 37.72 ± 3.08 | 40.15 ± 1.08 |
| a* | 10.58 ± 0.86 ^a | 4.9 ± 0.32 ^b | 5.39 ± 1.00 ^c | 5.1 ± 0.46 | 4.92 ± 0.78 |
| b* | 27.11 ± 2.58 ^a | 17.45 ± 1.92 ^b | 27.72 ± 0.66 ^c | 20.44 ± 1.88 | 21.55 ± 1.38 |
| Hardness (N) | 2.97 ± 0.05 ^a | 5.67 ± 0.07 ^b | 5.68 ± 0.03 ^c | 8.44 ± 0.42 | 9.4 ± 0.25 |
| Adhesiveness (J) | 0 ^a | 0 ^a | 0 ^a | 0 ^a | 0 ^a |
| Fracturability (N) | 3.53 ± 0.35 ^a | 5.64 ± 0.1 ^b | 5.65 ± 0.06 ^c | 3.08 ± 0.12 | 8.68 ± 0.11 |
| Cohesiveness | 0 ^a | 0.55 ± 0.06 ^b | 0.13 ± 0.06 ^c | 0.73 ± 0.15 | 0.53 ± 0.09 |
| Springiness (mm) | 0 ^a | 1.35 ± 0.09 ^b | 1.18 ± 0.02 ^c | 3.15 ± 0.05 | 3.69 ± 0.04 |
| Gumminess (N) | 0 ^a | 3.48 ± 0.03 ^b | 0.52 ± 0.06 ^c | 2.6 ± 0.2 | 5.77 ± 0.1 |
| Chewiness (J) | 0 ^a | 0 ^a | 0 ^a | 0 ^a | 0 ^a |

*Different small letters following the values in same row indicate differences for each attribute (P < 0.05).

Detection of Acrylamide using FTIR

An unsaturated amide commonly known as acrylamide is found in various thermally processed foods. At a high amount, it is considered as a neurotoxin and a potent carcinogenic. Foods containing high content of reducing sugars such as glucose and proteins specially rich in asparagine, amino acid, when heated at high

temperature more than 170°C produce acrylamide (**Gertz and Klostermann, 2002; Yadav et al., 2018**). It consists of -NO group as the functional side chain. The FTIR spectrum for asymmetric N-O is from 1500 – 1600 cm⁻¹ whereas for symmetric N-O the stretch is from 1300 – 1400 cm⁻¹. Also the FTIR stretch for N-H is found to be from 3350 – 3500 cm⁻¹ (**Pramanik et al., 2015**). It can be observed in Figure 1a, 1c and 1d that all the interferograms showed a peak for either -NO

group or for the N-H stretch. Since none of the interferograms showed peaks for both the stretch it can be concluded that the peaks observed are not because of acrylamide but are due to some protein moiety. In Figure 1b and 1e no peaks were observed for either -NO group or for N-H stretch, this can be due to protein denaturation in the treated samples. Further studies should be done to know better about the protein denaturation. The absence of acrylamide in the treated samples can be due to the increased mineral content of the product, since calcium content of the flour reduces the formation of Schiff's base responsible for the formation of acrylamide. Similar findings were reported by (Salazar et al., 2014) for maize tortilla. These authors observed a decrease of 52 and 36% in acrylamide content in tortilla chips, when a treatment of nixtamalization at lime concentrations of 1.5 and 2.0 g/100 g respectively was given to corn flour.

CONCLUSION

Based on the study it can be concluded that the nixtamalization treatment was efficient in increasing the ash, calcium content of the product. Protein gelatinization was seen with the reduction in the protein content. Since pearl millet is high in fat, the total fat content of the product showed an increase. There was decrease in the tannin and polyphenolic content of the sample which indicated the reduction in the antinutritional factor of the millet altogether. The increase in antioxidant scavenging activity was associated with the decrease of the antinutritional factors viz., phytic acid in the case of pearl millet. The result obtained for antioxidant scavenging activity, polyphenols and tannins were interdependent. The treatment was done to decrease the antinutritional factor while retaining the maximum antioxidant activity. Considering all the parameters under analysis the product sample made with lime concentration of 1.5% showed the best result. It gave the best overall result and was more acceptable in terms of colour and texture. Thus it can be affirmed that nixtamalization can be used to increase the mineral content of the grains and which in turn helps in lowering the acrylamide of the final fried product. With nixtamalization these problems can be solved, and the bioavailability can be increased. Thus, the amount of condensed tannins in pearl millet can be significantly reduced with increase in the antioxidant activity of the grains. More studies should be conducted on the processing of cereal grains for reduction in their antinutritional property and retention of nutrients. In under developed countries pearl millet can be very effective in eradicating diseases caused due to low quality nutrition and thus the use of these underutilised grains should be highly promoted and consumed on a larger scale.

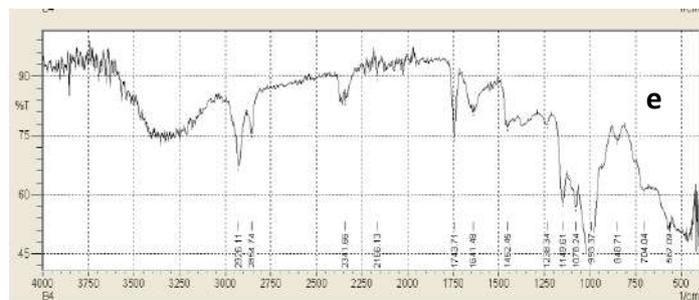
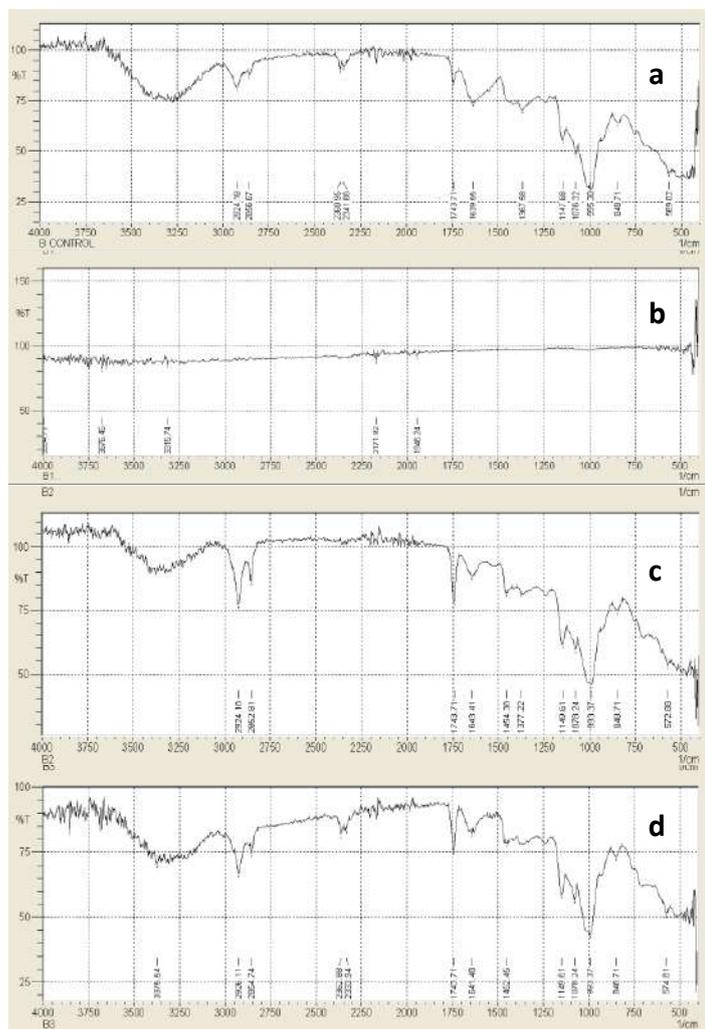


Figure 1 Effect of various a) 0% b) 0.5% c) 1% d) 1.5% and e) 2% lime concentrations on the acrylamide formation in crisp fried dough wafers

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Exploring the Cardiovascular Disease Risk Factor Perception and Barriers Faced among Working Women

Sir,

The United Nation Women has announced its theme for World International Women's Day as "Think equal, build smart, innovate for change" reemphasizing its earlier 2017 theme "Women in the Changing World of Work: Planet 50-50 by 2030" all emphasizing towards the gender equality and empowerment of women by advancing equal job opportunities for women.^[1] In India, nearly 31.2% of the labor force participation is provided by the females and working women are vulnerable to neglect their health due to family and work stress.^[2]

Cardiovascular disease (CVD) has become the leading cause of mortality in India among women.^[2] The GO RED for WOMEN campaign was started by the American Heart Association in view that women are more likely to underdiagnosed and undertreated than men and hence aims to encourage women to take care of their heart.^[3] A study done by Aswin K *et. al* to assess the cardiovascular disease risk factor profiling among group C employees of JIPMER Puducherry found that nearly 18.7% of the women employee were at the risk of developing CVD.^[4] The survey Visualizing the Heart Diseases in Indian Women (VEDNA) conducted among cardiologists by Heal Foundation Bangalore revealed that 83% of doctors believe that Indian women are ignorant about heart disease and they have also noted increased heart disease among the working population.^[5]

Majority of the CVD are caused by modifiable behavioral risk factors such as unhealthy diet, lack of physical activity, stress, overweight, raised blood pressure and blood sugar, consumption of tobacco and alcohol. In today's sedentary lifestyle scenario, the majority of working women are stressed in creating a work-life balance and are more prone to behavioral risk factors. The window of opportunity for secondary prevention exists among the family members of suspected CAD patients. Creating awareness about the risk factors of CAD amongst patients and their family members and counseling for modification may help in reducing the burden.^[6] Hence, researches need to be planned to explore the perception of risk factors for CVDs among working

women. The use of simple markers like Resting Heart rate (RHR) for the assessment of severity and complexity of CAD will benefit the general public especially working women in the future.^[7]

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Conflicts of interest

There are no conflicts of interest.

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Screening for respiratory morbidities and obstructive lung function among municipal waste handlers in Puducherry: A community-based cross-sectional study

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ABSTRACT

Background: Waste management is a societal problem because of its environmental impact and public health implications. Solid waste handlers have a high incidence of occupational health issues, with respiratory morbidities being the most common. **Aim:** To assess the prevalence of respiratory morbidities, obstructive lung pattern and its associated factors among municipal solid waste management workers in Puducherry. **Materials and Methods:** This community-based cross-sectional study was carried out in May and June 2018 among 264 solid waste management workers selected by simple random sampling. They underwent a semi-structured interview schedule capturing their sociodemographic characteristics, work profile, presence of respiratory symptoms, and morbidities followed by lung function tests using a portable spirometer. The data was entered using Epidata entry client and analyzed using SPSS (v16). **Results:** The mean age of the workers was 47.1 (± 8.87) years. The majority were females (85.6%), working as waste collectors (86%) on day duty (73.5%). More than two in five workers had either respiratory morbidity (42.8%) or obstructive lung pattern (44%). Higher age, occupation as waste collector, night shift duty, not using face mask on duty, and not receiving training on waste handling were the factors significantly associated with the respiratory morbidities and obstructive lung function. **Conclusion:** The prevalence of respiratory morbidities and obstructive lung disease were high among municipal solid waste handlers. Measures are needed to improve the work environment of waste handlers by ensuring the availability of protective gears and adequate training on work handling based on ergonomic principles.

Keywords: Lung function, occupational exposure, respiratory morbidities, solid waste, waste workers

Introduction

Municipal solid waste is generated because of economic production and consumption by individuals, commercial enterprises, institutions, markets, and businesses.^[1] Solid waste

management includes trash collection, separating recyclables, and processing commercial and industrial waste. Population and economic expansion have increased solid waste in both urban and rural regions.^[2] Waste management poses hazards at every stage: collection, transportation, recycling, and disposal, posing a growing environmental and public health problem.^[1,3]

In developing nations like India, the collected waste is seldom kept in covered containers and is instead deposited directly on the ground, where it must be either shoveled or cleaned up by hand. Workers, therefore, have significant direct contact with

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solid waste than their counterparts in high-income countries, who predominantly handle sealed plastic bags and covered dustbins.^[4] Furthermore, employees in solid waste management have reported terrible working conditions, with no access to drinking water or sanitation.^[5] All of these variables enhance worker exposure to infections, hazardous substances, and chemicals.^[6] Due to numerous risk factors, municipal solid waste employees have high rates of occupational health issues, especially respiratory morbidities such as chronic bronchitis and bronchial asthma.^[7] Additionally, bioaerosols from decomposing garbage cause irritation in the airways, causing respiratory issues.^[8]

Studies on the short- and long-term impacts of garbage exposure on public health have concentrated on finding any links between living near a landfill and unfavorable health effects.^[9] The danger presented to those directly engaged in garbage handling, particularly the impact on the respiratory system, has received less attention.^[4] Moreover, the screening for respiratory illness in these high-risk populations has not been part of any routine health services in primary care. Hence, the current study was an attempt to screen the municipal solid waste management workers in Puducherry for respiratory morbidities and obstructive lung function.

Objectives

1. To estimate the prevalence of respiratory morbidities among municipal solid waste management workers in Puducherry.
2. To determine the sociodemographic and work-related factors influencing respiratory morbidities and
3. To assess their obstructive lung function using spirometry.

Materials and Methods

The current research was a cross-sectional community-based analytical study conducted in the Puducherry district from May to June 2018. The district is one of four in the union territory of Puducherry in southern India, and it is made up of two administrative municipalities and five commune panchayats. Solid waste management workers who have been employed by Puducherry municipalities and communes for at least one year were included, while those who were absent on the day of the evaluation were excluded. A minimum sample size of 255 solid waste management workers were required considering the prevalence of respiratory morbidities among solid waste management workers as 21%,^[5] absolute precision of 5%, and 95% confidence interval. The required number of individuals were selected from the total list of eligible solid waste management workers working under Puducherry district by simple random sampling. Computer-generated random numbers were used.

Among the selected eligible solid waste workers, a semi-structured interview schedule was done capturing their sociodemographic characteristics and work-related information. The respiratory symptoms and morbidities were assessed using an adapted

version of the validated Medical Research Council Respiratory Questionnaire^[10] developed by Centre for Disease Control and Prevention (CDC), which screened for eight respiratory/cardiovascular illnesses and four respiratory symptoms in the last 3 months.

The interview was followed by respiratory assessment by trained physicians. Lung function tests were performed using a portable electronic spirometer following the American Thoracic Society (ATS) guidelines. All tests were performed by the same trained health care professional. The most important aspects of spirometry are the forced vital capacity (FVC) and the forced expiratory volume (FEV1) in one second. Spirometric airflow limitations are defined according to the Tiffenau Index as (FEV1/FVC) <70% or an FEV1 <80% of predicted values.^[11]

Permissions from Puducherry Municipality and informed consent of the workers were obtained. The workers were surveyed and tested at their agreed convenient time before the start of their shift. The information of the workers was kept anonymous from the stage of data collection. Institutional Human Ethical committee approval was obtained before starting the study.

The data collected was entered twice in Epidata Entry client (v4.2) to check for data entry errors and analyzed using SPSS (v16). The baseline sociodemographic characteristics and lung function of the participants were summarized. Pearson Chi-square or Fisher's exact tests were used to identify the various sociodemographic and work characteristics associated with the presence of respiratory illness and obstructive lung function among the solid waste management workers. The output was expressed as an odds ratio. To identify the predictors of respiratory illness and obstructive lung function, binomial logistic regression was used and expressed as an adjusted odds ratio. Statistical significance was set at *P* value less than 0.05.

Results

Sociodemographic and working condition characteristics

A total of 264 workers participated in this study. The mean age of the workers was 47.1 (± 8.87) years. The majority were females (85.6%), illiterate (65.9%), and belonging to lower middle class (45.8%) when classified according to modified BG Prasad's classification. Most of them were street sweepers or waste collectors (86%), working in day shifts (73.5%) and had more than 5 years' work experience [Table 1].

Occupational safety and behavioral factors

More than half of the workers used face mask (54.5%) and hand gloves (58.3%) regularly during their work shift. The majority did not receive any training on either waste handling (81.8%) or occupational safety (82.2%). Around one-tenth (10.6%) consumed alcohol, while nearly one-fourth (23.1%) consumed either one form of tobacco [Table 2].

Past respiratory symptoms and respiratory illness

Table 1: Sociodemographic and working condition characteristics of solid waste management workers in Puducherry (n=264)

| Variables | Summary Statistics |
|----------------------------------|--------------------|
| Age of Respondents, mean (±SD) | 47.1 (±8.87) years |
| Gender, n (%) | |
| Male | 38 (14.4%) |
| Female | 226 (85.6%) |
| Educational Qualification, n (%) | |
| Illiterate | 174 (65.9%) |
| Primary School | 55 (20.8%) |
| Secondary School | 22 (8.3%) |
| High School and above | 13 (4.9%) |
| Socioeconomic status, n (%) | |
| Upper class | 1 (0.4%) |
| Upper middle class | 26 (9.8%) |
| Middle class | 109 (41.3%) |
| Lower middle class | 121 (45.8%) |
| Lower class | 7 (2.7%) |
| Type of work, n (%) | |
| Street sweepers/waste collector | 227 (86%) |
| Lorry Drivers/Office staffs | 37 (14%) |
| Nature of work shift, n (%) | |
| Day | 194 (73.5%) |
| Night | 70 (26.5%) |
| Work experience, n (%) | |
| ≤5 years | 170 (64.4%) |
| >5 years | 94 (35.6%) |

Table 2: Utilization of personal protective equipment and behavioral status of solid waste management workers in Puducherry (n=264)

| Variables | Summary Statistics |
|--|--------------------|
| Use of face mask on duty, n (%) | |
| All the time | 144 (54.5%) |
| Some time | 61 (23.1%) |
| No | 59 (22.3%) |
| Use of hand gloves on duty, n (%) | |
| All the time | 154 (58.3%) |
| Some time | 52 (19.7%) |
| No | 58 (22%) |
| Occupational safety training done, n (%) | |
| Yes | 47 (17.8%) |
| No | 217 (82.2%) |
| Waste handling training done, n (%) | |
| Yes | 48 (18.2%) |
| No | 216 (81.8%) |
| Alcohol consumption, n (%) | |
| Yes | 28 (10.6%) |
| No | 236 (89.4%) |
| Tobacco consumption, n (%) | |
| Smoke form | 18 (6.8%) |
| Smokeless form | 43 (16.3%) |
| No | 203 (76.9%) |

The prevalence of phlegm, cough, breathlessness, and wheeze among the workers were 31.4%, 29.5%, 22.2%, and 17.1%, respectively. The overall prevalence of any one respiratory illness is 42.8% among the workers. Around 7.7% and 6.3% of the workers were known cases of bronchial asthma and chronic bronchitis, respectively. About 1.5% and 2.7% had history of tuberculosis and pneumonia [Figure 1].

Predictors of respiratory symptoms

Bivariate analysis revealed that higher age, occupation as street sweeper or waste collector, night shift duty, not using face mask on duty, and not receiving training on waste handling were the factors significantly associated with the presence of respiratory symptoms among the workers. Multivariate analysis revealed similar results as bivariate analysis except for waste handling training [Table 3].

Lung function and its predictors

The predicted lung function measured by spirometry among solid waste management workers is summarized in Table 4. The FEV₁/FVC ratio when categorized according to the American Thoracic Society (ATS) guidelines revealed that around 44% of the workers had obstructive lung pattern. Among the variables measured, higher age, female gender, occupation as street sweeper or waste collector, night shift duty, more than 5 years' work experience, not using face mask on duty, and not receiving training on waste handling were the factors significantly associated with obstructive lung pattern among solid waste management workers in Puducherry. Multivariate analysis revealed similar results as bivariate analysis except for waste handling training [Table 5].

Discussion

Most of the workers were in the age group of 35–50 years, illiterate, and belonging to lower middle class (45.8%) and were occupied as street sweepers or waste collectors. The baseline characteristics were similar compared to the study by Salve among waste workers in Mumbai where a majority of workers working as waste collectors and street sweepers were nonliterate, and two-third of them belonged to the scheduled caste category.^[12]

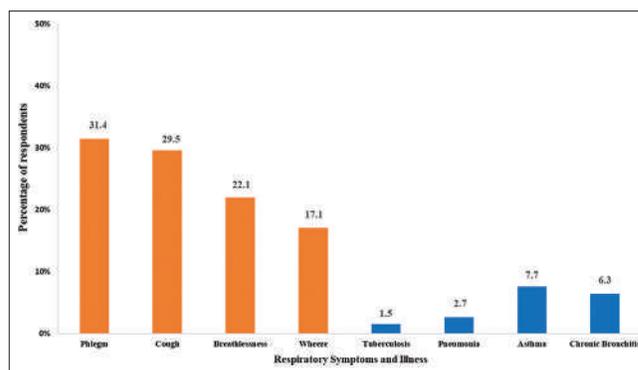


Figure 1: Distribution of past respiratory symptoms and respiratory illness among solid waste management workers in Puducherry (N = 264)

Table 3: Factors associated with respiratory symptoms among solid waste management workers in Puducherry (n=26)

| Variables | Total, n | Respiratory Illness present, n (%) | Odds ratio (95% CI) [†] | Adjusted odds ratio (95% CI) [‡] |
|------------------------------|----------|------------------------------------|----------------------------------|---|
| Age category | | | | |
| 21-35 years | 75 | 23 (30.6%) | Ref | Ref |
| 35-50 years | 105 | 53 (50.4%)* | 2.31 (1.24-4.29)* | 2.22 (1.15-4.09)* |
| >50 years | 84 | 51 (60.7%)* | 3.49 (1.81-6.75)* | 3.11 (1.69-6.44)* |
| Gender | | | | |
| Male | 38 | 15 (39.5%) | Ref | Ref |
| Female | 226 | 98 (43.4%) | 1.17 (0.58-2.37) | 1.09 (0.55-2.31) |
| Educational Qualification | | | | |
| Illiterate | 174 | 74 (42.5%) | 1.18 (0.37-3.77) | 1.09 (0.33-3.67) |
| Primary School | 55 | 26 (47.3%)* | 2.33 (0.73-7.43) | 2.31 (0.71-7.35) |
| Secondary School | 22 | 8 (36.4%)* | 1.49 (0.39-5.72) | 1.41 (0.36-5.68) |
| High School & above | 13 | 5 (38.5%) | Ref | Ref |
| Socioeconomic status | | | | |
| Upper and Upper middle | 26 | 9 (34.6%) | Ref | Ref |
| Middle | 109 | 45 (41.3%)* | 1.41 (0.58-3.41) | 1.29 (0.55-3.30) |
| Lower middle | 121 | 55 (45.5%)* | 1.67 (0.69-4.01) | 1.56 (0.65-3.93) |
| Lower class | 7 | 4 (57.1%)* | 2.67 (0.49-14.56) | 2.57 (0.45-14.46) |
| Type of work | | | | |
| Waste collector/sweepers | 227 | 107 (47.1%)* | 4.61 (1.85-11.47)* | 4.38 (1.72-11.2)* |
| Drivers/Office staffs | 37 | 6 (16.2%)* | Ref | Ref |
| Nature of work shift | | | | |
| Day | 194 | 68 (35.1%)* | Ref | Ref |
| Night | 70 | 45 (64.3%)* | 3.34 (1.88-5.90)* | 3.20 (1.75-5.74)* |
| Work experience | | | | |
| ≤5 years | 170 | 78 (45.9%)* | Ref | Ref |
| >5 years | 94 | 35 (37.2%)* | 0.70 (0.42-1.17) | 0.69 (0.41-1.15) |
| Use of face mask on duty | | | | |
| Yes | 205 | 71 (34.6%)* | Ref | Ref |
| No | 59 | 42 (71.2%)* | 4.66 (2.48-8.78)* | 4.50 (2.45-8.72)* |
| Occupational safety training | | | | |
| Yes | 47 | 24 (51.1%)* | Ref | Ref |
| No | 217 | 89 (41%)* | 0.67 (0.35-1.25) | 0.62 (0.33-1.23) |
| Waste handling training done | | | | |
| Yes | 48 | 13 (39.8%)* | Ref | Ref |
| No | 216 | 100 (56.3%)* | 2.32 (1.16-4.63)* | 2.20 (0.98-4.33) |
| Smoking | | | | |
| Yes | 47 | 34 (71.1%)* | 4.57 (2.28-9.17)* | 4.29 (2.21-9.03)* |
| No | 217 | 79 (36%)* | Ref | Ref |

*P<0.05; †Crude odds ratio by Chi-square test; ‡Adjusted odds ratio by Binomial logistic regression

Table 4: Percentage of predicted lung function measured by spirometry among solid waste management workers in Puducherry (n=264)

| Lung function parameters* | Summary Statistics, mean (±SD) |
|----------------------------------|--------------------------------|
| Vital capacity (VC) | 74.5 (±15.9) |
| Forced vital capacity (FVC) | 58.9 (±12.7) |
| Forced Expiratory Volume1 (FEV1) | 67.6 (±15.5) |
| FEV ₁ /FVC ratio | 69.9 (±14) |

*Measured using portable spirometer

A study conducted by Nagaraj *et al.*^[13] in Bangalore on street sweepers found maximum sweepers to be illiterate.

Traditionally, waste collection or cleaning public areas is considered a poor person's line of employment in India. Scheduled caste people were socially and economically

disadvantaged, and they were constantly forced to engage in the cruel practice of manual scavenging.^[14] Manual scavenging has been banned by law; however, individuals involved in cleaning duties have not been disengaged at this time. Their health is severely impacted by a lack of education, bad housing, and an inadequate nutrition.

Their immune system is further compromised by contextual vulnerabilities such as tobacco chewing, smoking, and frequent alcohol use, all of which contribute to additional health deterioration. The proportion of workers consuming tobacco and alcohol were 23% and 11%, respectively, in the present study. The proportion was low when compared to a similar population in Mumbai where more than 45% consumed either alcohol or tobacco.^[15] The decrease in substance abuse proportion may be attributed to the maximum participation of females in the

Table 5: Factors associated with obstructive lung pattern among solid waste management workers in Puducherry (n=264)

| Variables | Total, n | Obstructive lung function, n (%) | Odds ratio (95% CI) [†] | Adjusted odds ratio (95% CI) [‡] |
|-----------------------------------|----------|----------------------------------|----------------------------------|---|
| Age category | | | | |
| 21-35 years | 75 | 27 (36%) | Ref | Ref |
| 35-50 years | 105 | 40 (38.1%) | 1.09 (0.59-2.02) | 1.01 (0.55-1.99) |
| >50 years | 84 | 49 (58.3%) | 2.49 (1.31-4.72)* | 2.38 (1.25-4.62)* |
| Gender | | | | |
| Male | 38 | 8 (21.1%) | Ref | Ref |
| Female | 226 | 108 (47.8%) | 3.43 (1.51-7.81)* | 3.33 (1.43-7.7)* |
| Educational Qualification | | | | |
| Illiterate | 174 | 70 (40.2%) | 0.58 (0.19-1.79) | 0.61 (0.2-1.8) |
| Primary School | 55 | 29 (52.7%) | 0.96 (0.29-3.21) | 1.03 (0.33-3.35) |
| Secondary School | 22 | 10 (45.5%) | 0.71 (0.18-2.83) | 0.78 (0.22-2.91) |
| High School & above | 13 | 7 (53.8%) | Ref | Ref |
| Socioeconomic status | | | | |
| Upper and Upper middle | 26 | 0 | Ref | Ref |
| Middle | 109 | 10 (38.5%) | 1.41 (0.58-3.41) | 1.29 (0.55-3.30) |
| Lower middle | 121 | 41 (37.6%) | 1.67 (0.69-4.01) | 1.56 (0.65-3.93) |
| Lower class | 7 | 61 (50.4%) | 2.67 (0.49-14.56) | 2.57 (0.45-14.46) |
| Type of work | | 4 (57.1%) | | |
| Waste collector/sweepers | 227 | | 3.29 (1.44-7.51)* | 3.36 (1.48-7.55)* |
| Drivers/Office staffs | 37 | 108 (47.1%) | Ref | Ref |
| Nature of work shift | | 8 (16.2%) | | |
| Day | 194 | | Ref | Ref |
| Night | 70 | 77 (39.7%) | 1.91 (1.11-3.21)* | 1.86 (1.01-3.12)* |
| Work experience | | 39 (55.7%) | | |
| ≤5 years | 170 | | Ref | Ref |
| >5 years | 94 | 65 (38.2%) | 1.92 (1.15-3.19)* | 1.82 (0.98-3.09)* |
| Use of face mask on duty | | 51 (54.3%) | | |
| Yes | 205 | | Ref | Ref |
| No | 59 | 80 (39.1%) | 2.45 (1.35-4.43)* | 2.30 (1.21-4.19)* |
| Occupational safety training done | | 36 (61.1%) | | |
| Yes | 47 | | Ref | Ref |
| No | 217 | 27 (57.4%) | 0.52 (0.27-1.18) | 0.55 (0.29-1.23) |
| Waste handling training done | | 89 (41%) | | |
| Yes | 48 | | Ref | Ref |
| No | 216 | 28 (58.3%) | 0.51 (0.27-0.96)* | 0.56 (0.29-1.08) |
| Smoking | | 90 (41.6%) | | |
| Yes | 47 | 27 (36%) | 6.46 (3.05-13.7)* | 6.19 (2.95-13.36)* |
| No | 217 | 40 (38.1%) | Ref | Ref |

*P<0.05; †Crude odds ratio by Chi-square test; ‡Adjusted odds ratio by Binomial logistic regression

current study.

More than one-fifth of the workers did not use any personal protective equipment during their work shift contrary to the study in Ethiopia where 87.8% workers did not use any PPEs on duty. The majority of waste collectors were less adherent to health and safety measures.^[16] The main reasons for not using any protective clothing were their ignorance and poverty.^[17] The overall prevalence of acute respiratory symptoms among solid waste collectors in Puducherry was 42.8% with proportion of cough and wheeze being the highest. Similarly, the overall prevalence of respiratory symptoms among solid waste collectors in Ethiopia was 40.7% with a major proportion of cough and breathlessness.^[16] Jayakrishnan *et al.*^[4] found that 21% of waste management workers in Kerala had respiratory symptoms and illness.^[4] Chokhandre *et al.*^[18] reported that the prevalence

of respiratory symptoms was significantly higher among the waste-pickers (28%) compared to the control work group (15%). Particularly, the prevalence of dyspnea and chronic cough were found to be higher among the waste-pickers.

The occupation as solid waste handler, improper training, and inadequate use of personal protective equipment were significantly associated with respiratory morbidities and obstructive lung function. Similarly, Emiru *et al.*^[16] revealed that the absence of facemask on duty, sleeping disorder, and past illnesses were major contributing factors for respiratory symptoms to occur in Ethiopia. In Gambia, solid waste collectors who never used respiratory protective device had significantly higher prevalence of respiratory symptoms.^[19] In the current study, around 7.7% and 6.3% of the workers were known cases of bronchial asthma and chronic bronchitis, respectively. Sabde

and Zodpey in their study on street sweepers found upper respiratory tract infections (URTI) in 7.3%, followed by chronic bronchitis (5.9%) and bronchial asthma (1.8%).^[20]

Around 44% of the workers had obstructive lung pattern when assessed by spirometry in the study. Studies have reported higher respiratory morbidity among conservancy workers. Roopa *et al.*^[21] reported a higher prevalence of respiratory impairments (as established through pulmonary function tests) in conservancy workers working in solid waste management sector of Chennai, India. Hamid *et al.*^[22] evaluated the respiratory health of elementary workers and found that 46% of solid trash pickers had spirometry limitations. Van Kampen *et al.*^[23] found that, although compost workers' spirometry readings were within normal limits, their FVC percent predicted values were substantially lower than controls. All the previous studies had linked the obstructive pattern and high prevalence of respiratory symptoms to an inflammatory response of the airway caused by bioaerosol exposure and subsequent interaction with bacterial endotoxins and beta-glucans. On the contrary, few studies had found no significant reduction in the lung function parameters of the waste management workers on comparison with different control groups.^[24,25]

A limitation of this study is that a temporal relationship cannot be determined due to the cross-sectional design of our study. A further limitation is that data pertaining to specific bioaerosol, or chemical exposures were not available in this study. The lung function measurements were made on-site using a portable spirometer; hence, an objective assessment of static lung volumes was not possible. The diagnosis of obstruction was based on low FVC values, as total lung capacity could not be calculated. Because a specific exposure assessment and air pollution measurements were not part of this study, there is some uncertainty over the generalizability of our results.

It is critical that these waste management employees be covered by an occupational health-monitoring programmer that keeps them under frequent observation. Primary care physicians could be sensitized to these kinds of occupational hazards and could be trained to screen for respiratory morbidities routinely as part of the health profile. Additionally, the use of the hand-held spirometer could be a feasible option to detect obstructive lung function early at the primary care level. Longitudinal studies may be designed using this study's findings to evaluate chronic or permanent functional decline. Because epidemiological data from this sector is scarce, medical and occupation health institutes should be encouraged to research the health of conservancy employees. Aside from this, an environmentally sound garbage management system is needed.

Summary

The study found that two in five workers had either respiratory morbidity or obstructive lung pattern. Specific demographic factors such as occupation as solid waste handler, improper

training, and inadequate use of personal protective equipment were significantly associated with respiratory morbidities and obstructive lung function. Those identified with obstructive lung pattern were referred to higher centers for further management. An emphasis is placed on screening solid waste management employees for respiratory illness. Baseline health evaluations of solid waste management employees can help develop monitoring systems. A periodic pulmonary function test is also required to evaluate fitness to use respirators.

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Ethical Committee approval

Approved by the Institutional Human Ethics Committee of Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth, Puducherry.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Epidemiological profile of Chronic Kidney Disease (CKD) in patients attending a tertiary care hospital

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Abstract

Problem: The global prevalence of Chronic Kidney Disease (CKD) is steadily increasing due to the rise in lifestyle related diseases like diabetes and hypertension. Overall the current prevalence of CKD worldwide is estimated to be 229 per million populations with more than 100,000 people entering renal replacement therapy every year. **Methodology:** The objective of this study is to understand the clinico-epidemiological profile of patients who are presenting with CKD for analysing the risk factors which will help to improve the quality of their life. **Findings:** In this study, 85 CKD patients attending a tertiary care hospital were included and a detailed history was obtained in order to study their clinico-epidemiological profile. On assessment, it was found that CKD was more prevalent in males (65.8%) and people of low socio-economic background (87%), more common in agriculture workers (70.5%), and majority were of the age group of 40-49 years (24.7%) Diabetes and hypertension were also found to be major risk factors with 44.8% having diabetes and 61.1% having hypertension. **Conclusion:** It is strongly recommended to implement more schemes to provide affordable treatment to people of low socio-economic background and agriculture workers as they form a major population of people affected with CKD.

Keywords: 1.CKD, 2.clinical, 3.epidemiological

Introduction

Chronic Kidney Disease (CKD) refers to a gradual and progressive loss of kidney function which leads to End Stage Renal Disease (ESRD) (Sathyan *et al.*, 2016; Haileamlak, 2018; Hallan *et al.*, 2006). It can also be described as an anatomical or pathological lesion of the kidney lasting for duration of 3 months or more (Singh *et al.*, 2013). CKD is one of the major non communicable diseases on the rise among the present generation and is a global health problem in view of increasing number of patients, high mortality and morbidity in addition to the increased cost of treatment (Jha *et al.*, 2013; Jurkovitz *et al.*, 2008).

The occurrence of CKD in an individual is attributed to various factors such as Diabetes mellitus, hypertension, hyperlipidemia and the consumption of diet with a high salt intake (Sathyan *et al.*, 2016). The clinical course of CKD, if left untreated and undiagnosed at an early stage, leads to end stage renal disease which can be treated only by dialysis or by transplantation both of which are high cost options of therapy and therefore not feasible for countries with a lower socio-economic status and it also poses a major burden on the physical, psychological and economic conditions of the patient (Santosh and Georgi, 2013; Agarwal *et al.*, 2005).

CKD can be classified into 5 stages based on renal function which is assessed with the help of GFR (Sathyan *et al.*, 2016). Glomerular Filtration Rate (GFR) which is the rate of blood flow through the kidney is generally accepted as the best overall index of kidney function (Singh *et al.*, 2013; Levey *et al.*, 2000). Stage 1 CKD is defined as a normal GFR above 90ml/ min/ 1.73m². Stage 2 CKD is defined as GFR in the range of 60-89ml/ min/ 1.73m². Stage 3 CKD is defined as GFR in the range between 30-59ml/ min/ 1.73m². Stage 4 CKD is defined as GFR in the range of 15-29ml/ min/ 1.73m². Stage 5 CKD is defined as a GFR below 15ml/ min/ 1.73m² which is consistent with ESRD (Sathyan *et al.*, 2016).

Etiological diagnosis of CKD includes chronic glomerulonephritis, diabetic nephropathy, ADPKD, obstructive uropathy etc (Santosh and Georgi, 2013). CKD is referred to as a silent killer as most individuals don't recognize the symptoms of CKD until the damage has been done (Veerappan and Abraham, 2013). Early management of CKD by early screening for CKD in addition to spreading awareness about the risk factors of CKD and their prevention along with the advantages of early detection of minor presenting symptoms like oliguria, microalbuminuria can go a long way in helping curb the steady rise of CKD in present days (Haileamlak, 2018).

Low socio-economic status is associated with a higher prevalence and risk of CKD because of the metabolic risk factor, lack of awareness about the disease and its presenting symptoms, lack of a balanced and healthy diet and the high cost of treatment which deter them from seeking treatment for their condition (Sathyan *et al.*, 2016; Selvavinayagam, 2018). Also, since the treatment for ESRD is mainly provided via higher healthcare centres, there is reduced access to these facilities for the people of a low socio-economic background by the financial burden and the transport facilities (Selvavinayagam, 2018; Volkova *et al.*, 2008).

This study is aimed at understanding the clinico-epidemiological profile of patients presenting with CKD such as the demographic details along with a detailed history in order to provide a better understanding of the disease and its risk factors and to facilitate early detection and treatment of the disease which will help to improve the quality of life.

Materials and Methods

Type of study: Prospective and Observational study

Study settings: Department of General Medicine in a tertiary care hospital

Study population: Patients with CKD who attended Medicine Outpatients and Inpatients Department

Period of study: The study was conducted for a period of 2 months (June to July 2019)

Sample size: Initially 80 cases were proposed based on the overall prevalence rate. But as there was a high prevalence rate in the particular locality, the number of cases evaluated and studied was increased to 85.

Ethical Consideration

The study was started after getting approval from the institutional ethical committee (Ref: 636/TSRMMCH&RC/ME-1/2019-IEC No: 004 dated 17.07.2019) and the details of the patient were collected only after obtaining informed consent.

Newly diagnosed cases of CKD attending a tertiary care hospital were included in the study. Patients who did not give consent, patients below 18 years of age and patients already undergoing dialysis and treated for CKD were excluded.

Procedure

A comprehensive effort was made to assess and understand the epidemiology of CKD. A detailed case study proforma was prepared in order to collect and evaluate the personal details of the patient including details such as age, economic background, occupation, social habits, source of water for daily use and food habits along with an elaborate history of the patient's past and present illness and also mentioned about the family history of CKD. Only after informing the patients about the objectives of the study, assuring confidentiality of the data and obtaining their consent the proforma details were collected. Questions were asked in patient's native language to facilitate better understanding and in order to obtain a clear history. Assessment of the individual was also supplemented with the help of clinical examination and lab investigations. Final statistics was done by means of a descriptive analysis of the data collected.

Variable Terms

Diabetes: Self-reported history of diabetes in patients was confirmed by checking the blood glucose values and a random blood sugar >200mg/dl and fasting blood sugar >126mg/dl was confirmed to be a diabetic. History of medication was also verified with the help of medical records.

Blood Pressure: The blood pressure of patients was measured with the help of a mercury sphygmomanometer. It was also confirmed by asking a history of medication and verifying medical records. Systolic BP >140mmHg and Diastolic BP >90mmHg was considered to be hypertensive.

Body Mass Index (BMI): The BMI of the patient was assessed using the standard BMI formula: Weight (kg) / Height x Height (m²). The parameters followed were: underweight (<18.5); Normal (18.5 to 24.9); Overweight (25 to 29.9) and Obese (>30.0).

Results

Among the 85 cases studied, 65.8% were found to be males and 34.2% were found to be females and the details were interpreted in figure 1. On analysing the data collected, it was found that CKD was highly prevalent among people belonging to the age group of 40 – 49 years with 24.7% followed by people who belong to the age group of 60 – 69 years with 21.1% .CKD was least prevalent among the age group of 20 – 29 years with no case being recorded.

Around 7% of the population hail from an urban area, 5.9% hail from a semi- urban area and the remaining 87.05% which form the majority, hail from a rural area (Figure 2).The occupational distributions of the study population are divided into two categories including Agriculture and Non-Agriculture workers. Out of the two categories CKD was more prevalent among agriculture workers (70.5%) compared to non-agriculture workers (29.5%) (Figure 3).

The study population was questioned about their food habits, in which the majority (90.5%) said they consumed a mixed diet of both vegetarian and non-vegetarian food while 9.5% consumed a vegetarian diet (Figure 4).The social habits of the study population namely alcohol consumption, smoking and tobacco chewing were studied and the result was depicted in table 2.

The BMI of the study population was assessed with the help of the standard BMI formula and integrated data was impregnated in table 3. In this population study, 44.8% presented with Diabetes and 55.2% did not have a history of Diabetes; meanwhile, while analysing the hypertension, 61.1% presented with hypertension (Figure 5).

While analysing other co-morbid status, only 6 had a history of bronchial asthma; only 4 cases had undergone treatment for pulmonary tuberculosis; 6 had thyroid disorder; 10 had coronary artery disease (CAD); 7 patients had history of renal stones and 10 patients have the family history of chronic kidney disease (CKD) (Table 4).

Discussion

On completion of the study, it was found that out of 85 cases 65.8 and 34.2% were males and females respectively which are in accordance with a previous study showed out of 333 patients 65 and 35% were males and females respectively. In concordance with the India CKD registry study, it was noted that 68 and 32% were males and females respectively which implies that hormonal influence could play a role in the development of CKD (Volkova *et al.*, 2008; Satyan *et al.*, 2016).

Majority of the study population were in the age group of 40-49 years which corresponds to the reference study in which majority of the people were in the age group of 41-60 years and the India CKD registry study which had a mean age of 48.3±16.6 years which indicates that the decline in GFR progresses with age (Rajapurkar *et al.*, 2012; Satyan *et al.*, 2016).

More and near 87% people were from a rural background in the present study while other studies reflected nearly 84% people were from a rural background which implicates that low socio-economic status is one of the major factors of CKD due to the lesser awareness about CKD leading to people presenting at a later stage and consequently leading to unaffordable cost of treatment (Rajapurkar *et al.*, 2012; Satyan *et al.*, 2016).

Around 90% consumed a mixed diet of both vegetarian and non-vegetarian food and 78% people consumed water from the municipal street taps which implicates that dietary factors could play a role in the progression of CKD (Khalil, 2005; Veerappan and Abraham, 2013). Nearly 70% of the study population were agriculture workers which once again indicate that low socio-economic status is one of the major demographic factors for the prevalence of CKD. It could also implicate the constant exposure to pesticides as one of the factors which play a role in the progression of CKD.

In the present study, 36.5% were found to be alcoholics whereas in the study conducted by the India CKD registry, out of 333 cases 7% were found to be alcoholics (Rajapurkar *et al.*, 2012). This can be attributed to the increase in the trend of consumption of alcohol which has largely negative effects on the body overall (Levey *et al.*, 2000). Nearly 22% had a history of smoking in the present study compared to 33% and 32% in the India CKD registry study (Rajapurkar *et al.*, 2012; Satyan *et al.*, 2016). This implicates that even though awareness has been spread regarding the ill effects of smoking it still remains a trend and contributes to the progression of diseases. Also depicted with 15.3% were found to have a history of tobacco chewing.

While analysing the diabetic status, more than 44% were found in the present study which is in accordance with the India CKD registry study which showed that 40.7% were found to have Diabetes making DM one of the major factors of CKD. This shows that early screening for microalbuminuria and proteinuria can help in the earlier detection of CKD (Prasad *et al.*, 2012). Nearly 61% were found to have hypertension in the current study compared to 84.6% in the Sathyan *et al.* study and 71.1% in the India CKD registry study (Rajapurkar *et al.*, 2012; Satyan *et al.*, 2016). This showed that hypertension is also one of the major risk factors of CKD. It could also be due to hypertensive nephrosclerosis.

The history of bronchial asthma and tuberculosis were found to be 5.9 and 4.8% respectively and had undergone treatment for it. In the SEEK (Screening and Early Evaluation of Kidney Diseases) study conducted in 2013, 3.1% were found to have Tuberculosis (Santosh and Georgi, 2013).

Around 7% were found to have thyroid disease. 11.8% had CAD (Coronary Artery Disease) in the present study compared to the study in which 50.15% had CAD (Satyan *et al.*, 2016). Nearly 8% had a history of renal stones in the current study compared to 5.3% in the SEEK study (Santosh and Georgi, 2013). Nearly 12% had a family history of CKD. Thus when compared to other similar studies it was found that Diabetes and hypertension are the major risk factors of CKD. Other factors include hailing from a rural background and the occupation at risk was found to be agriculture workers. Therefore targeted screening can lead to the prevention of the rising incidence of CKD.

Only 85 cases were studied so it was not possible to gain more data for a more precise and accurate analysis of the risk factors. Also the study was limited only to patients attending a particular tertiary care hospital therefore the geographical variations of the risk factors could not be analysed.

Therefore it is recommended that future studies include a wider range of study population so that an in-depth analysis can be made.

On studying the clinico-epidemiological profile of patients presenting with CKD, it reveals that Diabetes and hypertension continue to be major risk factors for CKD. Detection of urine microalbuminuria and proteinuria, especially in patients with DM, helps to identify patients at risk of kidney disease at an early stage.

It is strongly recommended to implement more schemes to provide affordable treatment to people of low socio-economic background and agriculture workers as they form a major population of people affected with CKD. Also initiation of nation-wide programs to spread awareness among the public and the healthcare professionals regarding the prevalence, symptoms, risk factors and the advantage of early detection and treatment of CKD could help in reducing the incidence of CKD and its progression to end stage renal disease.

Screening of high risk individuals those with hypertension, diabetes mellitus, cardiovascular diseases and other risk factors and educating them about the benefits of lifestyle modification, physical exercise and abstinence from social habits like alcohol consumption and smoking will retard the progression to ESRD (Anupama and Uma, 2014). Upgrading facilities to provide better treatment will also retard the progression of CKD into end stage renal disease.

This study has the wide recommendations of screening of the high risk group facilitates prevention of CKD and its progression to ESRD; spreading awareness of CKD and its risk factors among the public and the medical community via implementation of nation-wide programs; upgrading facilities for treatment and initiation of schemes to provide more affordable treatment for people of low socio-economic background and agriculture workers is advised and further performing an extensive study in the field of the co-factors bringing about an outcome of CKD needs further progressive introspection over prolonged periods of time taking into account various other intricate factors accounted for in the disease process.

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Table 1: Age distribution of study population

| Age (in years) | Number of cases (%) |
|----------------|---------------------|
| 30 – 39 | 11 (12.9) |
| 40 – 49 | 21 (24.7) |
| 50 – 59 | 17 (20) |
| 60 – 69 | 18 (21.1) |
| 70 - 79 | 11 (12.9) |
| >80 | 7 (8.4) |

Table 2: Social Habits of the study population

| Social habits | Yes | | No | |
|---------------------|--------------|------------|--------------|------------|
| | No. of cases | Percentage | No. of cases | Percentage |
| Alcohol consumption | 31 | 36.5 | 54 | 63.5 |
| Smoking | 19 | 22.4 | 66 | 77.6 |
| Tobacco Chewing | 13 | 15.3 | 72 | 84.7 |

Table 3: BMI distribution of the study population

| BMI variables | Number of cases (%) |
|---------------|---------------------|
| Underweight | 7 (8.2) |
| Normal | 63 (74.1) |
| Overweight | 13 (15.2) |
| Obese | 2 (2.5) |

Table 4: Co-morbid status of the CKD patients (n=85)

| Comorbid status | Number of cases | Percentage |
|-------------------------|-----------------|------------|
| Bronchial asthma | 6 | 7.1 |
| Pulmonary tuberculosis | 4 | 4.7 |
| Thyroid disorders | 6 | 7.1 |
| Coronary artery disease | 10 | 11.8 |
| Renal stones | 7 | 8.2 |
| Family history of CKD | 10 | 11.8 |

Figure 1: Gender distribution of study population

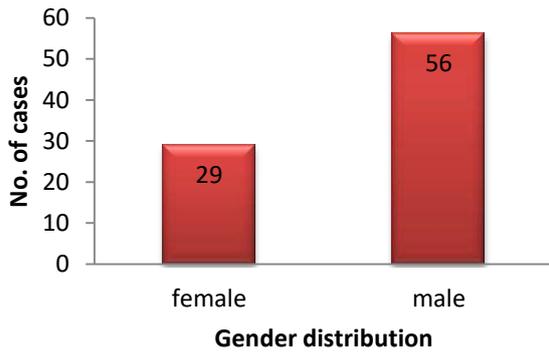


Figure 2: Distribution of Locality of study population

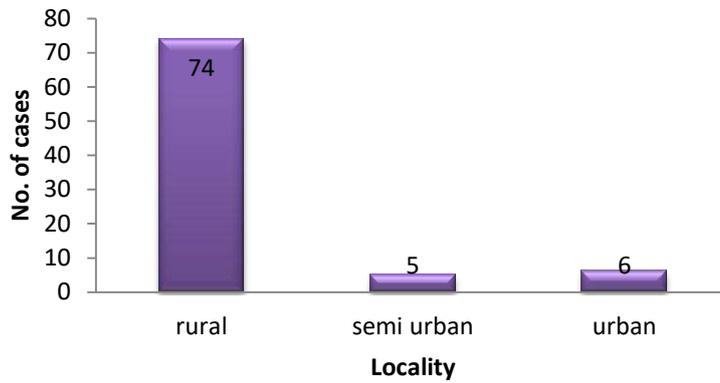


Figure 3: Occupational distribution of study population

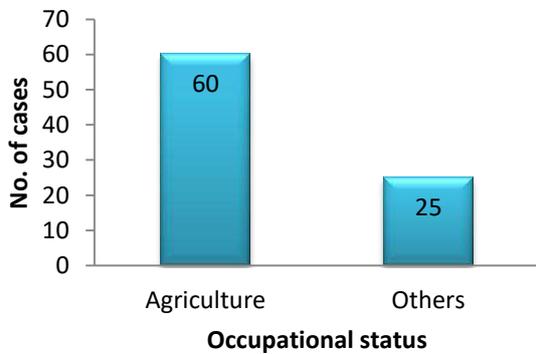


Figure 4: Food Habit of study population

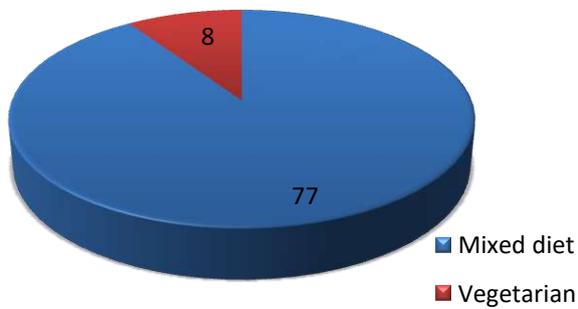
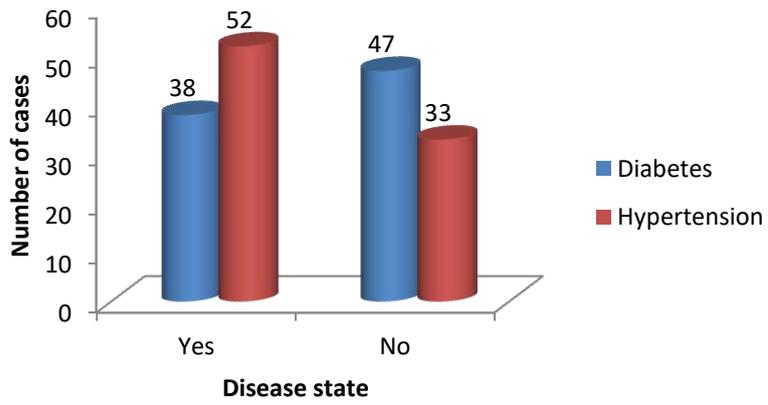


Figure 5: Prevalence of Diabetes and hypertension among cases included



Association of Nonalcoholic Fatty Liver Disease with Coronary Artery Disease in Type 2 Diabetes Mellitus: A Cross-Sectional Study from a Tertiary Care Medical College Hospital

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Abstract

Introduction: Nonalcoholic fatty liver disease (NAFLD) is a risk factor for increased morbidity, mortality, and cardiovascular disease. This study was done to assess the association of NAFLD with coronary artery disease (CAD) in type 2 diabetes mellitus. **Materials and Methods:** The study was done as a cross-sectional study in a tertiary care medical college hospital for 2 years among 218 adults patients of both sexes with type 2 diabetes mellitus. The study protocol was approved by the institutional ethics committee of the hospital. Age, sex, lifestyle, hypertension, personal history for smoking, and details of any previous CAD were recorded. Electrocardiogram (ECG) and ultrasonography of the abdomen were done. The association of NAFLD in type 2 diabetes mellitus patients with symptoms of angina according to modified rose and ECG changes using Minnesota codes was studied. Data collected were analyzed with Statistical Package for the Social Sciences (SPSS) version 20. **Results:** Of the total 218 diabetic patients, there were 92 (42.2%) were in the age group of 65–74 years. One hundred and forty-two (65.1%) had NAFLD and 76 (34.9%) had normal liver. Eighty-eight males and 54 female diabetic patients had NAFLD. Of the NAFLD patients 88 (58%) were smokers, 77 (54%) were obese, and 72 (51%) had hypertension. Low-density lipoprotein was increased in 132 (93%) patients with NAFLD. Angina symptoms according to modified rose questionnaire was present in 26 (18%) of NAFLD patients. Probable ST/T and Q/QS ECG changes according to Minnesota coding was present in 32 (22.53%) and in 26 (18.3%) of diabetic patients with NAFLD. **Conclusion:** There is significant association of coronary artery disease and cardiovascular risk factors with NAFLD in type 2 diabetes.

Keywords: Cardiovascular risk factors, coronary artery disease, diabetes, nonalcoholic fatty liver disease

INTRODUCTION

The most significant risk factors for nonalcoholic fatty liver disease (NAFLD) include the components of metabolic syndrome namely obesity, glucose intolerance or diabetes, hypertension, and dyslipidemia, particularly elevated triglycerides and low levels of high-density lipoprotein (HDL) cholesterol.^[1] NAFLD is becoming a major public health problem due to increasing prevalence of obesity and type 2 diabetes.^[2] The overall prevalence of NAFLD is 15%–40% in Western countries while 9%–40% in Asian countries.^[3] NAFLD in type 2 diabetes may be linked to increased coronary artery disease (CAD) risk, independent of the risk correlated

by the other components of the metabolic syndrome.^[4] In type 2 diabetic mellitus patients, up to 70% may have NAFLD.^[5] Ultrasonographic findings of bright liver, with increased echogenicity in comparison with the kidneys, vascular blurring, and deep attenuation, are suggestive of liver steatosis.^[6] Previous studies has shown strong association between NAFLD and cardiovascular diseases.^[7] Our study was conducted to estimate the magnitude of NAFLD as

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diagnosed by ultrasound examination of the liver and to assess the association between NAFLD and CAD in type 2 diabetes. Magnitude of CAD was assessed using Modified Rose Questionnaire^[8] and electrocardiogram (ECG) changes by Minnesota codes.^[9]

MATERIALS AND METHODS

Study design

The study was done as a comparative cross-sectional study.

Study setting

A study was done for 2 years from in a tertiary care medical college hospital.

Sample size

A total of 218 the adult patients of both sexes with type 2 diabetes mellitus, who gave informed written consent were taken up for the study. Patients with a history of type 1 diabetes mellitus, gestational diabetes mellitus, known case of liver disease, and chronic alcohol consumption were excluded from the study.

Ethical approval

The study protocol was approved by the Institutional Ethics Committee of the hospital (IECH/AVMCH/PG/SI No 19/2014).

Data collection and analysis

A detailed history including age, sex, lifestyle, symptoms of angina using modified rose questionnaire, hypertension, personal history for smoking, and details of any previous treatment were recorded. Detailed physical examination including anthropometric measurements and vital signs was done. All the necessary investigations such as HbA1c, fasting lipid profile, ECG, and ultrasound of the abdomen were done. The study group was divided into two subgroups based on ultrasonography finding of the liver, one group with NAFLD and the other with normal liver. The presence of CAD was assessed using the Modified Rose questionnaire and by ECG changes using Minnesota codes. Risk factors for CAD were also compared between diabetic patients with and without NAFLD. Data collected were analyzed with Statistical Package for the Social Sciences (International Business Machines Corporation. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

Modified Rose Questionnaire for angina

- A. Have you ever had pain or discomfort in your chest?
 1. Yes
 2. No
- B. Do you get this pain or discomfort when you walk up hill or hurry?
 1. Yes
 2. No
- C. Do you get it when you walk at an ordinary pace on the level?
 1. Yes
 2. No

- D. When you get any pain or discomfort in your chest, what do you do?
 1. Stop
 2. Slow down
 3. Continue at the same pace
 - E. Does it go away if you stand still?
 1. Yes
 2. No
- If yes
- F. How soon?
 1. 10 min or less
 2. More than 10 min
 - G. Where do you get this pain or discomfort
Mark the place with an X on the diagram
 - H. Have you ever had a severe pain across the front of your chest lasting for half an hour or more?
 1. Yes
 2. No

Resting 12-lead ECG was Minnesota coded

Probable CHD was defined as Minnesota coding

- 1.1–1.2 (large Q and QS waves) in all leads

Possible CHD as Minnesota coding

- 1.3 (small Q and QS) in all leads
- 4.1–4.4 (ST-T depression) in all leads
- 5.1–5.3 (flattened or inverted T waves) in all leads
- 7.1.1 (complete left bundle branch block) in all leads

RESULTS

Of the total 218 diabetic patients, there were 92 (42.2%) were in the age group of 65–74 years [Table 1]. One hundred and forty-two (65.1%) diabetic patients had NAFLD and 76 (34.9%) had normal liver [Table 2]. Male patients were 130 (59.6%) and females were 88 (40.4%). Of the total 142 NAFLD patients, 88 (62%) were males and 54 (38%) were female diabetic patients [Table 3]. Of the 142 NAFLD patients, 78 (55%) had grade 1 fatty liver [Table 4]. Of the NAFLD patients, 88 (58%) were smokers, 77 (54%) were obese and 72 (51%) had hypertension [Tables 5-7]. Low-density lipoprotein (LDL) was increased in 132 (93%) and HDL was decreased in 130 (92%) patients with NAFLD [Table 8]. Angina symptoms according to the modified rose questionnaire were present in 26 (18%) of NAFLD patients and were absent

Table 1: Distribution of age

| Age group | n (%) |
|-----------|-----------|
| 35-44 | 16 (7.3) |
| 45-54 | 76 (34.9) |
| 55-64 | 30 (13.8) |
| 65-74 | 92 (42.2) |
| ≥75 | 4 (1.8) |
| Total | 218 (100) |

Table 2: Age distribution in type 2 diabetes mellitus with nonalcoholic fatty liver disease

| Age group | NAFLD (%) | Normal liver (%) |
|--------------|------------|------------------|
| 35-44 | 12 | 4 |
| 45-54 | 46 | 30 |
| 55-64 | 20 | 10 |
| 65-74 | 62 | 30 |
| 75-85 | 2 | 2 |
| Total, n (%) | 142 (65.1) | 76 (34.9) |

NAFLD: Nonalcoholic fatty liver disease

Table 3: Sex distribution in type 2 diabetes mellitus patients with nonalcoholic fatty liver disease

| Gender | NAFLD, n (%) | Normal liver, n (%) |
|--------|--------------|---------------------|
| Male | 88 (62) | 42 (55) |
| Female | 54 (38) | 34 (45) |
| Total | 142 | 76 |

NAFLD: Nonalcoholic fatty liver disease

Table 4: Distribution of grade of nonalcoholic fatty liver disease (n=142)

| Grade | n (%) |
|---------|-----------|
| Grade 1 | 78 (55) |
| Grade 2 | 52 (36.6) |
| Grade 3 | 12 (8.4) |

in 72 (95%) of diabetic patients with normal liver [Table 9]. Probable ST/T and Q/QS ECG changes according to Minnesota coding were present in 32 (22.53%) and in 26 (18.3%) diabetic patients with NAFLD [Table 10]. Eighty-eight (62%) diabetic patients with NAFLD had CAD [Table 11].

DISCUSSION

NAFLD is defined as hepatic steatosis either by imaging or by histology in the absence of secondary hepatic steatosis such as alcohol consumption, use of steatogenic drugs, or hereditary disorder.^[1-3] The prevalence of NAFLD is increasing due to increasing prevalence of obesity and type 2 diabetes.^[1-3] NAFLD usually diagnosed with the help of ultrasonography of the liver is found to be linked to cardiovascular diseases including CAD and stroke.^[4-7] Our study was conducted on type 2 diabetes mellitus patients to find the association of NAFLD with CAD using the modified rose questionnaire^[8] and ECG changes by Minnesota codes.^[9] Among the 218 type 2 diabetes mellitus patients in our study, 59.6% were male. In our study, out of 218 type 2 diabetes mellitus patients, 65.1% of patients had NAFLD. The proportion of NAFLD in diabetes in our study was similar to the previous studies.^[5,10] NAFLD was seen more in males (62%) than females (38%). This indicates male gender as a risk factor for NAFLD.^[10,11] Of the NAFLD patients 55% had grade 1 fatty liver, 36.5% had grade 2, and 8.5% had 3 fatty liver. In our study, 88 patients were smokers in

Table 5: Association of nonalcoholic fatty liver disease in smokers with type 2 diabetes mellitus

| Liver | Smokers (88), n (%) | Nonsmokers (130), n (%) |
|---------------------|---------------------|-------------------------|
| NAFLD (n=142) | 82 (58) | 60 (42) |
| Normal liver (n=76) | 6 (8) | 70 (92) |

NAFLD: Nonalcoholic fatty liver disease

Table 6: Association nonalcoholic fatty liver disease with body mass index

| Liver | Increased (≥ 30), n (%) | Normal < 30 , n (%) |
|---------------------|--------------------------------|-----------------------|
| NAFLD (n=142) | 77 (54) | 65 (46) |
| Normal liver (n=76) | 22 (29) | 54 (71) |

NAFLD: Nonalcoholic fatty liver disease

Table 7: Association of nonalcoholic fatty liver disease in type 2 diabetes mellitus with hypertension

| Liver | Hypertensive, n (%) | Normotensive, n (%) |
|---------------------|---------------------|---------------------|
| NAFLD (n=142) | 72 (51) | 70 (49) |
| Normal liver (n=76) | 44 (58) | 32 (42) |

NAFLD: Nonalcoholic fatty liver disease

total and among NAFLD patients smokers were 58% patients and 8% of smokers had normal liver. This was significant as a study done by Zein *et al.* showed the significant bivariate associations between advanced liver fibrosis and age, diabetes, and smoking history.^[12] In our study, 116 patients were hypertensive in total, among them, 72 patients had NAFLD and 44 patients had normal liver.^[13] In our study, 99 patients were obese (body mass index ≥ 30) in total, among them, 77 (54%) had NAFLD and 22 had normal liver. This was similar to the study done by Bhatia *et al.*, which showed the prevalence parallels that of increasing rates of obesity and type 2 diabetes worldwide, with up to 95% of obese persons and 75% of diabetics likely to have NAFLD, which carries a higher risk of cardiovascular disease and mortality.^[13] The prevalence of NAFLD in subjects with metabolic syndrome is increased four-fold compared with those without the disease and 30% of NAFLD subjects have metabolic syndrome.^[14] In our study, 160 (73.4%) patients had elevated LDL levels in total, among them, 132 (82.5%) patients had NAFLD and 28 (17.5%) had normal liver. Serum triglycerides level was elevated in 150 (68.8%) patients in total, among them 124 (82.7%) patients had NAFLD and 26 (17.4%) had normal liver. In our study, 142 (65.1%) patients had decreased HDL levels, among them, 130 (91.5%) patients had NAFLD and 12 (8.5%) had normal liver. In a study done by Gaggini *et al.* dyslipidemia, hypercholesterolemia, hypertriglyceridemia, or both was been reported in 20% to 80% of cases associated with NAFLD.^[15] In another study done by Mellinger *et al.*, NAFLD was significantly associated with dyslipidemia and dysglycemia.^[16] The association of increased LDL, decreased HDL, elevated triglyceride with NAFLD was statistically

Table 8: Dyslipidemia and nonalcoholic fatty liver disease in type 2 diabetes mellitus

| | LDL | | Triglycerides | | HDL | |
|--------------|------------------|---------------|------------------|---------------|------------------|---------------|
| | Increased, n (%) | Normal, n (%) | Increased, n (%) | Normal, n (%) | Decreased, n (%) | Normal, n (%) |
| NAFLD | 132 (93) | 10 (7) | 124 (87) | 18 (13) | 131 (92) | 11 (8) |
| Normal liver | 28 (37) | 48 (63) | 26 (34) | 50 (66) | 13 (17) | 63 (83) |
| P | <0.0001 | | 0.0003 | | 0.002 | |

NAFLD: Nonalcoholic fatty liver disease, LDL: Low density lipoprotein, HDL: High density lipoprotein

Table 9: Symptoms of angina according to Modified Rose Questionnaire in type 2 diabetes mellitus patients

| Liver | Symptoms present, n (%) | No symptoms, n (%) |
|---------------------|-------------------------|--------------------|
| NAFLD (n=142) | 26 (18) | 116 (82) |
| Normal liver (n=76) | 4 (5) | 72 (95) |

NAFLD: Nonalcoholic fatty liver disease

Table 10: Electrocardiogram changes according to minnesota coding

| ECG | Minnesota codes | | | |
|---------------------|-----------------|----------|--------------|----------|
| | ST/T changes | | Q/QS changes | |
| | Probable | Possible | Probable | Possible |
| NAFLD (n=142) | 32 | 16 | 26 | 14 |
| Normal liver (n=76) | 6 | 0 | 4 | 0 |

ECG: Electrocardiogram, NAFLD: Nonalcoholic fatty liver disease

Table 11: Association of nonalcoholic fatty liver disease with coronary artery disease in type 2 diabetes mellitus

| Liver | CAD present, n (%) | No CAD, n (%) | P |
|---------------------|--------------------|---------------|--------|
| NAFLD (n=142) | 88 (62) | 54 (38) | 0.0001 |
| Normal liver (n=76) | 10 (14) | 66 (86) | |

CAD: Coronary artery disease, NAFLD: Nonalcoholic fatty liver disease

significant in our study similar to the previous studies.^[15-17] In our study, symptoms of angina according to modified rose questionnaire were present in 26 (18%) of the NAFLD patients.^[17] In our study, of the 142 patients who had NAFLD, 88 (62%) patients had CAD as identified by ECG changes according to Minnesota coding, which is found to be statically significant. Taking both the modified rose questionnaire and Minnesota coding, 114 (80%) had CAD in NAFLD diabetic patients when compared to 14 (18%) in diabetic patients with normal liver. Previous studies also showed a significant association of CAD in type 2 diabetes mellitus patients with NAFLD.^[17-20]

CONCLUSION

Our study showed a significant association of NAFLD in type 2 diabetes mellitus patients. There is also a significant association of CAD with NAFLD in type 2 diabetes mellitus patients. NAFLD is also significantly associated with cardiovascular risk factors. The presence of NAFLD in type 2 diabetes mellitus

should be considered as a strong coronary risk factor and all patients with type 2 diabetes mellitus should be screened for NAFLD, so that appropriate primary prevention for CAD may be initiated.

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Conflicts of interest

There are no conflicts of interest.

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Prevalence of microbes in unpacked dried fruits – a public health alarm

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Abstract

Problem: Food borne infections and intoxications were the principal cause leading to various gastrointestinal disorders including diarrhea with devastating and fatal health consequences. Investigations related to microbial identification provided a clear picture for ensuring the food safety and this study has been limited to dried fruits only. **Design/ Methodology:** The objective of this study is to design for isolating *E. coli*, *Salmonella* sp primarily and other possible microbes from the unpacked dried fruits available in the study area. The details about the dry fruits were collected. All the unpacked dried fruits samples were analyzed for their physical properties and further analyzed for pH, moisture content and water activity. The initial qualitative screening of the dried fruits was microscopically assessed by wet mounting. Further, the samples were inoculated on appropriate culture media and the colonies were assessed for the presence of bacterial and fungal species. **Findings:** In this study, the bacterial species like *Staphylococcus* dominating followed by *E. coli*, *Salmonella*, *Bacillus cereus*, *Pseudomonas* and *Shigella*. Among fungal species, *Aspergillus*, *Rhizopus* and *Penicillium* are the possible isolates in this study. No spoilage state of the dried fruits was found. **Conclusion:** By this study, the vendors were educated appropriately in order to minimize the microbial load in dried fruits and also recommended the health authorities to inspect such unpacked fruits frequently and necessary measures will be taken to curtail the spread of food borne infections.

Keywords: 1.Unpacked dried fruits, 2.microbial contamination, 3.health issues

Introduction

Salmonella sp and *Escherichia coli* are the leading causes of food borne illnesses. Consumers have to be protected from food borne illnesses that are caused by *E. coli*. It is estimated that *Salmonella* causes more food borne illnesses than any other bacteria. *Salmonella* and *E. coli* are the common food borne pathogens affecting millions of people annually. Food borne illnesses are the principal cause leading to diarrhea or debilitating and fatal health consequences (Todd, 2014). Globally *Salmonella* is estimated to cause one million cases of food borne illnesses annually, accounting for 28 and 35% of all hospitalizations and deaths respectively. The contaminated low moisture foods (LMFs) are important vehicles for these outbreaks,

accounting for 21% of investigated *Salmonella* outbreaks reported by the Centers for Disease control and prevention (CDC) (CDC, 2019).

Worldwide LMFs were involved in numerous outbreaks, with *E. coli* and *Salmonella* infections that are responsible for 49 and 53% respectively; while an overwhelming majority (83%) of food borne outbreaks associated with LMFs between 2007 and 2018 involved *Salmonella* alone (Jayeola *et al.*, 2022). Since dried fruits have a water activity (aW) below 0.85, they are considered as low moisture foods (Larry and Beuchat *et al.*, 2013). Unlike many other LMFs, dried fruits are characterized by a unique combination of traits, including low water activity and pH, high sugar content, and exhibiting antimicrobial phenolic compounds. Dried fruits proved an increased concern as vehicles of food borne pathogens implicating outbreaks of *Salmonella* and *E. coli* infections (Gruzdev *et al.*, 2011).

The survival of *E. coli* and *Salmonella* on dried fruits is considerably lower than that on other LMFs (Beuchat *et al.*, 2013). Nonetheless, *Salmonella* and other food borne pathogens could still be recovered from certain dried fruits for weeks or months, especially variant temperatures, thoughts of non-contamination by spoilage microbes and storing for long time suggesting that the presence of microbes on dried fruit that raises food safety and public health concerns.

Food borne illnesses are highly infectious and caused by food borne pathogens that are disturbing the gastrointestinal system through contaminated food and food products. These incidences of food borne diseases were underreported and there is a difficulty in the establishment of causative agent of food contamination, hence the illness and fatality has created a burden to public health. Globally the health threats are endangering the public and it is enhanced by unsafe food. All food handlers and consumers must understand the public health importance and they must play their role by following standard hygienic practices.

Unpacked open air exposed and long stored dried fruits are having more number of microbial pathogens and they are more infectious than packed dried fruits. Food borne pathogens from dried fruits are reported in some studies which has raised a serious public health concern (Witthun *et al.*, 2002). Several factors including the type of fruits, drying methods and storage time and temperatures are involved for the survival of common food borne pathogens on dried fruits (Sushumna *et al.*, 2022). In order to curtail the food spoilages in dried fruits and related infections, there should be a good collaboration between policy makers, industries, sales persons, consumers to ensure safety of the food products.

These type of experimental analysis are not taken place much in research in order to determine the presence of infectious microbes on the surface of unpacked dried fruits. The harvested fresh fruits dried within a week have very less or no microbial load; thus inclusion of dried fruits which are not packed and are stored in the market for intermittent and long period. The details of the unpacked dried fruits provide vast information related to type of dried fruit, method, display, store and type of consumers. The unpacked dried fruits are subjected to analyze the water activity level for determining the source and type of microbes (Beuchat *et al.*, 2013). Microbiological investigations provide a clear picture to all stake holders for ensuring the food safety. This experimental study has been limited to dried fruits alone and analyzed from a single city.

This study creates awareness about the presence of infectious microbes in the unpacked dried fruits which leads to food poisoning and intoxications. Hence, the objective of the current study is designed to isolate *E. coli*, *Salmonella* sp primarily and other possible microbes from the unpacked dried fruits.

Materials and Methods

Study Design: This is designed for the purpose of analyzing the presence or absence of microbial (both bacterial and fungal) entities in the unpacked dried fruits by standard Experimental study.

Type of the Study: This is a prospective observational study carried out in the single zone of Central Tamilnadu.

Sample Size: Five samples per dried fruits were included [Tutti frutti (dried sweetened raw papaya), cherry, dates, raisins and figs). Overall, 25 samples (each five samples) were subjected for physical, chemical and hydration characterization, and microbiological investigations.

Inclusion criteria: Dried [unpacked and packed (control)] fruits

Exclusion criteria: Fresh fruits; dried within a week

Demographic details

The details about the dry fruits including period and source of harvesting, reason for unpacking, frequency of sales, type of customers and cost were collected. These data were highly useful for understanding the route of entry, proliferation status and appropriate environment for growth and development, pathogens responsible for various gastrointestinal diseases and disorders, the mode of curtailing the spread of infection.

Physico-chemical properties

All the unpacked dried fruits samples were analyzed for their physical properties based on the visual observations and further subjected to analyze pH and moisture (%) using Hanna – H1 8314 membrane pH meter. For moisture determination, 10 grams of all the test samples were weighed and dried at 105°C in furnace till reaching a fixed dry weight, and then water content was determined by comparing the weight before and after furnace drying (Abekhti *et al.*, 2013).

In general, the aW of various samples were calculated from the difference in weight between the fresh and dried fruits and expressed in gram per gram of the dried fruits (Marzec *et al.*, 2010). But in this study, we use the dried fruits directly, thus comparing with fresh fruits were not possible. The test dried fruits were then dried in desiccators at 25°C until the water activity (a_w) approximated (Jayeola *et al.*, 2022) and aW was performed using All food, Pan India aW instrument (at 25°C ± 2°C with high accuracy of ±0.003aW). The controlled humidity (0%) and temperature (25°C) was maintained until use for further quality comparison (Marzec *et al.*, 2020).

Direct Microscopy

For initial microbiological qualitative screening, the dried fruit samples were further marinated into small pieces and soaked in the sterile distilled water and the water samples will be microscopically assessed by wet mount microscopy. The quality of the sterile distilled water was confirmed by screening the same by wet mount microscopy before processing.

Culturing

Further, the samples were smashed and the pulp samples were inoculated on Nutrient, MacConkey, Eosin methylene blue, *Salmonella Shigella* (SS) agar and Sabaroud's Dextrose agar plates and incubated appropriately. The colonies were assessed for the presence of *E. coli* and *Salmonella* sp. from respective selective agar plates and possibilities of other bacterial species from Nutrient and MacConkey agar plates. Further, the isolates were impregnated for determining genus and species level using microscopy, biochemical tests and other specific confirmatory tests. In this study, total aerobic viable bacterial load were enumerated in colony count of dried unpacked fruits whose limit of detection is 30 colony forming units (CFU)/ gram (g) (Abekhti *et al.*, 2013; Lani *et al.*, 2019).

For quality checking and maintaining negative control, the sterile distilled water used for smashing the samples were also plated. All the samples were inoculated in subjective agar plates in duplicate for fungal identification and triplicate for bacteria, and the mean values of bacterial counts were recorded as CFU/g (Neha and Surbhi, 2021). All the data were analyzed statistically and reported as mean and standard

deviation with coefficient of variance (CV) and p-value less than 0.05 is considered to be statistically significant.

Results

The basic information about the details of source and period of collection, reason for unpacking, frequency of sales, type of customers were collected and impregnated in table 1. Accordingly, most of the vendors collected the dried fruits (5 different dried fruits from 5 different locations) from other wholesale vendors and very few were interested to visit the harvesting site and collect the fresh fruits and performed the drying mechanism in their area. All the vendors informed that they won't discard any dried fruits even after expiry; and they are selling the expired dried fruits in low cost and large quantity.

The physical properties based on the visual observations of all the dried fruits subjected for this investigation were described for its texture, size, watery nature and colour. By nature, each and every fresh and dried fruits having its own physical properties; if deviations found, then it may not be considered for consumption and the detailed physical properties verses location of collection of the dried fruits were depicted in table 2.

The higher mean pH value was observed among tutti frutti (6.26) followed by dates and figs with 4.48 and 3.94 respectively and the p value showed significant. The higher mean percentage moisture was recorded as 28.12% (cherry) followed by dates (22.7%) and raisins (18.56%). While determining the water activity levels, it was identified that the cherry (1.06) showed greater value compared with other dried fruits and the detailed statistical description of various fruits were tabulated (Table 3).

The direct microscopic observations of various dried fruits (n=25) showed presence of mono (as cocci and as motile bacilli) [13 (52%)], di (cocci and motile bacilli) [9 (36%)] and poly (cocci, motile and non motile bacilli) [3 (12%)] bacterial combinations among 25 samples of dried fruits isolated from 5 different locations. The sterile distilled water used for culture media preparations showed no microscopic cells and debris. All the packed dried fruits are not shown microbial shades except tutti frutti; the microscopic observation of tutti frutti showed some bacterial cells (trace of cocci). The figure 1 explained the detailed description of the wet mount microscopy.

Out of five locations included in this study, the location one and five which are semiurban areas showed maximum number of isolates of 12 and 11 respectively; where *Staphylococcus* dominated with 4 samples (except tutti frutti in location one and Cherry in location five) followed by *Salmonella* with 3. In location 3, *Staphylococcus* dominated except in figs followed *E. coli* and *Salmonella*. In the location 4, *Shigella* species isolated which was not isolated in any location. No growth was identified in the dates of location 2.

Among dried fruits included in this study, tutti frutti showed maximum isolation of *Salmonella* with 4 possibilities followed by *Pseudomonas* and *Staphylococcus*. No other fruits showed *Shigella* growth except tutti frutti of location 4. All the samples showed *E. coli* growth in Cherry followed by *Staphylococcus* and *Salmonella*. While processing figs from five locations, *Bacillus cereus* isolated from three locations except 1 and 5. *Salmonella* growth dominated with all five samples in Raisins followed by *Staphylococcus*. The date samples showed maximum isolates of *Staphylococcus* followed by *Pseudomonas*.

Staphylococcus was considered as a dominating isolates with 16 samples. *Bacillus cereus* isolates (n=6) were possible in figs followed by raisins and tutti frutti; dates and cherry were free from *B. cereus*. Eleven isolates of *Salmonella* recorded (five raisins samples, four tutti frutti and two cherry). Isolates of *Shigella* species and *Shigella dysenteriae* are possible only in tutti frutti, other samples were free from *Shigella*. All five cherry samples had *E. coli* followed by figs, no *E. coli* found among tutti frutti. Out of 25 samples, *Pseudomonas* isolation was possible among five samples.

The sterile distilled water had no microbial growth in any of the culture plates. Further, the microscopically identified packed tutti frutti showed bacterial growth and confirmed as *Staphylococcus* sp,

which is a normal skin flora. No fungal growth observed. The data variations of bacterial isolates among dried fruits samples verses location, samples and bacteria were depicted in table 4 and figure 2.

The monofungal isolation was possible with *Aspergillus* species in location 1; difungal (*Aspergillus* and *Rhizopus*) was found among three locations and one location had trifungal (*Aspergillus*, *Penicillium* and *Rhizopus*). No fungal growth was observed while processing tutti frutti; whereas *Aspergillus* and *Rhizopus* were isolated from cherry, figs and dates; and only *Aspergillus* isolated from Raisins. Out of 20 fungal isolates, *Aspergillus* sp. (n=13; 65%) followed by *Rhizopus* sp. (n=6; 30%) and *Penicillium* sp. (n=1; 5%) (Table 5 and Figure 3).

The growth of the bacterial isolates was determined by CFUs/g of the five dried fruit samples which were collected from five different locations. The bacterial CFUs were found lesser in all samples except in tutti frutti; only one sample of dates showed nil growth and the detailed counts of CFU were pictured in figure 4.

Discussion

This study is highly useful for understanding the route of entry, proliferation status and appropriate environment for growth and development, pathogens responsible for various gastrointestinal diseases and disorders, the mode of curtailing the spread of infection especially with dried fruits. While interviewing with the vendors, they told that they purchased from the whole sale market and not having any ideas of method of drying. Commercially, the pretreatment process of dried fruits remove the humidity faster before they dry naturally; this is processed by physical and chemical methods which retain the colors, aroma and nutritional levels. By Microbiological point of view, the processes takes place for drying is to ensure hygiene by curtailing microbial entry, growth and reactions (Kaveh *et al.*, 2020; Alp and Bulantekin, 2021).

In this study, none of the vendors are trained for antimicrobial maintenance of dried fruits. The knowledge about the expiry, spoilage state, maintenance strategies, storage methods, risk of contamination, reusable steps and time of disposal are lacking among vendors. After drying, the fruits are to be preserved to extend the shelf-life, which is largely useful to ensure the quality and safety. Canning, drying, freezing, freeze drying, inert gases, irradiation, preservatives, spray drying, sugar crystallization and vacuum packing are the common mode of drying the fruits (Yadav and Singh, 2014).

The physical observations of the test dried fruits included in this study were described to understand the colour, size, texture and watery nature. Among them, texture of dried fruits is considered as the important quality that attributes the food products thereby, soft to hard, shrunked and sticky observed. No studies so far highlighted the physical characteristics as a whole. The changes takes place in the dehydrated fruits affects structure. The size of the dried fruits is ranged from square to irregular. In common, the drying of fruits takes place by frictional properties between the fruits and surface materials; thus the structure varied (Keramat *et al.*, 2008).

The water content of the dried fruits plays a vital role in the colonization of microbes. The pretreatment process while air drying may reduce water content from 30 to 70% (Yadav *et al.*, 2014) provides a maximum dried environment to reduce the microbial entry. In nature, low and high contents of dry mass and tannins respectively are most important while drying fruits (Agata *et al.*, 2010).

The pH of the dried fruits provides the picture of acidic and alkaline state, thereby the type, source and variance of the microbes analyzed. In this study, the mean highest pH level was identified among tutti frutti (6.26 ± 0.42) and minimum level was 3.7 ± 0.35 among cherry. This indicated that the tutti frutii is high source of bacterial spoilage and cherry with fungal colonization leads to consumer health risk. Bacterial cells lodging on the surface of dried fruits are directly exposing to internal tissues with high acidic environment showed minimal, also making it to difficult for attributing the pH level as a factor that are largely influencing the inactivation rate of bacteria (Alp and Bulantekin, 2021); but fungal growth is encourageable.

The moisture content and water activity of the dried fruits included in this study showed maximum of 28.12 ± 2.58 and 1.06 ± 0.23 among cherry respectively; whereas the lesser had found among figs with

15.34±1.74 (moisture content) and 0.7±0.09 (aW). The data were statistically significant ($p=0.05$) for moisture content conversely, insignificant for aW ($p=0.02$). The moisture content is an important indication for dried fruits, thereby the lesser moisture content curtail the microbial growth (Miranda *et al.*, 2011). Additionally, the size, structure, color, flavor, texture, aeration and nutritional value of the dried fruits are determined by influencing the major parameters including aW (Radojcin *et al.*, 2021). While storing and displaying the unpacked dried fruits for selling, the vendors are not much concentrating on the moisture that leads to acquiring microbes from the environment. The storage and proper maintenance including temperature of the dried fruits are inversely proportional to shelf life and quality of the product (Romeo *et al.*, 2010).

The wet mount microscopy of dried fruits included in the study showed certain bacterial cells ranging from trace to numerous. The tutti frutti showed some bacterial cells likely to be trace of cocci mainly due to the unhygienic handling contamination. Education is required among the vendors for maintaining the hygienic and microbe free unpacked dried fruits for public health importance. The culture plates dominating with *Staphylococcus* followed by *E. coli*, *Salmonella*, *Bacillus cereus*, *Pseudomonas* and *Shigella* as described in table 4. The same type of observation was also made by other studies (Witthuhn *et al.*, 2005; Zhuosheng *et al.*, 2021; Akbas and Ozdemir, 2008). Scanty data about *Pseudomonas* and *Shigella* isolation observed in some dried fruits (Ntuli *et al.*, 2017).

Generally, fruits are rich in carbohydrates, fibers, proteins, essential vitamins and minerals supporting the human health in various aspects. Now a days, all age groups and genders are much concentrating on balanced diet; thereby consumption of fruits and vegetables increasing; leads to reducing the nutritional deficiencies and risk of various diseases (Tango *et al.*, 2018). The increasing demand of dried fruits and their products are continuing due to non-availability of the fresh fruits throughout the year; but researchers and nutritionists always thinking about the comparativeness of nutritional values of fresh fruits and its dried conditions. Despite the entire nutrition is not available in the dried fruits, the passion of modern dish, storage, long period of usage and easy transportation motivates to prefer dried fruits.

Naturally, the fresh and raw fruits are largely contaminated with various saprophytic and pathogenic microorganisms such as *E. coli*, *Staphylococcus aureus*, and *Salmonella* spp (Buyukunal *et al.*, 2015). While the same fruits dried by various methods, the colonization of same microorganism are questionable. The factors that influencing the microbial contamination in dried fruits are usually done when contact with dust, soil, and wastewater during harvest and post-harvest periods, method of drying, complete or partial drying, storage methods, logistics and vendor display (Buyukunal *et al.*, 2015).

The colonized microbes on dried fruits may cause serious diseases including abdominal distress, nausea and vomiting, diarrhea with or without fever and headache, abdominal cramps, lethargy, low blood pressure and pulse rate, and even death (Alp and Bulantekin, 2021). In fresh fruits, the environment for microbial growth is suitable thereby the colonization and stress behavior of the microbes are neutral, whereas in dried fruits, the stress environments for the microbes are high, thus making them more infectious while consuming that provide more option for colonization, thereby infection state increased.

Generally, microflora in vegetables is associated with bacterial groups while the fruit flora generally consists of yeasts and molds. Dried fruits provide shelter for pathogenic microbes where it can survive even at low water activity (Finn *et al.*, 2013). Bacteria are considered as the confronted cell which can survive at various stress environments including diverse temperatures, higher osmolality and acidic pH (Singh *et al.*, 2017). In response to the dry environment, the microbes accumulate biofilm formation, filamentation and osmoprotectant molecules (glutamate, potassium chloride and trehalose) (Chitrakar *et al.*, 2019; Finn *et al.*, 2013).

In this study, *Aspergillus* isolates dominated followed by *Rhizopus* and *Penicillium* (Figure 3); correlated the previous study highlighted the various species of *Aspergillus* (*A. flavus* and *A. niger* and *A. fumigatus*). The uncontrolled and unlicensed marketing of unpacked dried fruits in developing countries like

India provide lodgment for various fungal pathogens that cause systemic infections along with aflatoxin and other fungal toxins leads to major clinical complications (Abbas *et al.*, 2004; Abbas *et al.*, 2019). *Penicillium* spp., were isolated from Dates and second predominance of *Rhizopus* spp. showed the possibilities of fungal entry when the dried fruits are unpacked which was documented in previous reports (Rossetto *et al.*, 2005; Abbas *et al.*, 2019).

This laboratory based experimental study highlighted the presence of bacterial and fungal contamination in the test dried fruits which are defined as rich source of nutrients even though the growth factors are minimally available. This cause mild to severe health issues while consuming the contaminated dried fruits. In this study, the spoilage state of the dried fruits is not found. Care should be taken while purchasing such unpacked dried fruits for consumption. The surface cleaning of the unpacked dried fruits also suggested for consumers. Vendors have to be educated appropriately in order to minimize the microbial load in the products and also teach them about the personal and environmental hygiene. This study recommends the health authorities to inspect such unpacked fruits available in local markets frequently and take necessary measures to curtail the spread of food borne infections.

Conclusion

Six bacterial isolates (*Staphylococcus*, *E. coli*, *Salmonella*, *B. cereus*, *Pseudomonas* and *Shigella*) were possible in this study by screening the five different dried fruits collected from five locations of the study area. Among fungus, *Aspergillus*, *Rhizopus* and *Penicillium* were isolated. Further, this work is extended with various unpacked dried fruits throughout the region in order to explore more possible microbial species associated with food borne infections and intoxications.

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Table 1: Categorical response among vendors (n=5)

| Category | Response | Number (%) |
|----------------------|-------------------------------------|------------|
| Source of harvesting | Purchase from other vendors | 4 (80) |
| | Direct harvesting and drying by own | 1 (20) |
| Reason for unpacking | No facility | 4 (80) |
| | Costly | 4 (80) |
| | Easy to handle | 5 (100) |
| | Easy to display | 5 (100) |
| Frequency of sales | More than 10 customers/ day | 4 (80) |
| | Predominantly during evening time | 5 (100) |
| Type of customers | Upper middle class | 3 (60) |
| | Middle class | 4 (80) |
| | Lower middle class | 5 (100) |
| | Lower class | 5 (100) |
| | Below poverty line | 5 (100) |
| | Children | 5 (100) |
| Period of collection | 1 to 2 weeks | 2 (40) |
| | 2 to 4 weeks | 3 (60) |
| | 4 to 6 weeks | 3 (60) |
| | 6 to 8 weeks | 2 (40) |

Figure 1: Wet mount microscopic observations of dried fruits

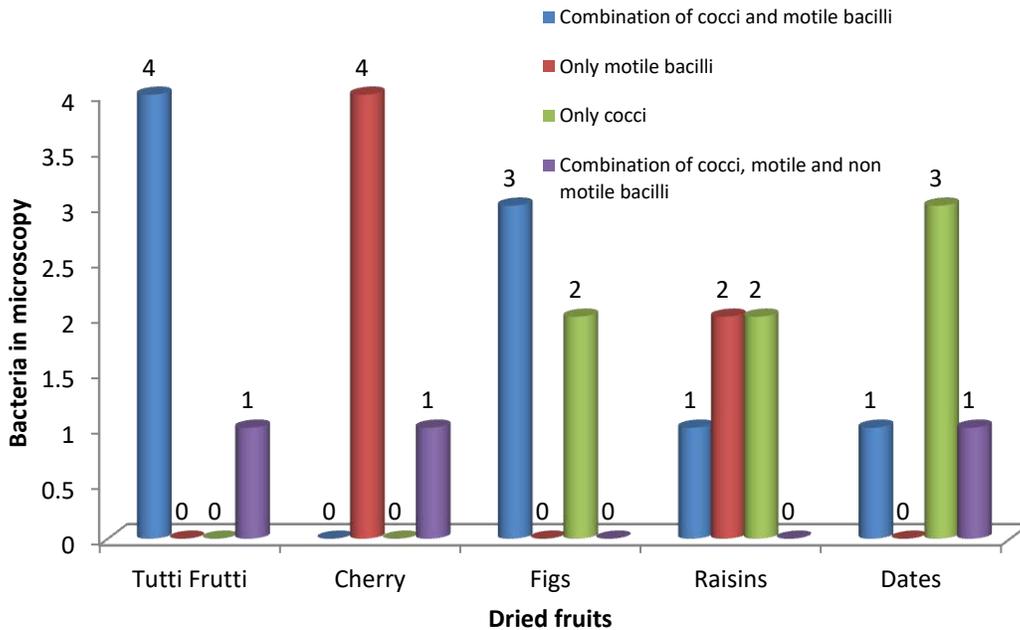


Table 2: Location wise physical properties of dried fruits

| Samples | Physical properties of dried fruits verses location (in numbers) | | | | | | | | | | | | | | | | | | | |
|--------------|--|---|-----|---|---|----------------|-----|-------|-------|-----|---------------|-----|---|-----|-----|--------|-----|-----|-----|---|
| | Texture | | | | | Size and shape | | | | | Watery nature | | | | | Colour | | | | |
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Tutti Frutti | a | a | a | a | a | a,c | a,c | b,c,d | a,b,c | c,d | a | a | a | a | a | a | a | a | a | a |
| Cherry | b | b | b | b | b | b,e | a | b | b | a | b | b | b | b | b | b | b | b | b | b |
| Figs | c | c | c | c | c | a,e | a,f | a,b,f | a,f | a,e | c | c | c | c | c | c | d | d,e | d,e | c |
| Raisins | d | d | d | d | d | a | a,b | b | b | a | a,b | a,b | c | a,b | a,b | d | f | f | d | e |
| Dates | e | e | e,f | d | e | b | a | a | a | a | c | b | b | b | b | f | e,f | e | e,f | e |

[**Location:** 1 - Semiurban area; 2 - Rural -collected during festival time; 3 - Urban - crowd area; 4 - Urban - high crown area; 5 - Semiurban area; **Texture:** a - little hard, b - soft, seeds are removed and fruit cut in the middle, c - peeled dried and sliced into fragments, d - dry but slightly sticky, e - shrunked with seeds and f - shiny; **Size and Shape:** a - smaller, b - medium, c - square, d - rectangle, e - round, f - uneven; **Watery nature:** a - sticky, b - watery and pulpy, c - completely dried; **Color:** a - artificial colors, b - red, c - buff and brown, d - pale brown, e - dark brown, f - black]

Figure 2: Possible bacterial isolates from dried fruit samples (n=25) from various locations (n=5)

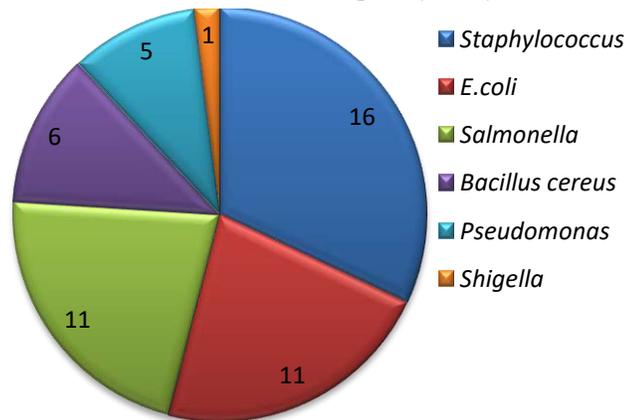


Table 3: Comparative analysis of mean value of pH and water activity of dried fruit samples

| Category | Mean±SD and Coefficient of variance (CV) of dried fruit samples in five locations | | | | | |
|---------------------|---|------------------------|-------------------------|------------------------|-----------------------|---------|
| | Tutti Frutti | Cherry | Figs | Raisins | Dates | P value |
| pH | 6.26±0.42 and 6.64 | 3.7±0.35 and 9.56 | 3.94±0.43 and 11.0 | 3.86±0.18 and 4.71 | 4.48±0.3 and 6.77 | 0.002* |
| Moisture (%) | 17.56±0.76 and 4.35 | 28.12±2.58 and 9.18 | 15.34±1.74 and 11.34 | 18.56±1.02 and 5.50 | 22.7±0.97 and 4.26 | 0.05* |
| Water activity (aW) | 0.8±0.06 and 7.75 | 1.06±0.23 and 22.15 | 0.7±0.09 and 13.45 | 0.84±0.06 and 7.05 | 0.82±0.06 and 7.48 | 0.02** |

[*Significant and **not significant]

Figure 3: Possible Fungal isolates

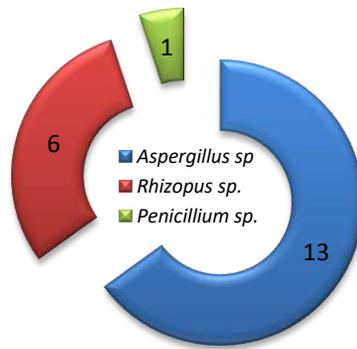


Table 4: Bacterial isolation from dried fruit samples

| Samples | Location wise possible bacterial isolates | | | | |
|--------------|---|-------------------------|---|--------------------------------------|---|
| | 1 | 2 | 3 | 4 | 5 |
| Tutti Frutti | Salmonella, Bacillus cereus | Salmonella, Pseudomonas | Pseudomonas, Staphylococcus, Salmonella | Shigella sp, S. dysenteriae | Salmonella, Staphylococcus |
| Cherry | E. coli, Salmonella, Staphylococcus | E. coli, Staphylococcus | E. coli, Staphylococcus | E. coli, Staphylococcus, Pseudomonas | E. coli, Salmonella |
| Figs | E. coli, Staphylococcus | Bacillus cereus | E. coli, Bacillus cereus | E. coli, Bacillus cereus | E. coli, Staphylococcus |
| Raisins | Salmonella, Bacillus cereus, Staphylococcus | E. coli, Salmonella | Salmonella, Staphylococcus | Salmonella, Staphylococcus | Bacillus cereus, Staphylococcus, Salmonella |
| Dates | Pseudomonas, Staphylococcus | Nil | E. coli, Staphylococcus | Staphylococcus | Pseudomonas, Staphylococcus |

[Location: 1 – Semiurban area; 2 – Rural –collected during festival time; 3 – Urban – crowd area; 4 – Urban – high crown area; 5 – Semiurban area]

Figure 4: Determination of bacterial CFUs in culture plates

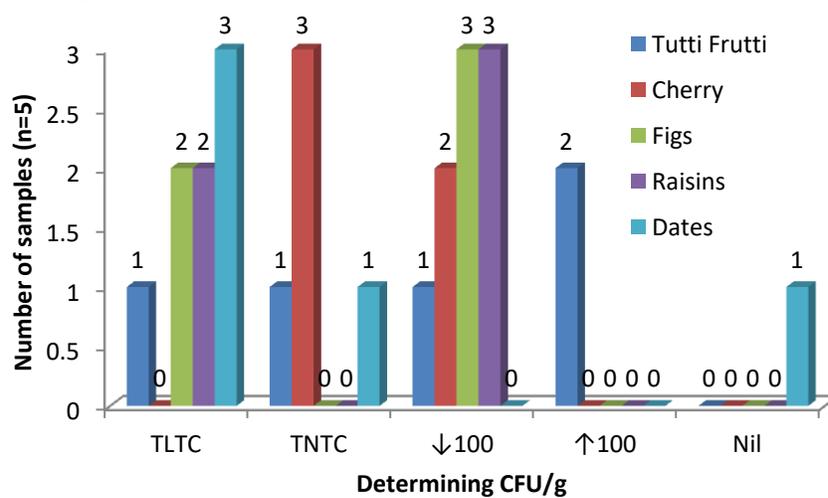


Table 5: Fungal isolation from dried fruit samples

| Samples | Location wise possible fungal isolates | | | | |
|--------------|--|----------------|----------------|---|--------------------------------|
| | 1 | 2 | 3 | 4 | 5 |
| Tutti Frutti | Nil | Nil | Nil | Nil | Nil |
| Cherry | Aspergillus sp | Aspergillus sp | Nil | Rhizopus sp, Aspergillus sp | Aspergillus sp |
| Figs | Aspergillus sp | Aspergillus sp | Aspergillus sp | Rhizopus sp | Rhizopus sp, Aspergillus sp |
| Raisins | Nil | Rhizopus sp | Rhizopus sp | Aspergillus sp | Aspergillus sp |
| Dates | Aspergillus sp | Aspergillus sp | Nil | Aspergillus sp, Rhizopus sp, Penicillium sp | Nil |

[**Location:** 1 – Semiurban area; 2 – Rural –collected during festival time; 3 – Urban – crowd area; 4 – Urban – high crown area; 5 – Semiurban area]

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Effectiveness of inclusion of chlorhexidine cleansing in pre-operative preparation of C-Section in reducing postpartum endometritis: A comparative study in tertiary care institute

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Abstract

Background: Infectious morbidity is one of the commonest cause, contributing to almost 15% of maternal mortality. Vaginal cleansing with 1% chlorhexidine solution before C section can be an economical and affordable source of infection control.

Aim: To evaluate the effect of vaginal chlorhexidine cleansing before C-section and its role in reduction of postpartum genital tract infections.

Objective: To include 1% chlorhexidine cleansing as a routine procedure pre-operatively in all C-sections.

Methods: 50 AN patients taken for C- section during the study period(01.12.2020 to 06.06.2021) in TSRMMCH&RC were divided into two groups A and B. Group A –taken as control group was given the routine pre-operative skin preparation ,medications and prophylactic antibiotics. Group –B was the interventional group in which patients in addition were given vaginal cleansing with 0.5% chlorhexidine for 30 seconds before and after catheterisation with 2 separate swab sticks.

Inclusion Criteria: 1) All women undergoing C section. 2) Those who give willingness to participate in study and for follow up till 2 weeks postpartum.

Exclusion Criteria: 1) Patients with H/O sensitivity to chlorhexidine 2) H/o skin infection /genital infection.

Results: The statistical analysis was done with the software-SPSS version 16. The following observations were taken for analysis- 1) post-partum fever 2) foul smelling lochia 3) Induration or infection at the incision site 4) need for prolonged antibiotics and prolonged hospital stay.

Conclusion: Chlorhexidine swab cleansing before C section was found to reduce post-partum endometritis in C-section patients and can be included as a cost-effective and an efficient infection control method.

Keywords: Pre-operative preparation, chlorhexidine, endometritis

Introduction

Direct maternal infections around the time of childbirth are responsible for around a tenth of all maternal deaths worldwide. Women who get peripartum infections are more likely to have significant morbidity and long-term impairments including chronic pelvic pain and Secondary infertility due to anatomical distortions. An estimated 1 million new borns die each year as a result of this [1].

Pre-existing maternal illnesses (e.g., malnutrition, diabetes, obesity, severe anaemia, bacterial vaginosis, and group B streptococcus infections), as well as prelabour membrane rupture and multiple births. Multiple vaginal examinations, manual placenta removal, operational vaginal birth, and caesarean section enhance the risk of maternal peripartum infections. As a result, initiatives to minimise maternal peripartum infections and their short- and long-term effects have focused on infection prevention and control [2].

Cesarean section is the most important risk factor for endometritis, as it occurs in 11% of sections following labour and 3% of electives. The incidence is 2-9 % more in C-section than labour naturalis [3, 5]. Prolonged hospitalisation, sepsis, peritonitis, and intrapelvic abscess are all consequences of endometritis [4].

Post-operative endometritis is principally due to ascending pathogens from the vagina and cervical canal. The altered pathological flora is usually polymicrobial and resistant to the usual prophylactic antibiotics used [6, 7, 8].

Cleaning the vaginal canal before surgery is not a new scientific topic. Vaginal washing was used before abdominal hysterectomy in the early 1970s, and it was found to lower the number of vaginal bacteria and the rate of post-operative infection [9]. Later, researchers looked at whether pre-cesarean cleaning using antiseptics could reduce post-cesarean infection. Antiseptics were employed in a variety of forms (e.g., povidone-iodine, metronidazole, and chlorhexidine), procedures (e.g., vaginal washing or skin scrub), and types (e.g., solutions or wipes) [10-13]. According to a comprehensive review, vaginal washing with chlorhexidine is quite safe, inexpensive, and simple to use [14]. Preparing the vaginal area with an antiseptic chemical could be viewed as intrusive procedure. In this study, adequate care has been taken for educating women about the benefits of vaginal preparation and safeguarding their safety and preserving the privacy. The method and timing of vaginal preparation should be used in a way that ensures the best results and in this study it is combined during Pre-operative preparation.

The disinfection is rapid, within ten minutes, making it potentially useful immediately before the caesarean delivery. More well-designed randomised clinical studies, according to the review's authors, are needed to evaluate that safe and valuable method. The authors of the current study were motivated by this to investigate the impact of preoperative vaginal washing with chlorhexidine antiseptic on post-caesarean section infection morbidity. Additionally, the American College of Obstetricians and Gynaecologists (ACOG) recently updated practice recommendations in September 2018 for infection prophylaxis on labor and delivery [7].

Aim: The purpose of this study is to see if preoperative vaginal cleaning with a 0.5% chlorhexidine gluconate solution can reduce the risk of postoperative caesarean section maternal infectious morbidities, specifically endometritis.

Objective: To recommend vaginal cleansing with chlorhexidine antiseptic solution as an accompaniment to the abdominal skin scrub and prophylactic antibiotic therapy, immediately prior to C-section for lowering endometrial exposure to bacteria.

Methodology

The study incorporated 50 AN patients who were scheduled for a C-section during the study period (01.12.2020 to 06.06.2021) into two groups A and B by Random sampling. Each pregnant woman scheduled for a C-section was interviewed when she arrived in the operation room waiting area. The study's goal and nature were defined, and eligible pregnant women giving informed written consent, only is included the group's assignment was determined at the same time. The standard pre-operative skin preparation, medicines, and prophylactic antibiotics were administered to both Group A, the control and Group -B, intervention group. Patients in Group -B received vaginal washing with chlorhexidine. A sterile bowl with 30 mL chlorhexidine 0.5 percent antiseptic solution with 2 sterile gauzes was used for vaginal disinfection. The area between the

vaginal apex and the introitus was cleansed in a clockwise direction. The vaginal apex was cleansed first, then the four vaginal fornices, and finally the introitus, for 30 seconds before and after Foley catheterization.

Inclusion criteria

- 1) All women undergoing C section during the study period.
- 2) Those who give willingness to participate in study and for follow up till 2 weeks postpartum.

Exclusion criteria: Patients with

1. H/O sensitivity to chlorhexidine
2. H/O skin infection /genital infection.
3. H/O immune compromised status like Diabetes.
4. H/O Antepartum risk factors like Anaemia, Premature membrane rupture, Antepartum
5. Haemorrhage.
6. H/O prolonged intra-operative period & intra-op bleeding.

Definition of outcomes measured:

- 1) **Fever:** defined by a temperature of 38 degrees Celsius or higher, omitting the first day following caesarean delivery and any other causes of fever such as mastitis, urinary tract infection, or tonsillitis.
- 2) Lower abdominal pain and fundal tenderness, as well as one or more of rebound tenderness, tenderness with cervical motion, adnexal tenderness, foul-smelling lochia, and fever, were all signs of endometritis.
- 3) **Infection at the Site of incision:** It was detected by the appearance of erythema or the disruption of an abdominal incision, as well as purulent discharge from the incision site, which need antibiotics and wound care.

Data collection & Analysis of variables

Maternal age, weight and height with BMI, gestational age at the time of delivery, and parity were among the demographic factors studied. Details of the LSCS procedure, including the indication, duration, and length of stay in the hospital were also taken into contemplation. SPSS for Windows version 20.0 was used for all statistical analyses .The mean and standard deviation of all continuous values were calculated. Numbers and percentages were used to express categorical data. For two variables with continuous data, the comparisons were made using the Student's t-test. When comparing variables with categorical data, the Chi-square test was utilised. The threshold for statistical significance was established at $p < 0.05$.

Table 1: Gravida Distribution in both Groups

| | Group | Frequency | Percent |
|---|-------------------------|-----------|---------|
| A | Primi | 11 | 44.0 |
| | 2 nd Gravida | 13 | 52.0 |
| | 3 rd Gravida | 1 | 4.0 |
| B | Primi | 7 | 28.0 |
| | 2 nd Gravida | 17 | 68.0 |
| | 3 rd Gravida | 1 | 4.0 |

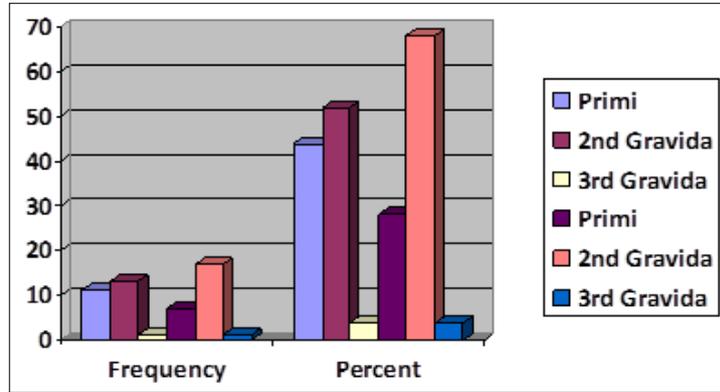


Fig 1: Gravidity Distribution in both Groups

Table 2: Age wise distribution

| Group | Age | Frequency | Percent |
|-------|---------|-----------|---------|
| A | 21 - 25 | 13 | 52.0 |
| | 26 - 30 | 8 | 32.0 |
| | >=31 | 4 | 16.0 |
| B | 21 - 25 | 8 | 32.0 |
| | 26 - 30 | 9 | 36.0 |
| | >=31 | 5 | 20.0 |

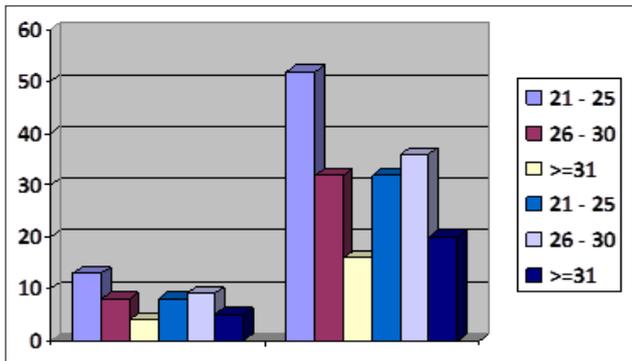


Fig 2: Age wise distribution

Table 1 shows the fundamental characteristics of the women who took part in the study. The research group had a mean age of 25.8 years, while the usual care group had a mean age of 26.7 years. In terms of maternal age, parity, gestational age at delivery, birth weight, indication, and duration of the CS, there were no statistically significant differences between the two groups, indicating that they were matched and homogeneous.

Table 3: Comparison of outcomes

| Variable | Intervention Group | | Control Group | | P value | RR or CI |
|----------------------|--------------------|---------|---------------|---------|---------|-------------------------|
| | Frequency | Percent | Frequency | Percent | | |
| Postpartum fever | 4 | 16% | 11 | 44% | 0.002 | 4.125 (1.092 to 15.585) |
| Foul smelling lochia | 3 | 12% | 5 | 20% | 0.440 | 1.833 (.387 to 8.674) |
| Wound infection | 1 | 4% | 10 | 40% | 0.031 | 16 (1.855 to 137.974) |

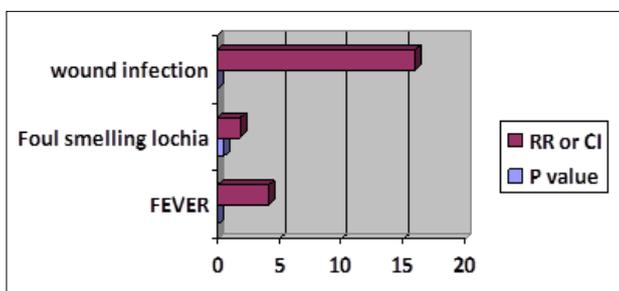


Fig 3: Statistical significance in outcomes

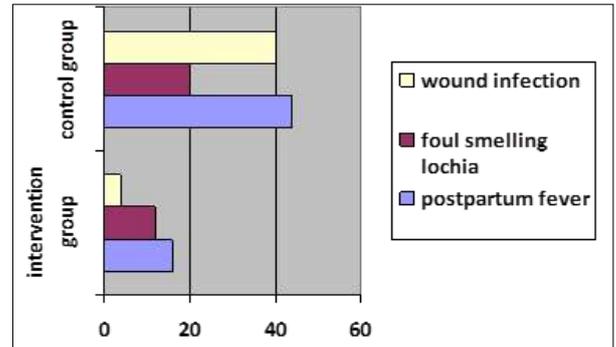


Fig 4: Comparison of infectious morbidities in both group

As shown in Figure 2. The endometritis rate, which was 16 percent in the interventional group versus 44 percent in the usual care group ($p = .002$), revealed statistically significant reduction in overall post Caesarean infectious morbidity (Figure 2). Wound infection (40 percent vs 4 percent; $p = 0.03$) and surgical site infection (40 percent vs 4 percent; $p = 0.03$) rates were vastly shorter in the interventional group than in the standard care group. The average length of hospital stay in the study and usual care groups was nearly identical (8.0 vs. 8.1 days), with no statistically significant difference.

Discussion

The influence of preoperative vaginal scrub with chlorhexidine wipes on post-caesarean infectious morbidities in 219 women undergoing elective CS at Suez Canal University Hospital in Ismailia, Egypt, was assessed in a randomised controlled trial, which validated the findings of the current study [15, 16]. Tewfik and coauthors (2015) found that vaginal cleaning with chlorhexidine solution before elective C-section reduced total post-LSCS infection morbidity in 47 women at Ain Shams University Maternity Hospital in Egypt. Chlorhexidine antiseptic's antibacterial action against a variety of biotrophic pathogens, including those connected to peripartum infections, may explain its favourable impact in decreasing cumulative infectious morbidity. It also has a longer delayed effect than other antiseptics, making it superior [18, 19]. This can be attributed to the small sample size.

In the present research, there was no substantial statistical distinction in hospital stay time between the two groups. Similarly, Ahmad and colleagues found no statistically significant difference between the two groups in terms of hospital stay length.

Chlorhexidine is well tolerated and has few adverse effects, according to the latest study. Chlorhexidine vaginal wipes at concentrations less than 1%, according to Wilson *et al.* (2004), do not induce unpleasant skin reactions and are not destroyed by organic molecules such as sweat [20-22]. Despite the fact that the

current investigators revealed that the study group patients had a lower rate of wound infection and febrile morbidity, the difference was small and inconsequential. Ahmed and coauthors found a non-significant decline in rates of febrile morbidity and wound infection when contrasting the chlorhexidine vaginal washing group to the control group [15]. The similarities in methodology between the current study and Ahmad *et al.* study may explain the agreement between the two analyses. Both studies were designed as randomised controlled trials, and the inclusion criteria were similar in both. Tewfik *et al.* (2015), on the other hand, discovered that the reduction in febrile morbidity among the vaginal scrub using chlorhexidine group is statistically significant, which could be due to the lower sample size.

Conclusion

When compared to saline or without cleansing before caesarean delivery, vaginal preparation with chlorhexidine solution minimises the incidence of post-caesarean endometritis. Chlorhexidine cleansing being a simple and relatively affordable intervention, can be given as an exhortation in maintaining Asepsis during C-section.

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Comparative study of blood loss in C-section with usage of intravenous oxytocin and intramuscular methergine-in a tertiary care hospital

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ABSTRACT:

BACK GROUND :Post partum haemorrhage is a life threatening condition contributing to majority of maternal mortality-commonest cause is uterine atonicity(12 %)-signifying the active management and the use of prophylactic uterotonics¹. **AIM**: To compare the efficacy of intra-venous Oxytocin and intra muscular methergine in control of Postpartum Haemorrhage. **OBJECTIVE** :To substantiate the use of Oxytocin as the first line uterotonic. **METHODS** :100 AN Mothers taken for LSCS during the study period (from 01.12.2020 to 07.06.2021) in Trichy SRMMCH&RC -were included in the study. **Inclusion criteria** : Those with- Duration of surgery \leq 2 hours, patients giving written consent, BMI \leq 30. **Exclusion criteria**: Duration of surgery $>$ 2hours, patients with PROM, patients not giving consent, known sensitivity to oxytocin and methergine. previous H/O bleeding disorders. BMI $>$ 30., Previous H/O post partum haemorrhage, H/O antepartum haemorrhage. Patients were divided into two groups each containing 50, based on random sampling. Group A was given 10 units intravenous Oxytocin and Group B was given 0.2 mg intramuscular methergine after delivery of placenta and outcomes were studied on the basis of blood loss and fall of Haemoglobin and PCV. **RESULTS** : After statistical analysis with SPSS Version 16-No significant differences was noted among the 2 groups, except for significant fall of PCV in oxytocin group than methergine group. **CONCLUSION** : Both drugs are equally efficacious in controlling postpartum haemorrhage as prophylactic uterotonics. Considering the significant side effects of methergine with quickest onset of action –oxytocin is equally effective with better safety profile.

KEY WORDS :Postpartum haemorrhage, Atonic PPH, Uterotonics.

INTRODUCTION:

Obstetric haemorrhage is a major cause of maternal mortality with an incidence of 7 per 1000 deliveries among the critically ill post partum patients 35 % cause is due to postpartum haemorrhage¹

The golden period of management in Post partum haemorrhage is often missed because the bleeding may be concealed and may get diluted with the mixing of amniotic fluid making accurate measurement difficult and delayed. The physiological changes in pregnancy like increased cardiac output and increased blood volume at term, mask the quantity and magnitude of blood loss. The earliest sign of acute blood loss-Tachycardia presents only after 30 % - 40 % of circulating volume is lost which leads to delayed recognition and treatment. The uteroplacental blood flow is 800-900 ml at term ,amounting to brisk and rapid haemorrhage difficult to control.Among the causes like trauma to the genital tract,retained products of conception,abnormally adherent placenta ,bleeding disorders,placental anomalies,acute uterine inversion –Atonicity contributes to 25 %- 50%.²

The regular and swift implementation of the Active management in third stage of labour has lead to substantial reduction of post partum haemorrhage to 60 %.

The three indissociable actions in this are (1)Administration of prophylactic uterotonic (2)Cord clamping and cutting (3)controlled cord traction.⁵

This necessitates the need for Active management of third stage of labour with prophylactic uterotonics,early cord clamping and controlled cord traction Oxytocin stimulates contractility of uterine muscles acting through sodium channel in 2 minutes after intravenous injection and effect persists for 30-60 minutes without any significant side effects. Intramuscular Methergine is an ergot alkaloid acting directly on serotonin receptors of smooth muscles of uterine blood vessels causing immediate vasoconstriction preventing blood loss within 2 minutes of administration with significant side effects.

Methergine is a conventional uterotonic used extensively ,considered it as a second line drug because of its vexatious side effects like vomiting ,hypertension ,hour glass constriction of uterus and retained products and the worst one-cardio respiratory arrest.This study aims to add up to the significance and substantiation to the use of Intravenous oxytocin as the first line drug in the management of PPH.

METHODS :

This was a prospective interventional comparative study conducted in the Department of Obstetrics and Gynaecology at Trichy SRM Medical college hospital and research centre ,Irungalur,Trichy between 01.12.2020 to 07.06.2021.The sample size taken was 100 with 50 in Group-A and 50 in Group B .

Inclusion criteria:

- 1)All singleton pregnancy with Gestational age > 37 weeks.
- 2) AN patients with intact membranes
- 3) All patients taken for LSCS-including elective and emergencies
- 4) Duration of surgery </= 2 hours .
- 5) BMI </= 30 and
- 6)Those giving willingness and consent for the study.

Exclusion criteria :

- 1)Multifetal gestation,
- 2)Duration of surgery > 2 hours
- 3)Previous H/O antepartum haemorrhage ,Postpartumhaemorrhage ,bleeding disorders
- 4)BMI>30

5)patients with known sensitivity to oxytocin and methergine

6)patients not giving consent

7)Patients with absolute contraindications to methergine like –heart disease, Rh negative pregnancy hypertensive disorder ,pre-eclampsia and those with peripheral vascular diseases.

The 100 AN patients taken for LSCS during the study period in the Institute is divided into 2 groups –A and B according to the random sampling .The following variables were taken into consideration 1)volume of blood loss in ml .2)fall or difference in Haemoglobin and Packed cell volume. 3)Duration of 3rd stage of labour 4) need for 2nd line drugs to control PPH

5)need for blood transfusion .After delivery of the baby ,time was given for spontaneous placental separation and the time was measured.For Group-A patients ,intravenous 10 U Oxytocin was given and the blood loss in ml was measured through separate suctioning .For Group-B patients intramuscular 0.2 mg methergine was given and the same process of blood loss measurement was done.

This study was discussed in the Institutional ethics committee and Institutional Research board in Trichy SRM Medical college and research centre and was approved .

RESULTS :

TABLE-1: Age wise distribution

| AGE | FREQUENCY | PERCENTAGE |
|--------------|-----------|------------|
| <20 yrs | 8 | 8.0 |
| 21-25 | 42 | 42.0 |
| 26-30 | 34 | 34.0 |
| 31 and above | 16 | 16.0 |

Age distribution was from 18 – 32 years . Out of the 100 subjects 45 (50%) were in the age group of 18-25 years. 34 % were between 26-30 years, and 16% were above 31 years

Table 1 Age wise distribution

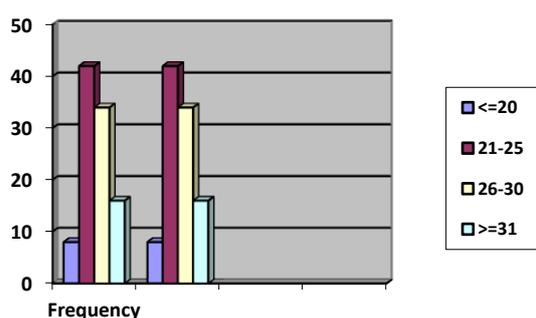


TABLE-2 :Age distribution in Oxytocin Group

| AGE range | Frequency | Percent |
|-----------|-----------|---------|
| <=20 | 5 | 10.0 |
| 21-25 | 17 | 34.0 |
| 26-30 | 18 | 36.0 |
| >=31 | 10 | 20.0 |

TABLE-3: Methergine Group-Agewise Distribution

| | Frequency | Percent |
|-------|-----------|---------|
| <=20 | 3 | 6.0 |
| 21-25 | 25 | 50.0 |
| 26-30 | 16 | 32.0 |
| >=31 | 6 | 12.0 |
| Valid | | |

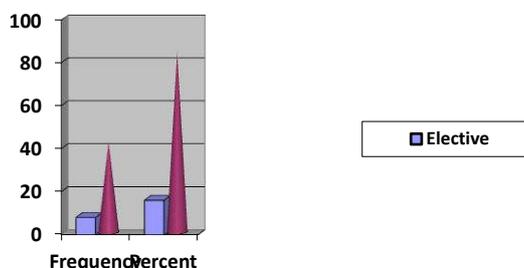
TABLE-4 Gravidia distribution in Oxytocin group

| | Frequency | Percent |
|---------------|-----------|---------|
| Primi gravida | 14 | 28.0 |
| Multi gravida | 36 | 72.0 |
| Total | 50 | 100.0 |

TABLE-5 Gravidia distribution in Methergine Group

| | Frequency | Percent |
|--------------|-----------|---------|
| Primigravida | 13 | 26.0 |
| Multigravida | 37 | 74.0 |
| Total | 50 | 100.0 |

In both the groups ,majority were Multigravida

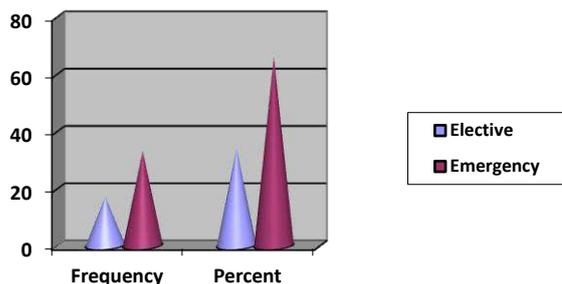
**Figure 2 Type of surgery in oxytocin Group****Table-6 Surgery type-Distribution in Oxytocin group**

| | Frequency | Percent |
|-----------|-----------|---------|
| Elective | 17 | 34.0 |
| Emergency | 33 | 66.0 |
| Total | 50 | 100.0 |

Table-7 Surgery Type in methergine group

| | Frequency | Percent |
|-----------|-----------|---------|
| Elective | 8 | 16.0 |
| Emergency | 42 | 84.0 |

| | | |
|-------|----|-------|
| Total | 50 | 100.0 |
|-------|----|-------|

Table 2-Surgery type in Methergine group

In Group A 66%(33)were emergency c sections and 34%(17) were elective.

In Group-B 84% (42)were Emergencies and 16%(8)were planned.

TABLE- 8 DESCRIPTIVE STATISTICS

| | Minimum | Maximum | Mean | Std. Deviation |
|----------------------|---------|---------|-------|----------------|
| Third stage duration | 3.0 | 5.2 | 4.0 | 0.4 |
| Blood loss | 240 | 320 | 280.4 | 23.6 |
| Pre OP Hb | 10.0 | 11.6 | 10.77 | 0.4 |
| Post OP Hb | 9.5 | 11.0 | 10.13 | 0.3 |
| Pre OP HCT | 28 | 33.2 | 29.7 | 1.3 |
| Post Op HCT | 26 | 30.0 | 27.6 | 1.3 |

TABLE-9: Comparison of different variables in both groups

| | Group | Mean | Std. Deviation | T | P value |
|----------------------|-----------|-------|----------------|------|---------|
| Blood loss | Oxytocin | 278.8 | 22.6 | -.67 | 0.5 |
| | methergin | 282.0 | 24.6 | | |
| Third stage duration | Oxytocin | 4.0 | .45 | .59 | 0.5 |
| | methergin | 3.9 | .48 | | |
| Pre OP Hb | Oxytocin | 10.7 | .42 | -.97 | 0.3 |
| | methergin | 10.8 | .43 | | |
| Post OP Hb | Oxytocin | 10.1 | .29 | -.71 | 0.4 |
| | methergin | 10.1 | .35 | | |
| Hb difference | Oxytocin | .62 | .42 | -.41 | 0.6 |
| | methergin | .66 | .48 | | |
| Pre OP HCT | Oxytocin | 30.0 | 1.4 | 2.3 | 0.02 |
| | methergin | 29.4 | 1.2 | | |
| Post Op HCT | Oxytocin | 27.9 | 1.1 | 2.1 | 0.03 |
| | methergin | 27.42 | 1.3 | | |
| HCT difference | Oxytocin | 2.12 | 1.4 | .23 | 0.8 |

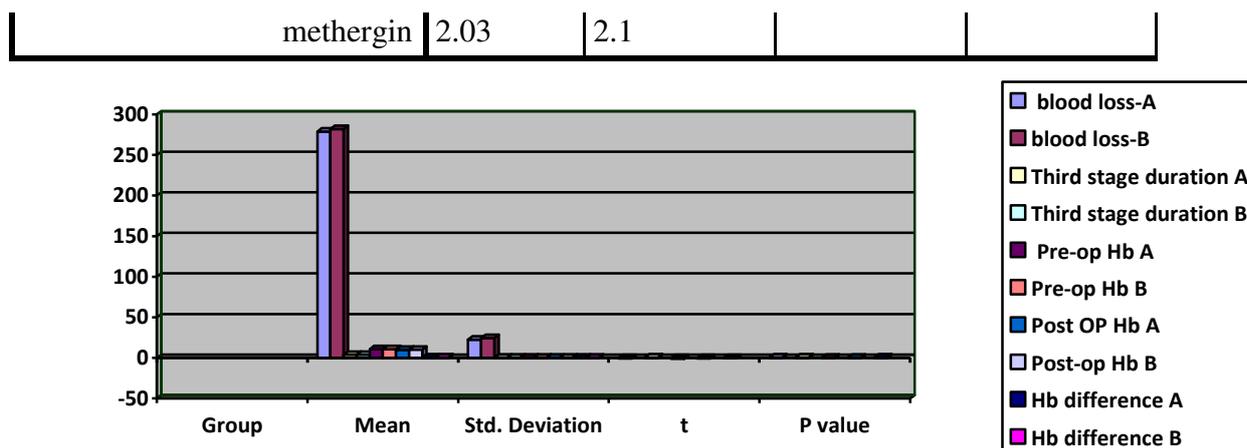


Figure 2-Comparison of variables

The Mean duration of third stage of labour among the group A was 4.034 ± 0.455 minutes and the mean duration in the B group is 3.978 ± 0.488 minutes. Comparison between the blood losses in the third stage of labour between the two groups is shown (Table 9). The mean Pre-operative Hb% in the A group was 10.73 ± 0.423 and the mean Hb% in the B group was 10.82 ± 0.437 . The mean Hb% 24hrs after delivery in the Oxytocin (A) group is 10.108 ± 0.2919 % and in the oxytocin group is 10.82 ± 0.437 gms% (Table 9).

The mean PCV in the A group was 30.0806 ± 1.4773 and the mean PCV in the group B was 29.456 ± 1.2455 % The mean PCV 24hrs after delivery in the group A was 27.964 ± 1.1783 and the mean PCV 24 hrs after delivery in the B group was 27.42 ± 1.3716 (Table 9).

Out of 100 cases in the study- 4 (4%) women needed additional oxytocic in the form of Intravenous 20 u Oxytocin . The remaining 96 (96%) did not need any additional oxytocics .

Independent Samples Test

| | | t-test for Equality of Means | | |
|---------------------------------|---------|------------------------------|---------|-----------------|
| | | Degrees of freedom | P value | Mean Difference |
| Third stage duration in minutes | A group | 96 | 0.4 | .06 |
| | B group | 95.9 | 0.4 | .06 |
| Hb difference | A group | 96 | 0.6 | 0.03 |
| | B group | 89.042 | 0.6 | 0.03 |
| PCV difference | A group | 96 | 0.6 | 0.14 |
| | B group | 84.231 | 0.6 | 0.14 |

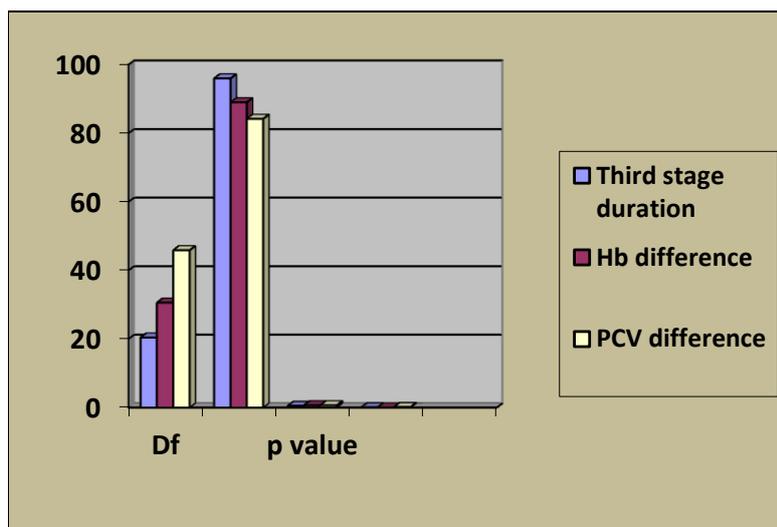


Figure 3-comparison between Group A and B-t Test for equality of means.

DISCUSSION:

The World Health Organization (WHO) statistics suggest that worldwide, 25% of maternal deaths are due to PPH, accounting for more than 100,000 maternal deaths per year. The death of these mothers has serious implications for the newborn and any other members of the family.¹⁰ The best choice of uterotonic will be the one which can be used in all levels of maternity care, which must be affordable at a low cost, should be without stringent storage conditions, easily accessible and with easy and simple administration technique.

Both oxytocin and methergine almost fits for all the above reason-with an additional advantage of oxytocin over methergine is its wide range of safety profile in place of methergine –which has certain absolute and relative contra-indications. In our study both were equally effective in controlling blood loss and fall of Haemoglobin from the pre-operative value. In Group-B, the fall in Haematocrit is lesser than Group-A, indicating the increased potency of methergine over Oxytocin, which was proved by statistical significance. In both the groups, there was no post-natal anaemia and need for blood transfusion.

In a study conducted by Adhikari S et al there was no statistical significance between the mean fall in both groups.⁴ In the present study, the mean fall in Hb in the Oxytocin group was $0.628 \pm 0.428\%$ and in the Methergine group it was $0.66 \pm 0.428\%$. The p value is 0.678, statistically insignificant.

In the study by Adhikari S, et al there was no statistical significance between the mean fall in PCV between the methergine group and the oxytocin group.⁴ In the present study the mean fall in PCV in the two groups was 2.122 ± 1.406 in the Oxytocin group and 2.036 ± 2.187 in the methergine group with a p value of 0.234 which is statistically insignificant.

In the study conducted by Adhikari S, et al the incidence of use of additional oxytocics was almost the same though statistically insignificant. The incidence of PPH was higher in oxytocin group compared to methylergometrine group but this did not reach statistical significance.⁴

In the present study only 4 (8%) women of the oxytocin group required the use of additional oxytocics and none of the women of either groups required exploration of the uterus or blood

transfusion. In the study by Adhikari S, et al the adverse effects of oxytocin were mild and they subsided spontaneously. Also the mean fall of PCV was statistically not significant, in the present study⁴

In our study 12 patients (24%) in Group-B had vomiting and 6 (12%) patients had increased blood pressure, while Group-A patients who were given Oxytocin alone did not have any side effects. The intravenous route is technically easy to administer during C-section and can be used in patients with contra-indications for methergine. Also, the additional advantage of Oxytocin, is whenever there is a need for additional uterotonic, dose escalation can be done without significant side effects.

Limitations: The main limitation is small sample size and external validity. Large sample studies, considering the various Antenatal risk factors like –anaemia complicating pregnancy, multiple pregnancy, pre-eclampsia, H/O previous surgeries on uterus, Fibroid complicating pregnancy –are imperative to advocate the same in future

SUMMARY : It is seen that intravenous oxytocin is as effective as intra-muscular methergine in lessening the blood loss after placental separation and thereby alleviating the incidence of post-partum haemorrhage. The plasma half life is 1-6 minutes and the clinical response is rapid after intravenous infusion almost as close as to the onset of action of methergine¹¹.

CONCLUSION :

- Oxytocin remains the first line uterotonic for the management of Post Partum Haemorrhage.
- This study has unveiled that both the uterotonics- Oxytocin and methergine were impartially worthwhile in the prevention and management of post-partum haemorrhage.
- The cost of Oxytocin is also relatively low and easily available.
- It can also be used in emergencies –like imminent delivery, when patient is received in 2nd stage without the knowledge of patient's previous history, whereas the other commonly used uterotonics (T.misoprostol, Inj. Carboprost, Inj. Tranaexamic acid) needs to be used with caution.

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Diagnostic accuracy of hemoglobin estimation by point of care devices – A comparative study with the automated hematology analyzer

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Abstract

Hemoglobin (Hb) estimation for the assessment of anemia can be done by various methods. Each of these methods has their own advantages and limitations. The present study was conducted to assess the accuracy of the commonly used point of care devices – Hemo Cue 301 and Handy span, a portable hemo calorimeter to assess haemoglobin against the automated hematology analyzer Coulter 780. Hb was assessed in fifty blood samples by utilizing the Hemo Cue, Handy span and Automated hematology analyzer Coulter 780. The results by Hemo Cue and Handy span devices were comparable with that obtained by the automated hematology analyzer Coulter 780. Hence it was derived that the Point of care devices such as Hemo Cue and portable hemo calorimetry (Handy span) are as accurate as the Automated hematology analyzer Coulter 780.

Keywords: Hemo globin, Cyan met hemo globin, Automated methods, Hemo Cue, Handy span, Point of care devices (POCD).

Introduction

Hemoglobin estimation is the most frequent laboratory investigation requested in clinical practice. Different methods are used to estimate the hemoglobin in the blood.

Besides the use of traditional laboratory resources, the diagnosis of anemia can also be accomplished by assessing hemoglobin (Hb) concentration with Point-of-care testing (POCT) devices such as the Hemo Cue, portable hemo calorimetry (Handy span) test systems. In several situations, these devices might suitably replace traditional laboratory testing, including several areas of health care where a very rapid hemoglobin measurement might be required to make immediate therapeutic decisions.

For instance, it could be imperative in patients who require critical venous access, especially neonates and those undergoing chemotherapy due to the low amount of blood required by these devices, as well as in natural disasters or in sports medicine.

Aims and objectives

The aims and objectives of the current study are to

➤ compare the haemoglobin values obtained by automated hematology analyzer Coulter 780 with the point of care devices (POCD) such as Hemo cue and portable hemo calorimetry (Handy span) and to evaluate the diagnostic accuracy of these Point of care devices.

Materials and methods

Hemoglobin was estimated from the venous and capillary blood samples collected from fifty adults using Hemo cue, Handy span and Automated hematology analyzer Coulter 780 method.

Hemocuehb 301 method

The fingertip is pricked with a sterile lance after cleaning the finger with 70% alcohol. Wipe away first 2-3 drops of blood. Fill the microcuvette in one continuous process with 10 microliters of blood. Place the filled cuvette in the cuvette holder. After 10 seconds the hemoglobin measurement is made. This is a photoelectric method based on the determination of azide methemoglobin.



Fig 1: Hemocue HB 301

Handy span method

The hemoglobin level is estimated using alkali-haematin method. The blood is diluted using an alkaline solution containing a non-ionic detergent at pH 13.0. This converts all hemoglobin derivatives into a stable end product, alkali-hematin. The absorbance maxima of alkali-hematin is at 575nm. Absorbance of alkali hematin is directly proportional to hemo globin concentration in blood.

$$\text{Hemoglobin concentration (gm/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$



Fig 2: Handy span.

Automated hematology analyzer coulter 780

The lytic reagent rapidly and simultaneously destroys the erythrocytes and converts a substantial proportion of the hemoglobin to a stable pigment. The absorbance of the pigment is directly proportional to the hemoglobin concentration of the sample. The accuracy of this method equals that of the hemoglobin cyanide method, the reference method of choice hemoglobinometry recommended by the international committee for standardization in hematology.



Fig 3: Coulter 780

The results obtained by the three methods were compared using appropriate statistical methods.

Results

Frequency Table

Age

| Particulars | No. of respondents | Percentage |
|-------------|--------------------|------------|
| 21 to 30yrs | 9 | 18.0 |
| 31 to 40yrs | 5 | 10.0 |
| 41 to 50yrs | 12 | 24.0 |
| 51 to 60yrs | 15 | 30.0 |
| 61 to 70yrs | 9 | 18.0 |
| Total | 50 | 100.0 |

One third (30 per cent) of the respondents were in 51 to 60yrs of age group.

Sex

| Particulars | No. of respondents | Percentage |
|-------------|--------------------|------------|
| Male | 33 | 66.0 |
| Female | 17 | 34.0 |
| Total | 50 | 100.0 |

Majority (66 per cent) of the respondents were male.

One-way ANOVA difference between age of the respondents and their method of Auto, Hemocue and Handy Span

| Age | n | Mean | S. D | SS | Df | MS | Statistical inference |
|----------------|----|---------|---------|--------|----|--------|---|
| Auto | | | | | | | |
| Between Groups | | | | 80.102 | 4 | 20.026 | F=2.593 .049>0.05 Not Significant |
| 21 to 30yrs | 9 | 12.6778 | 2.23594 | | | | |
| 31 to 40yrs | 5 | 14.7800 | .95760 | | | | |
| 41 to 50yrs | 12 | 11.5250 | 2.86773 | | | | |

| | | | | | | | |
|----------------|----|---------|---------|---------|----|--------|-------------------------------------|
| 51 to 60yrs | 15 | 10.6733 | 2.97453 | | | | |
| 61 to 70yrs | 9 | 12.9667 | 3.34664 | | | | |
| Within Groups | | | | 347.595 | 45 | 7.724 | |
| Hemocue | | | | | | | |
| Between Groups | | | | 88.477 | 4 | 22.119 | |
| 21 to 30yrs | 9 | 13.3444 | 2.23725 | | | | F=2.887 .033<0.05 Significant |
| 31 to 40yrs | 5 | 15.3600 | 1.04547 | | | | |
| 41 to 50yrs | 12 | 12.1500 | 2.83148 | | | | |
| 51 to 60yrs | 15 | 11.1200 | 2.99647 | | | | |
| 61 to 70yrs | 9 | 13.6667 | 3.28748 | | | | |
| Within Groups | | | | 344.768 | 45 | 7.662 | |
| Span | | | | | | | |
| Between Groups | | | | 63.072 | 4 | 15.768 | |
| 21 to 30yrs | 9 | 12.0889 | 2.29970 | | | | |
| 31 to 40yrs | 5 | 14.5400 | 1.07378 | | | | |
| 41 to 50yrs | 12 | 11.7750 | 2.88164 | | | | |
| 51 to 60yrs | 15 | 10.8267 | 2.74447 | | | | |
| 61 to 70yrs | 9 | 13.0333 | 3.85681 | | | | |
| Within Groups | | | | 362.713 | 45 | 8.060 | |

Statistical test: One way ANOVA 'f' test was used the above table

The above table auto methods of mean ± S.D values from 21 to 30yrs age group (n=9) 12.6778 ± 2.23594, 31 to 40yrs age group (n=5) 14.7800 ± 0.95760, 41 to 50yrs age group (n=12) 11.52.50 ± 2.86773, 51 to 60yrs age

group (n=15) 10.6733 ± 2.97453 and remaining 61 to 70yrs age group (n=9) 12.9667 ± 3.34664. Therefore, there is no significant difference between age of the respondents and their auto methods. Hence, the calculated value greater than table value (0.049>0.05)

T-Test

| | n | Mean | S. D | Statistical inference |
|---------|----|---------|---------|---|
| Auto | | | | |
| Male | 33 | 13.1091 | 2.78336 | t=3.987 df=48 0.000<0.05 Significant |
| Female | 17 | 10.0294 | 2.14148 | |
| Hemocue | | | | |
| Male | 33 | 13.7061 | 2.79586 | t=3.998 df=48 0.000<0.05 Significant |
| Female | 17 | 10.6000 | 2.16304 | |
| Span | | | | |

| | | | | |
|--------|----|---------|---------|---|
| Male | 33 | 13.0939 | 2.77409 | t=3.983 df=48 0.000<0.05 Significant |
| Female | 17 | 10.0235 | 2.14695 | |

Paired Sample 't' test

| | n | Mean | S. D | Correlation | Statistical inference | Mean | S. D | t | df | Statistical inference |
|---------|----|---------|---------|-------------|------------------------|--------|--------|---------|----|---------------------------|
| Auto | 50 | 12.0620 | 2.95441 | .997 | 0.000<0.05 Significant | -.5880 | .24714 | -16.824 | 49 | 0.000<0.05 Significant |
| Hemocue | 50 | 12.6500 | 2.97351 | | | | | | | |
| | n | Mean | S. D | Correlation | Statistical inference | Mean | S. D | t | df | Statistical inference |
| Auto | 50 | 12.0620 | 2.95441 | .975 | 0.000<0.05 Significant | .0120 | .66567 | .127 | 49 | .899>0.05 Not Significant |
| Span | 50 | 12.0500 | 2.94779 | | | | | | | |
| | n | Mean | S. D | Correlation | Statistical inference | Mean | S. D | t | df | Statistical inference |
| Hemocue | 50 | 12.6500 | 2.97351 | .973 | 0.000<0.05 Significant | .6000 | .69429 | 6.111 | 49 | 0.000<0.05 Significant |
| Span | 50 | 12.0500 | 2.94779 | | | | | | | |

Descriptive Statistics

| | n | Min. | Max. | Mean | S.D |
|---------|----|------|-------|---------|---------|
| Age | 50 | 21 | 70 | 47.36 | 14.659 |
| Auto | 50 | 6.00 | 19.10 | 12.0620 | 2.95441 |
| Hemocue | 50 | 6.40 | 19.90 | 12.6500 | 2.97351 |
| Span | 50 | 6.20 | 19.70 | 12.0500 | 2.94779 |

Karl Pearson coefficient correlation relationship between auto, haemacu and their span

| | Mean | S. D | Auto | Haemacu | Span |
|---------|---------|---------|----------|----------|----------|
| Auto | 12.0620 | 2.95441 | 1 | .997(**) | .975(**) |
| Hemocue | 12.6500 | 2.97351 | .997(**) | 1 | .973(**) |
| Span | 12.0500 | 2.94779 | .975(**) | .973(**) | 1 |
| n | | | 50 | 50 | 50 |

** Correlation is significant at the 0.01 level

Statistical test: Karl Pearson coefficient correlation test was used the above hypothesis table

The above table indicates that there is a significant relationship between automated, Hemocue and handy

span. Hence, the calculated value is less than table value (p**<0.05). So the research hypothesis is accepted.

Discussion

There are several reasons supporting the use of POCD devices, including those for Hb assessment, in clinical

and laboratory practice. First, saving time is critical in several areas of health care, where a very rapid Hb measurement might be required to make immediate therapeutic decisions.

This might happen in any health care context where the clinical laboratory is too far, making turnaround time incompatible with a fast triage (e.g., in decentralized health care facilities with no support of a clinical laboratory unit within a network or those organized according to a hub-and-spoke model), or in hospital units where the fastest possible turnaround time from shipping a sample for Hb assessment to the core laboratory might still be insufficient (e.g., critical hemorrhages in the operating room, intensive care patients)^{9,10}.

The availability of POCD for Hb assessment is also valuable due to the low amount of blood required by these devices in patients requiring critical venous access, especially neonates and those undergoing chemotherapy.

The use of POCD devices also represents the best option in the unfortunate circumstance of natural disasters, where there is a compelling need to convey laboratory technologies that can be easily transported, installed, and appropriately used outside the traditional laboratory environment.

Sports medicine is another ideal context for POCD, where rapid test results might guide the application of specific training regimens and testing when carried out with rigorous preanalytical and analytical requirements⁸.

In all these situations, POCD devices such as the Hemo Cue and Handy span might yield accurate Hb results within seconds, with a small amount of sample required and thereby less discomfort^{9,11}.

Our study reveals that there is no significant difference in the results obtained on statistical analysis of hemoglobin estimation by the above three methods-Hemo Cue ,handy span and automated hematology analyzer coulter 780 .

Diagnostic accuracy of Hemoglobin estimation by point of care devices with Automated Hematology Analyzer Method has been proved in our study. Therefore, anyone of these methods can be used depending upon the accessibility and cost effectivity.

Conclusion

It was concluded that the Hemoglobin (Hb) estimation done using point of care devices - Hemocue Hb 301 and handy span in spite of the different working principles were found to correlate with the standard Automated hematology analyzer coulter 780 method.

It is therefore recommended that for small samples and preliminary parameters like hemoglobin POCD methods can be employed since these are cost effective, easy in operation, requires less training and feasible to be used in field work. Moreover these PCOD methods have also been proven to have good diagnostic accuracy,

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Cytopathological Spectrum of Thyroid Lesions – A Hospital Based Study

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Introduction

Diseases of the thyroid gland are commonly encountered in clinical practice and comprise a spectrum of entities which cause systemic diseases like autoimmune thyroiditis a localised lesion such as colloid goiter or a tumor mass. Prompt accurate diagnosis and early management help in reducing the morbidity and mortality associated with the thyroid pathology. The significance of the study lies in bringing to focus the change in scenario of the variation in thyroid pathology in the recent years. This is an attempt to evaluate the spectrum of various thyroid lesions using cytopathology which is a simple and non-interventional method of study.

Aims and Objectives

1. To evaluate the morphology of the thyroid lesions by fine needle aspiration cytology in adult patients with swelling of the thyroid gland.
2. To study the distribution of lesions according to age and sex.
3. To correlate cytomorphological features of the thyroid gland lesions with histopathological features wherever possible.

4. To evaluate sensitivity, specificity and diagnostic accuracy of different lesions.

Source and Data for the Material and Methods

The present study was conducted in the Department of Pathology Chennai Medical College Hospital and Research Centre, Irungalur.

Duration of Study: Two year prospective study.

Sample Size: 100 cases

Inclusion Criteria: Adult patients with palpable thyroid gland enlargement attending the out and in -patient department in our hospital.

Exclusion Criteria: Children with thyroid enlargement below 12 years of age.

Materials and Method: After explaining about the procedure and getting his/her written consent, the Fine needle aspiration of the thyroid gland was performed. The smears were stained using Hematoxylin and Eosin, Giemsa and Papanicolaou stains and interpreted using Bethesda System of Reporting Thyroid Cytopathology (BSRTC).

Results and Discussion

Table 1: Distribution according to Bethesda classification in FNAC (n=100)

| Bethesda category | Frequency | Percent |
|-------------------|-----------|---------|
| 1 (ND/UNS) | 5 | 5.0 |
| 2 (BN) | 82 | 82.0 |
| 3 (AUS/FLUS) | 2 | 2.0 |
| 4 (FN/SFN) | 4 | 4.0 |
| 5 (SM) | 4 | 4.0 |
| 6 (M) | 3 | 3.0 |
| Total | 100 | 100.0 |

Comments: About 82% of the subjects belonged to category 2 in Bethesda classification of Cytopathological examination while 4% each were in category 4 and category 5. Malignant lesions (category 6) were seen in 3% and 2% of subjects were classified as category 3 with atypia of undetermined significance.

Fig 1: Distribution according to Bethesda classification in FNAC (n=100)

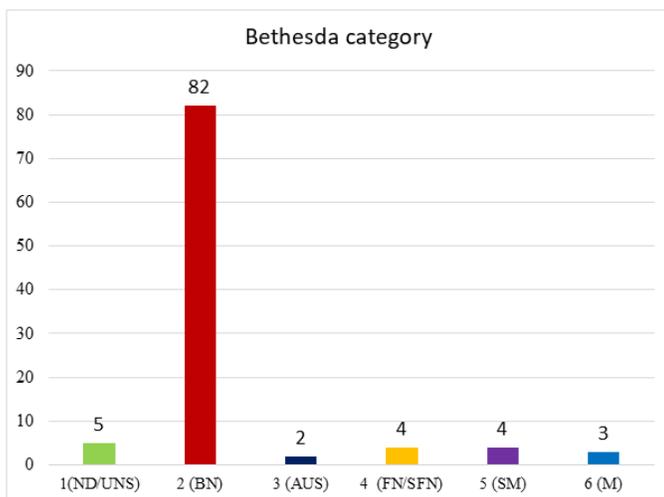


Table 2: Distribution of the thyroid lesions according to age and Bethesda classification in FNAC (n=100)

| Age group | Bethesda category | | | | | | Total |
|----------------|-------------------|----|---|---|---|---|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| 11 -20 years | 1 | 5 | 0 | 0 | 0 | 0 | 6 |
| 21 to 30 years | 0 | 12 | 0 | 2 | 0 | 2 | 16 |
| 31 - 40 years | 2 | 26 | 1 | 1 | 1 | 0 | 31 |
| 41 - 50 years | 1 | 23 | 1 | 1 | 1 | 0 | 27 |
| 51 - 60 years | 1 | 11 | 0 | 0 | 0 | 1 | 13 |
| >60 years | 0 | 5 | 0 | 0 | 2 | 0 | 7 |
| Total | 5 | 82 | 2 | 4 | 4 | 3 | 100 |

Comments: Majority of the thyroid lesions belonged to category 2 (82%) in Bethesda classification of Cytopathological examination which was common between 31 to 50 years (49%).

Table 3: Distribution of the individual thyroid lesions according to Bethesda classification in FNAC (n=100)

| Bethesda category | Number of cases n (%) | Cytological diagnosis | Number of cases n (%) |
|-------------------|-----------------------|--|-----------------------|
| 1 | 5 (5) | ND/UNS | 5 (5) |
| 2 | 82 (82) | Colloid goiter/ Multinodular goitre | 50 (50) |
| | | Autoimmune thyroiditis (hashimoto's thyroiditis) | 30 (30) |
| | | Granulomatous thyroiditis | 2 (2) |
| 3 | 2 (2) | Atypia of undetermined significance (AUS) | 2 (2) |
| 4 | 4 (4) | Follicular neoplasm | 2 (2) |
| | | Suspicious of Follicular neoplasm | 2 (2) |
| 5 | 4 (4) | Suspicious of papillary carcinoma | 3 (3) |
| | | Suspicious of Malignancy | 1 (1) |
| 6 | 3 (3) | Papillary carcinoma | 2 (2) |
| | | Poorly differentiated carcinoma | 1 (1) |
| Total | 100 (100) | | 100 (100) |

Comments: Colloid goiter/ Multinodular goiter was the commonest lesion (50%) followed by hashimoto's thyroiditis (30%). Category 4, 5 and 6 together accounted for 11% only.

Fig 2: Distribution of the individual thyroid lesions according to Bethesda classification in FNAC (n=100)

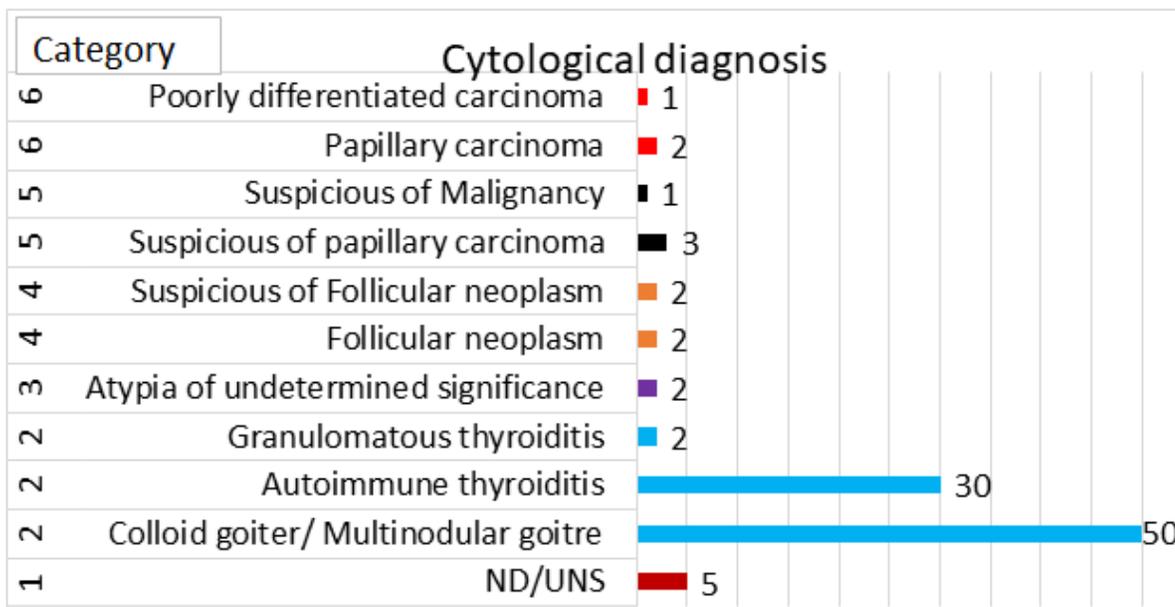


Table 4: Comparison of cytological diagnosis with various studies (n=100)

| Bethesda category (%) | Research study | | | | | |
|-----------------------|----------------|----------------|--------------|-------------|------------------|-----------------|
| | Current study | Prathima et al | Bhagat et al | Sinna et al | Shankar SP et al | Mondal SK et al |
| 1 (ND/UNS) | 5 | 11.7 | 5.6 | 7.1 | 10.7 | 1.2 |
| 2 (BN) | 82 | 77.5 | 87.5 | 33.1 | 81.6 | 87.5 |
| 3 (AUS) | 2 | 1.12 | 15 | 13.5 | 1.2 | 1 |
| 4 (FN/SFN) | 4 | 3.9 | 3.1 | 16.5 | 1.7 | 4.2 |
| 5 (SM) | 4 | 2.2 | 0.6 | 10.1 | 2 | 1.4 |
| 6 (M) | 3 | 3.3 | 3.1 | 19.5 | 2.7 | 4.7 |

Comments: The high proportion of category 2 lesions observed in the current study was comparable with almost all the studies except for Sinna et al. The proportion of follicular and papillary neoplasm also correlated with the findings of other study.

Fig 3: Comparison of cytological diagnosis with various studies (n=100)

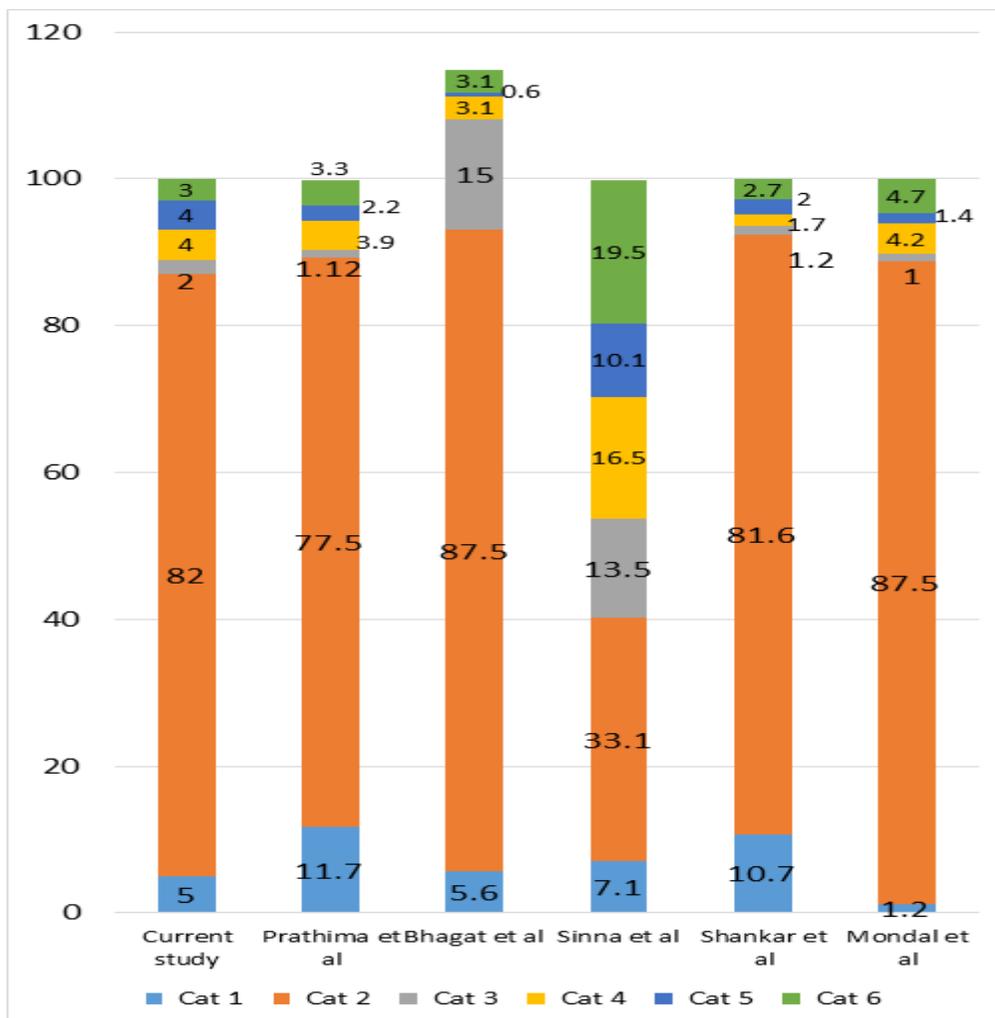


Table 5 Distribution of the individual thyroid lesions subjected to histopathology according to Bethesda classification in FNAC

| Bethesda category | Number of cases in FNAC n (%) | Histopathology done n (%) |
|-------------------|----------------------------------|------------------------------|
| 1 (ND/UNS) | 5 (5) | 0 (0) |
| 2 (BN) | 82 (82) | 20 (74.1) |
| 3 (AUS/FLUS) | 2 (2) | 1 (3.7) |
| 4 (FN/SFN) | 4 (4) | 2 (7.4) |
| 5 (SM) | 4 (4) | 2 (7.4) |
| 6 (M) | 3 (3) | 2 (7.4) |
| Total | 100 (100) | 27 (100) |

Comments: The high proportion of category 2 lesions accounted for high portion (74.1%) of the thyroid lesions subjected to histopathology. Two lesions each from category 4,5 and 6 were subjected to histopathology.

Table 6: Distribution of the thyroid lesions according to histopathology & Bethesda classification in FNAC (n=27)

| Bethesda category | No. of cases n (%) | Cytological spectrum of lesions | Number of cases n (%) |
|-------------------|-----------------------|--|--------------------------|
| 2 | 20 (74.1) | Colloid goiter/ Multinodular goiter | 15 (55.6) |
| | | Autoimmune thyroiditis (hashimoto's thyroiditis) | 2 (7.4) |
| | | Papillary carcinoma | 1 (3.7) |
| | | Follicular adenoma | 1 (3.7) |
| | | Hyperplastic colloid nodule | 1 (3.7) |
| 3 | 1 (3.7) | Follicular carcinoma | 1 (3.7) |
| 4 | 2 (7.4) | Colloid goiter/ Multinodular goitre | 1 (3.7) |
| | | Papillary carcinoma | 1 (3.7) |
| 5 | 2 (7.4) | Papillary carcinoma | 2 (7.4) |
| 6 | 2 (7.4) | Papillary carcinoma | 2 (7.4) |
| Total | 27 (100) | | 27 (100) |

Comments: Atleast one malignant lesion was found in thyroid lesions subjected to histopathology from all the Bethesda categories except category 1 as no lesion in that category was subjected to histopathological examination.

Table 7: Relation between cytological and Histological diagnosis (n=26)

| Category | Histopathology | | Total |
|----------|----------------|----------------|-------|
| | Neoplastic | Non-neoplastic | |
| 2 | 18 (FN) | 2 (TN) | 20 |
| 4 | 2 (TP) | 0 (FP) | 2 |
| 5 | 2 (TP) | 0 (FP) | 2 |
| 6 | 2 (TP) | 0 (FP) | 2 |
| Total | 24 | 2 | 26 |

Sensitivity = $6/24 = 25\%$, Specificity = $2/2 = 100\%$

Positive predictive value (PPV) = $6/6 = 100\%$

Negative predictive value (NPV) = $2/20 = 10\%$

Comments: In the current study, specificity and positive predictive value of cytological diagnosis according to Bethesda system was 100% but the sensitivity and negative predictive value was 25% and only 10% respectively. This is primarily because of the occurrence of high number of false negatives in category 2 of cytological diagnosis. Hence, it can be stated that Subjects reported to be suspicious of follicular neoplasm (cat 4), suspicious of malignancy (Cat 5) and malignant lesions (Cat 6) almost always had neoplastic lesions in histological diagnosis. Furthermore, the sample size is relatively small to generalize these results.

The result of this study is representative of the thyroid pathology presenting in this study population .

- A total of 100 cases with thyroid enlargement were subjected to fine needle aspiration cytology. Out of these, twenty seven cases had histopathological evaluation of their surgically removed thyroid.
- Using the gold standard technique - Fine Needle aspiration cytology which is a simple, safe, cost effective technique the cytopathological spectrum of the thyroid lesions was categorised using the standardized Bethesda system of reporting thyroid cytopathology and correlated with respective histopathological findings wherever possible.
- Age of the patients ranged from 18 to 80 years with a mean age being 41.3 years.
- Majority of the patients were females. Females-87% and Males 13% with a female to male ratio of 6.7 : 1 respectively.

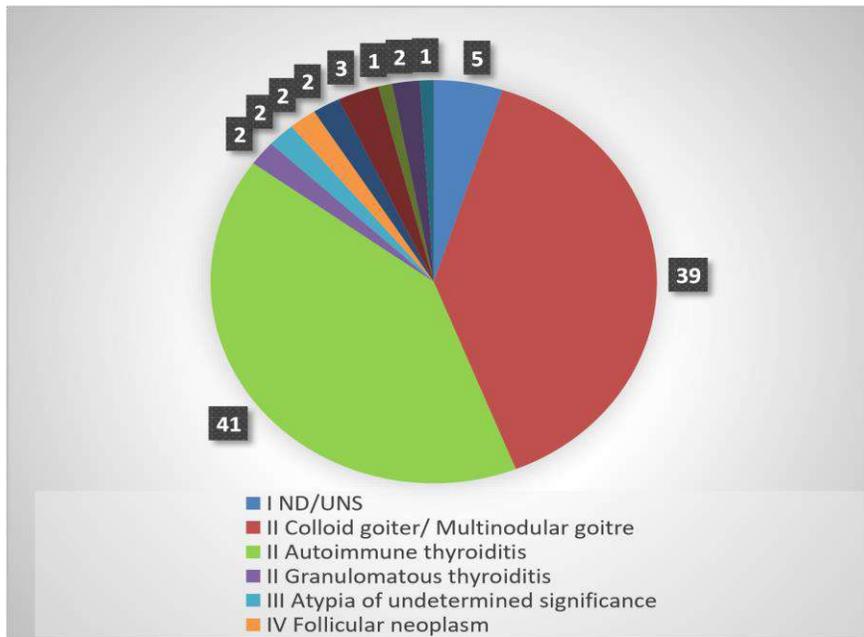
The cytological spectrum of the thyroid lesions in this study population as categorised using Bethesda system of reporting was as follows:

| | | |
|---|-----|---------|
| Colloid Goitre / Nodular colloid Goitre | 39% | CAT-II |
| Hashimoto thyroiditis | 30% | CAT-II |
| Lymphocytic thyroiditis | 11% | CAT-II |
| Granulomatous thyroiditis | 2% | CAT-II |
| Atypia of undetermined significance | 2% | CAT-III |

| | | |
|-------------------------------------|----|--------|
| Suspicious of Follicular neoplasm | 2% | CAT-IV |
| Suspicious of Papillary carcinoma - | 3% | CAT-IV |
| Follicular neoplasms | 2% | CAT-IV |
| Suspicious of malignancy | 1% | CAT-V |
| Papillary carcinoma | 2% | CAT-VI |
| Poorly Differentiated carcinoma | 1% | CAT-VI |

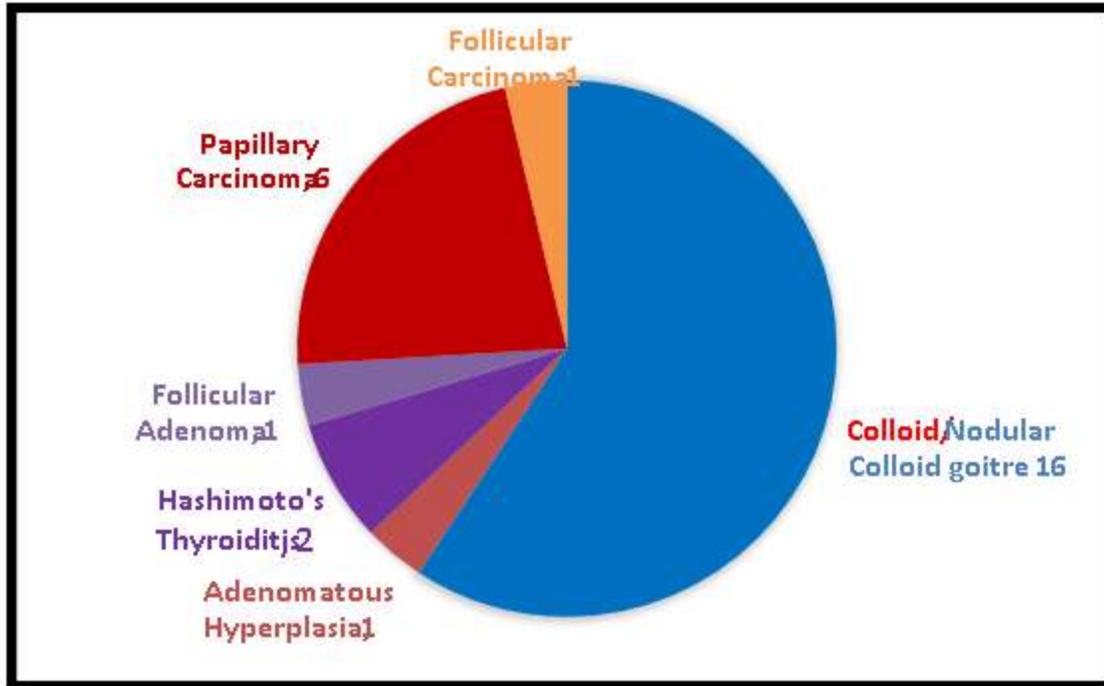
- In the present study , Autoimmune thyroiditis (41%) was found to be the commonest thyroid pathology to be diagnosed cytological, it was followed by Colloid goiter (39%).
- On cytology we reported one case as” Suspicious of Papillary carcinoma” which on histopathology was confirmed to be “Warthin tumor-like variant of Papillary Carcinoma” a very rare entity.
- Another interesting observation was of a case with long standing history of thyroid enlargement which was reported as “Follicular lesion of undetermined significance” on FNAC but turned out to be “Follicular carcinoma with Hurthle cell change” on histopathology.
- Among the malignant lesions reported on cytology, Follicular and Papillary neoplasms were of equal proportion.

Fig 4: Spectrum of thyroid lesions in FNAC (n=100)



- Out of the 100 cases subjected to thyroid cytology, 27 patients had surgical resection of their thyroid which revealed a spectrum of lesions on histopathology : Colloid goitre/Nodular colloid goiter, Adenomatous hyperplasia, Hashimoto’s thyroiditis , Follicular adenoma, Follicular carcinoma and Papillary carcinoma.

Fig. 5: Spectrum of thyroid lesions on Histopathology (n=27)



- Among the thyroid lesions confirmed by histopathology, Colloid / Nodular colloid goitre was the commonest (59%) followed by Papillary carcinoma (22%) and Hashimoto thyroiditis 7.4% respectively.
- Risk of malignancy on surgical resection for FNA thyroid diagnostic categories using TBSRTC was found to be as follows:
 - CAT-I -0%, CAT II -5%, CAT IV-50% , CAT III,V and VI 100%.
 - Cytological and histopathological correlation revealed that 81% of the lesions were concordant.
 - The specificity and the positive predictive value was 100%.
 - Sensitivity and negative predictive value was 25% and 10%. This is primarily because of the occurrence of high number of false negatives in category II on cytological diagnosis. Furthermore, the sample size is too small which is the limitation of our study.

Conclusion

The present study concludes that in our study population, Benign lesions of the thyroid are the most common based on Fine needle aspiration cytology with Autoimmune (Hashimoto's) thyroiditis being the predominant pathology reported here.

This study brings to focus the recent change in the scenario of the cytological spectrum of thyroid lesions i.e a shift from Iodine-deficient Colloid goitres to Autoimmune-based Thyroiditis.

In view of the fact that Hashimoto thyroiditis shows marked clustering with other auto immune diseases, it is strongly recommended that all cases with thyroid enlargement should undergo Autoantibody study along with the routine thyroid hormonal screening, clinical follow up and intermittent FNAC to reduce the incidence of associated morbidity.

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Histomorphological Evaluation Of Invasive Ductal Carcinoma Of Breast In A Tertiary Care Centre-India

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Conflicts of Interest: Nil

Abstract

In India, breast cancer is the most common cancer affecting the female population. Invasive ductal carcinoma is the most common histological subtype prevalent in India.^[1] Prognostic factors studied through routine histopathological examination are tumour size, histological grade and lymph node metastasis.

Objectives: To study the prognostic factors like tumour size, histological grade and lymph node metastasis status in the study samples.

Materials And Methods: Study was conducted in the Department of Pathology, Government Stanley Medical College between November 2016 to April 2017. 108 Modified Radical Mastectomy (MRM) specimens with the histopathological diagnosis of Invasive Ductal Carcinoma of breast were enrolled. Clinical details like age and tumour laterality were collected from the histopathology requisition forms. Formalin fixed paraffin embedded tissue blocks were stained with haematoxylin and eosin stain to study the tumour size, histological grade, lymph node metastasis and margin status.

Results: Mean age of this study population was 53 years. Out of 108 cases, 21 cases belonged to Grade I, 65 cases belonged to Grade II, 22 cases were of Grade III tumours. 18 cases were of tumour size less than 2 cm, 68 cases belonged to tumour size of 2-5cm, 22 cases belonged to tumour size of more than 5 cm. 48 cases had less than 3 nodal metastasis and 60 cases had 4 to 9 positive lymph node metastasis. 3 cases had microscopically positive resected margins.

Conclusion: To conclude this study through histopathological examination, the study population had better prognosis with lower tumour grade, lesser tumour size and lymph node metastasis.

Keywords: Histomorphology, Invasive ductal carcinoma, Prognostic factors

Introduction

In recent times there is an increase in incidence of breast cancer among the Indian female population.^[1] According to the WHO classification, there are various histological subtypes of breast cancer. Among the various histological subtypes, Invasive ductal carcinoma is the most common subtype in our country ^[2]. Histomorphological

examination of the breast cancer remains the gold standard in analysing the prognostic factors. The salient prognostic factors which can be studied by routine histopathological examination are the tumour size, tumour grade and lymph node metastasis. The main objective of this study is to analyse the tumour grade, tumour size and nodal metastasis in the study population. Early diagnosis and the treatment can

reduce the mortality and morbidity among the breast cancer patients.

Materials And Methods

After obtaining Institutional Ethical Committee clearance this study was conducted in Government Stanley medical college, a tertiary care centre in Chennai, Tamil Nadu, between November 2016 to April 2017. It was a descriptive prospective and retrospective study. **Inclusion Criteria:** Total of 108 modified radical mastectomy specimens with the histopathological diagnosis of Invasive Ductal Carcinoma were included in this study. **Exclusion Criteria:** All other histopathological subtypes, cases with the history of neoadjuvant chemotherapy, were excluded from the study. Clinical details like age and tumour laterality were collected from the histopathology requisition form. All the specimens were formalin fixed, meticulously grossed to detect the tumour size. Bits were taken, processed manually and their corresponding paraffin embedded blocks were made. Haematoxylin and Eosin stained slides were prepared from the paraffin embedded blocks.

Microscopic examination of all the slides were done to the analyse the tumour grade, the lymph node metastasis and resected margin positivity status. Tumours were graded by Nottingham modification of Scarff Bloom Richardson Grading System. All the data were entered in Microsoft Excel spreadsheet (windows10) and analysed statistically.

Results

Mean age of this study population was 53 years, with the youngest case being 36 years old. 19 % of the cases (20 cases) were aged below 45 years and 81 % (88 cases) were aged above 45 years of age. Out of 108 cases, 74 cases (69%) were right sided breast cancer, whereas 34 cases (31 %) were left sided breast cancer. 18 cases (17 %) had tumour of size less than 2 cm, 68 cases (63%) were of tumour size more than 2cm but less than 5 cm and 22 cases (20%) had more than 5 cm tumour size. [Table 1]. Among the 108 cases, 21 cases (19%) belonged to Histological Grade I tumour, 65 cases (61%) belonged to Histological Grade II tumour [Figure 1], 22 cases (20 %) were of Histological Grade III tumours. [Figure 2], [Table 2].

Table 1: Distribution of 108 cases based on Tumour size

| Tumour Size | Number of cases |
|-----------------------------------|-----------------|
| Less than 2 cm | 18(17%) |
| More than 2 cm and less than 5 cm | 68(63%) |
| More than 5 cm | 22(20%) |

Table 2: Distribution of 108 cases based on Histological Grades

| Histological Grade | Number of cases |
|--------------------|-----------------|
| Grade I | 21(19%) |
| Grade II | 65(61%) |
| Grade III | 22(20%) |

48 cases (45%) had nodal metastasis in less than 3 lymph nodes and 60 cases (55 %) had nodal metastasis in 4 to 9 lymph nodes. [Figure 3], [Table 3]. Out of 108 specimens, only 3 cases had positive resected margins.

Table 3: Distribution of 108 cases based on Lymph node metastasis

| Number of positive lymph nodes | Number of cases |
|--------------------------------|-----------------|
| 1 to 3 nodes | 48(45%) |

| | |
|--------------------|---------|
| 4 to 9 nodes | 60(55%) |
| More than 10 nodes | 0(0%) |

Figure 1: Histopathological image of Grade II tumour.

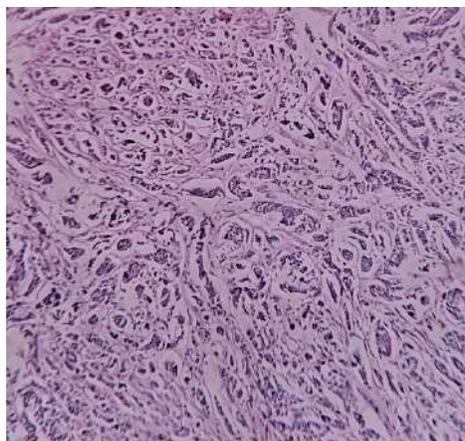


Figure 2: Histopathological image of Grade III tumour

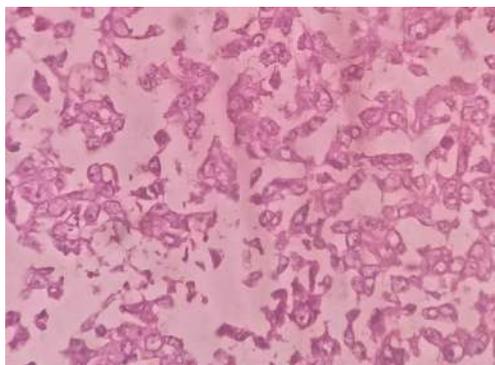
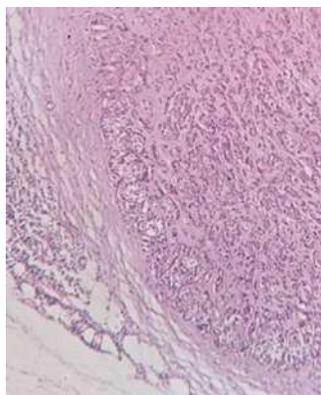


Figure 3: Histopathological image of lymph node with metastatic carcinomatous deposit



Discussion:

In this study the mean age of the study population was 53 years, with 81% of cases (88) were above 45 years. This shows that in the present study, majority

of the cases were in perimenopausal and in postmenopausal state. This was in concordance with the study done by Shetty and Kusuma K N et al ^[3] and Pradhan A et al ^[4].But this was in contrast with

other Indian studies which were carried over by Jitendra Singh Nigam *et al* [5] and A Goel *et al* [6] were the mean age of the study was below 45 years of age.

In the present study, majority of the cases were of tumour size more than 2cm but less than or equal to 5 cm (pT2 Stage of pTNM staging, AJCC 8th edition). [7] This result was similar to the studies done by Lavanya N *et al* [8], Lakmini *et al* [9], Shukla A *et al* [10] and Shetty and Kusuma K N *et al* [3]. This shows that in India, majority of the breast cancer patients presents with T2 stage.

In our study, majority of the tumour 65 cases (61%) were of Histological Grade II Tumour (moderately differentiated) which coincided with the other Indian studies by Manjunatha YA *et al* [11], Reddy *et al* [12] and Dr Lavanya N *et al* [8]. This shows that majority of the Indian breast cancer patient presents with moderately differentiated tumours (Grade II).

In this study, 55% of tumours (60 cases) belonged to N2 involving 4 to 9 lymph nodes. This is in contrast with the other studies done in Indian women by Dr Lavanya N *et al* [8], in Chinese women by Lin-Wei Wang *et al* [13] and in Iranian women by Afsharfard A *et al* [14] were majority of the cases had metastasis in less than 3 lymph nodes. This shows that our present study population had higher nodal metastasis.

In our study out of 108 specimens, only 3 specimens had tumour positive surgical resected margin. Rest of the specimens showed negative surgical resected margins. Thus our study population belonged to surgically resectable stage of breast cancer which in turn implies good prognosis.

Conclusion:

In the present study, mean age of the study population was 53 years. This implies that in the study population, breast cancer were common among perimenopausal, postmenopausal women than the women of reproductive age group. This study population presented with the surgically resectable tumour with lesser tumour size (T2), lower Histological Grade (II) and Nodal metastasis (N2). Thus our study population have comparatively better prognosis than population with non resectable tumour of high tumour grade and nodal metastasis. Breast cancer awareness and motivation for breast self-examination can lead to early presentation of cancer

cases to the clinicians. Early diagnosis and treatment can reduce the morbidity and mortality of breast cancer. To conclude, this study highlights the importance of histomorphological examination of mastectomy specimens for not only diagnosing the cancer but also in establishing the prognostic indicators like tumour grade, tumour size and lymph node metastasis.

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RESEARCH ARTICLE

Prevalence of pregabalin prescribing trends from various outpatient department: A prospective observational study from a tertiary care teaching hospital at South India

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ABSTRACT

Background: We assessed utilization patterns, profile of diagnoses, and fixed dose combination (FDC) with pregabalin in patients with various indications. **Aims and Objectives:** The aim of the study was to estimate the prevalence of pregabalin prescribing pattern among the patients attending the various outpatient departments (OPDs) of a tertiary care hospital at Trichy in South India. **Materials and Methods:** A hospital-based prospective observational study was planned and conducted over a period of 12 months. Patients who attended the various OPDs were included in the study. Prescriptions were collected from the respective consulting departments and pharmacy. **Results:** A total of 2490 patients prescription were analyzed. There was female preponderance (54.22%). Majority (45.74%) were in the age group of 51–64 years. General medicine was the highest (41.36%) visited patients. Neuropathic pain was the most prevalent disease condition among the various age group (57.67%), and predominant between 40 and 65 years (55.86%). Total of 12,220 drugs were prescribed, most frequently prescribed drugs belong to category of plain pregabalin 75 mg (53.37%) with female predominance, with maximum prescription from the medicine department (40%). Among the FDC, pregabalin 75 mg with nortriptyline 10 mg was the highest prescribed drug by the orthopedic department (41%). Extended release pregabalin 75 mg (13.04%) and low dose pregabalin 50 mg (2.19%) were also prescribed in our study. **Conclusion:** The prevalence of pregabalin prescription was maximum to neuropathic pain followed by radicular pain, trigeminal neuralgia, claudication, and herpetic neuralgia. Plain pregabalin 75 mg is preferred by most of the departments, few preferred extended release of 75 mg. Among the various FDC, pregabalin 75 mg with nortriptyline 10 mg and with methylcobalamine 750 mcg were prescribed by many departments.

KEY WORDS: Pregabalin; Prevalence; Prescribing Trends; Fixed Dose Combination

INTRODUCTION

Current trend in prescription pattern has been changing according to existence and arrival of newer drugs. The

adherence and pattern of prescription various distantly among different ethnicity. Many a time it is influenced by both communicable and non-communicable disease, its prevalence, environmental influences, socioeconomic status, and multiple patient's characteristics.

Pregabalin is a newer generation gabapentinoid, synthesized four decades ago and the molecule was initially developed as an adjuvant antiepileptic drug. Moreover, nearly two decades ago after its release, the off-label prescriptions for many communicable and non-communicable disease conditions have been preferred about 90% of its use than epilepsy.^[1]

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Documental evidence for the treatment of painful diabetic neuropathy and postherpetic neuralgia (PHN) is preferred by pregabalin and it is one of the earlier drug approved by the USFDA at 2004.^[2] Mechanism of Pregabalin endeavor its analgesic action and causes inhibition of excitatory neurotransmitters in the pain pathways of both central and peripheral nervous system through antagonistic activity at the voltage gated Ca²⁺ channels where it binds to the alpha-2- delta subunit of presynaptic voltage-gated calcium channels.^[2,3] The drug pregabalin is inclined towards for its long duration of action than gabapentin due to convalescent pharmacokinetic profile.

For the treatment of generalized anxiety disorder and Resistant partial epilepsy, pregabalin is approved as an add on drug therapy.^[4] Furthermore, in epilepsy there is 50% curtailment in seizures incidence been reported for patients using pregabalin as an accoutrement drug therapy.^[5] Besides, existing pregabalin trials are of short duration and long term trials are inadequate to fully characterize to accommodate in the therapy.^[5]

Nevertheless, a few western and other clinical trials were of relatively short durations and involved a population of highly selected patients, which could reflect in the real-world practice, may not be adequate and bias may occur without doubt. Considering the importance of pregabalin, the majority of the pivotal trials were carried out in western population and patients of Asian descent were under-presented.^[6] On evaluating the previous review of literature, the present study designed and aimed at evaluating the prevalence of pregabalin in the general population suffering from various disease conditions and the preference of particular drug dose and fixed dose combination (FDC) especially in South India.

Pregabalin has many indications and there is reluctant usage on how drugs are used in current clinical practice. Our concord methods designed to assess the utilization patterns, profile of diagnoses and FDC with pregabalin in patients with various indications.

MATERIALS AND METHODS

A prospective, observational, and cross-sectional hospital-based study, involving patients of one and the other gender were included in our study. It was planned and conducted over a period of 12 months from January 2021 to December 2021 at Trichy SRM Medical College Hospital and Research Centre, a 950 bed tertiary care teaching hospital, Trichy, India. The study conducted after obtaining institutional ethical clearance from institutional ethical committee No: TSRMMCH&RC/ME1/2020/093.

Patients of twain gender aged above 18 years attended the various outpatient departments (OPDs) and pharmacy

were identified and included in our comprehensive study. Prescriptions were collected from the individual consulting rooms of various OPDs, pharmacy and from medical record room at the end of each working day. Finally, the prescription information obtained was transferred into a detailed data sheet.

For our detailed study, the data was collected in a pro forma which was structured and concorded, which include the age, sex, department, diagnosis, prescribed drugs, and their dosages. All the following data were analyzed under the various headings such as, demographic details, prevalence of disease conditions, comorbid illness, department wise distribution of patients, category of drugs, mostly frequently prescribed drugs and FDC.

Statistical Analysis

The collected data were entered and analyzed using Microsoft Excel 2019. Chi-square test was performed for significance using SPSS Statistics version 20 software.

RESULTS

A total of 2490 patients prescription were included and analyzed in our study in the 12 month period. Among the 2490 patients, 1140 (45.78%) were male and 1350 (54.22%) were female, giving a female predominance which is exhibited in Table 1. Our demographic data paraded that the most of the patients were in the age group of 51–64 years (45.74%), followed by age group of 36–50 years were (29.16%), 65–80 years were (11.97%), and 18–35 years were (10.84%); the truncated number of patients were in the age group of 81–90 years (2.29%) disported in Figure 1.

Table 2 displayed the department wise distribution of patients, in that the maximum number of patients visited to general medicine OPD (1030; 41.36%), followed by orthopedic OPD

Table 1: Gender distribution of patients

| Gender | No of patients | Percentage |
|--------|----------------|------------|
| Male | 1140 | 45.78 |
| Female | 1350 | 54.22 |

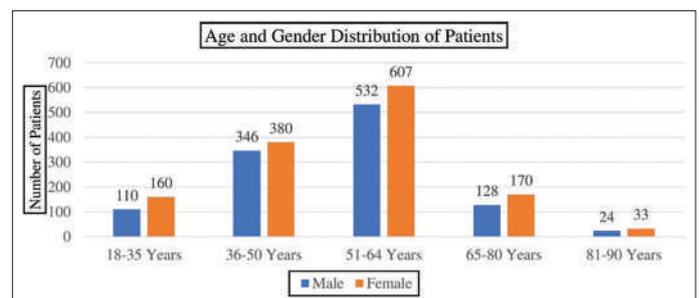


Figure 1: Demographic data

(860; 34.54%), and neurology OPD (185; 7.43%). The lowest number of patients visited to nephrology OPD (55; 2.21%).

The disease condition prevalent among these 2490 patients was belong to predominantly suffering from neuropathic pain, almost 1436 (57.67%) patients with maximum at the age group of 40–65 years (55.86%) ensuing by elderly age group. Diseases related to radicular pain were the second most conspire for attending the hospital (985; 39.55%), ensuing by trigeminal neuralgia (31; 1.24%), and claudication disorder (28; 1.12%). The least frequently encountered was herpetic neuralgia (10; 0.4%), which is flaunted in Table 3.

Among the frequently prescribed drug, the maximum prescriptions belong to plain pregabalin 75 mg (919; 36.90%) with female predominance followed by FDC of pregabalin 75 mg with nortriptyline 10 mg (828; 33.25%) and FDC of pregabalin 75 mg with methylcobalamin 750 mcg (609; 24.45%) which is flaunted in Figure 2. Extended release

Table 2: Department wise distribution of patients

| Department | No of patients <i>n=2490, n (%)</i> |
|------------------|-------------------------------------|
| General medicine | 1030 (41.36) |
| Orthopedics | 860 (34.54) |
| Neurology | 185 (7.43) |
| Neurosurgery | 160 (6.43) |
| Cardiology | 120 (4.82) |
| General surgery | 80 (3.21) |
| Nephrology | 55 (2.21) |

Table 3: Prevalence of disease condition among the various age group

| Disease/condition | Total (<i>n=2490</i>) <i>n(%)</i> | < 40 Years (<i>n=275</i>), <i>n (%)</i> | 40 to 65 years (<i>n=1860</i>), <i>n (%)</i> | >65 years (<i>n=355</i>), <i>n(%)</i> | <i>P</i> -value (Chi-square test) |
|----------------------|-------------------------------------|---|--|---|-----------------------------------|
| Neuropathy pain | 1436 (57.67) | 178 (64.73) | 1039 (55.86) | 219 (61.69) | 0.002** |
| Radicular pain | 985 (39.55) | 90 (32.73) | 789 (42.42) | 106 (29.86) | 0.001** |
| Trigeminal neuralgia | 31 (1.24) | 02 (0.73) | 09 (0.48) | 20 (5.63) | 0.001** |
| Claudication | 28 (1.12) | 04 (1.45) | 18 (0.97) | 06 (1.69) | 0.4 |
| Herpetic neuralgia | 10 (0.4) | 01 (0.36) | 05 (0.27) | 04 (1.13) | 0.06 |

***P*<0.05 significant value

preparation of pregabalin 75 mg and low dose pregabalin 50 mg was also preferred in our consideration.

Total of 12,220 drugs prescribed to 2490 patients, the highest prescribed drugs belong to the category of plain pregabalin 75 mg (53.37%) followed by FDC with pregabalin, which is flashed in Figure 3.

Among the department wise distribution of drugs, general medicine department prescribed the maximum FDC (1437; 57.71%) of pregabalin 75 mg with nortriptyline 10 mg and Methylcobalamin 750 mcg when compared to plain pregabalin 75 mg (919; 36.90%) which is exhibited in Table 4.

DISCUSSION

In this elaborative long duration study, out of 2490 patients, supplemental female patients (54.22%) visited the outpatient department and majority of them were in the age group of 51–64 years (45.74%), ensuing by age group of 36–50 years were (29.16%). Patients who were sort out the outpatient department, majority were consulted the general medicine department (41.36%) ensuing by orthopedics department (34.54%) and neurology department (7.43). Neurosurgery, cardiology, and general surgery department were also preferred by the patient due to comorbid condition. Considering the overall seven departments, our study shows that the least consulted department was the nephrology outpatients (2.21%). Considering the developing nation like India, the

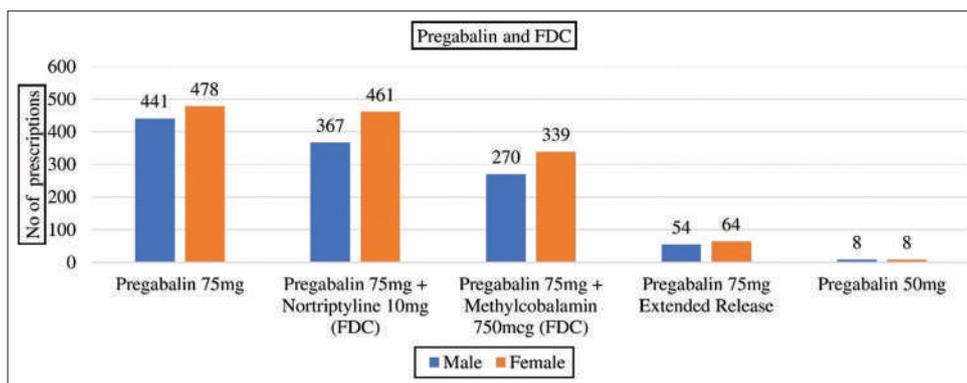


Figure 2: Demographic details of frequently prescribed drugs

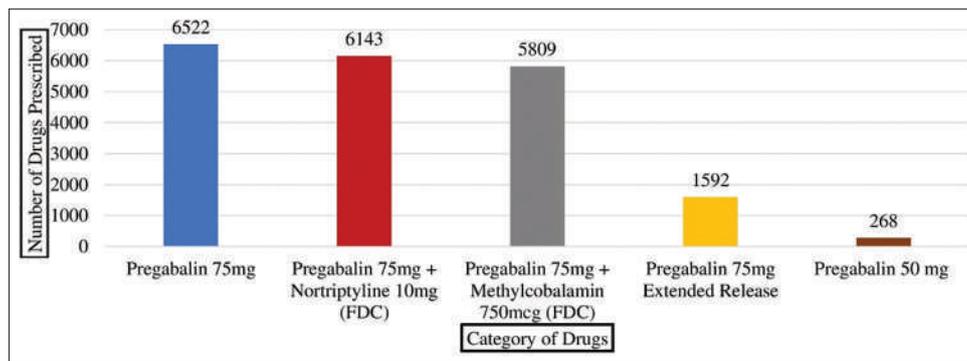


Figure 3: Total number of individual drugs prescribed

Table 4: Department wise distribution of drugs

| Name of the drug | n=2490, n(%) | General medicine n=1030, n (%) | Orthopaedics n=860, n (%) | Neurology n=185, n (%) | Neurosurgery n=160, n (%) | Cardiology n=120, n (%) | General Surgery n=80, n (%) | Nephrology n=55, n (%) |
|---|--------------|--------------------------------|---------------------------|------------------------|---------------------------|-------------------------|-----------------------------|------------------------|
| Pregabalin 75 mg | 919 (36.90) | 420 (40.77) | 256 (29.77) | 69 (37.30) | 78 (48.75) | 56 (46.67) | 28 (35%) | 12 (21.82) |
| Pregabalin 75 mg+Nortriptyline 10 mg (FDC) | 828 (33.25) | 327 (31.75) | 353 (41.05) | 48 (25.95) | 43 (26.88) | 21 (17.5) | 19 (23.75) | 17 (30.91) |
| Pregabalin 75 mg+Methylcobalamine 750 mcg (FDC) | 609 (24.46) | 241 (23.40) | 214 (24.88) | 51 (27.57) | 30 (18.75) | 35 (29.17) | 23 (28.75) | 15 (27.27) |
| Pregabalin 75 mg Extended Release | 118 (4.74) | 42 (4.08) | 25 (2.91) | 17 (9.18) | 9 (5.62) | 8 (6.67) | 10 (12.5) | 7 (12.73) |
| Pregabalin 50 mg | 16 (0.64) | – | 12 (1.40) | – | – | – | – | 4 (7.27) |

recent trend of non-communicable disease comorbidities has been a threat to all the general practitioners and epidemic spurt of communicable disease attack the general population. Its prime time to decide the right drug at right time at right dosage of pregabalin to prevent morbidity and mortality.

The prevalence of disease condition among the various age group revealed that, most of the patient visited the hospital for neuropathy pain (57.67%) followed by radicular pain (39.55%), trigeminal neuralgia (1.24%), and claudication (1.12%), with least infected herpetic neuralgia (0.4). In our present study, for different disease a total of 12220 drugs were prescribed to a total of 2490 patients. The most concur prescribed drugs in this elated study were belong to pregabalin 75 mg with female predominance of 478 prescription and male patient of 441 prescription was prescribed by once daily dosage of pregabalin in the night. The second most prescribed drug was FDC of pregabalin 75 mg with nortriptyline 10 mg once daily in the night as a preferred drug with female predominance of 461 prescriptions and 367 prescriptions for male patients. The FDC of pregabalin 75 mg with methylcobalamin 750 mcg were also prescribed for 339 female patients and 270 male patients as a once daily dosage.

As per the department wise distribution of drugs, it reveals that the FDC of pregabalin 75 mg with either nortriptyline

or methylcobalamin 750 mcg were highly preferred for all the disease condition related to the department visited by the patients for their comorbidities. Among the various department, orthopedics was the highest prescribed FDC of pregabalin 75 mg with nortriptyline 10 mg when compared to general medicine and other departments, Whereas the FDC of pregabalin 75 mg with methylcobalamin 750 mcg was highly prescribed by general medicine department when compared to orthopedic and other departments. Plain pregabalin 75 mg as an individual drug was overall prescribed by all the departments, especially by the general medicine followed by orthopedics, neurology, neurosurgery, cardiology, general surgery, and nephrology.

Our study reveals an another interesting finding of extended release pregabalin 75 mg was also preferred by most of the department for their drug therapy, preferred by physician and surgeons with female predominance of 64 prescriptions and 54 belongs to male patients. Even the lowest dosage of 50 mg pregabalin also prescribed to 16 patients by the department of orthopedics and nephrology, which was the least prescribed drugs in our study, whereas the other department not used the low dose pregabalin. In our observation, all the drugs were prescribed as a once daily dosage in the night time.

Regarding the age and gender distribution, analyzing our finding we were able to get a similar studies on persistence

of pregabalin treatment in Taiwan: A nation-wide population-based study confirms female preponderance and median age group of 60 years.^[7,8] Moreover the situation is different in a few more western countries and this is clearly shown by some Saudi pharmaceutical journal and other studies among the pregabalin uses where a predominantly male population.^[9] Considering the age group of our study population, most of the non-communicable disease risk factors and its complication may occur at median age of 60 years, could be the highest preponderance. Even elderly and young population were need of pregabalin therapy for their comorbidities. This can be persuasive, as majority of the patients were suffering from neuropathic pain, radicular pain, trigeminal neuralgia, claudication, and herpetic neuralgia from various disease comorbidities, they consulted the particular outpatient department.

All the diseased condition were more prevalent in the age group of 40–65 years ensuing by elderly age group of above 65 years and few belong to the age group of < 40 years, with statistically significant for neuropathy pain, radicular pain, and trigeminal neuralgia. Yen-feng wang *et al.* shows similar prevalence of neuropathic pain and herpetic neuralgia.^[7] Dutta *et al.* by drug utilization pattern in a pain clinic shows female predominance.^[10] Jena *et al* shows similar female predominance.^[11] Extended release pregabalin 75 mg were preferred and prescribed in this study, one western study by Yasmin and Onkar also studied extended release pregabalin.^[12]

Khajuria *et al* in his two different elaborative study, comparison of nortriptyline and pregabalin on efficacy and safety on sleep quality of life in PHN proves FDC of pregabalin 75 mg and nortriptyline 10 mg combination would be an ideal choice.^[13,14] Our earlier study conducted in orthopedic department also justified the use of pregabalin 75 mg^[15] and study from Holbech *et al.* for the treatment of neuropathic pain in combination support the usage.^[16] Another two expert recommendations based on a Danish and Delphi process also supports the similar initiation.^[16,17] A study from Jha *et al.* on the effect of pregabalin with fixed-drug combination in PHN, imbibe the FDC and early initiation^[18] and an another study from Sharma *et al.* comparative study of methylcobalamin plus pregabalin in patients of painful diabetic neuropathy provides valuable importance of this combination.^[19] A study from Italy by Romano *et al.* on the topic antineuropathic and antinociceptive combination in patients with chronic low back pain and pregabalin for neuropathic pain in primary care settings recommendations for dosing and titration support our study as a FDC in early initiation. Rainer *et al.* from Japan studied the latest safety evidence and clinical implications for the management of neuropathic pain and a study from London Rickels *et al.* imbibed the support for the use of pregabalin in various clinical condition.^[20-23]

Strength and Limitation of the Study

Even though our study is an elaborative, it is subjected to some limitations. We studied only patients attending the

OPD and the duration was almost 12 months. The study would have been considered more strength, if we included the patients admitted in wards as inpatients from various clinical departments. As this is the first detailed elaborative well framed study from south India, we need to have many multicentric trails across various parts of India to know more information from different ethnicity. Future study may concentrate on regular follow-up of the patients for few years may reveal a lot of clinical information regarding their adverse events, cost effectiveness, and adherence to the drug therapy.

CONCLUSION

Our elaborative, structured, and long-term study divulge much more new information to all practitioners irrespective of the specialty, the prescribing practice of drug avail oneself of to facilitate the rational use of pregabalin and its FDC. To assess our detailed drug utilization study for the pregabalin in prevalence of various disease conditions, we used well designed method and data. As per the data's from various clinical departments the present study, it will be a surprise for all the practicing physicians, surgeons, and others to prescribe pregabalin in the management of neuropathy pain and other various clinical disorders. Enhancing the future study it will definitely inspire other researchers to do further research in this area. Overall study reveals that all the department prefer either plain pregabalin 75 mg or FDC of pregabalin 75 mg as their preferred choice for neuropathy pain followed by radicular pain, trigeminal neuralgia, claudication, and herpetic neuralgia.

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Anti-inflammatory and Anti-ulcer Activity of *Morus indica* Linn

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ABSTRACT

Anti-inflammatory and anti-ulcer activities of the ethanol and aqueous extract of the shade dried aerial parts of *Morus indica* Linn. were studied in wister rats using the carrageenan induced left hind paw edema method and aspirin induced ulcer method. The ethanolic and aqueous extracts (100 and 250 mg/kg) produced the inhibition of carrageenan induced rat paw edema and also found to be effective in aspirin induced gastric ulcer. The results indicated that the ethanolic and aqueous extracts produced significant ($P < 0.001$) anti-inflammatory and anti-ulcer activity when compared with the standard and untreated control.

Keywords: *Morus indica*; ethanolic and aqueous extracts; Anti-inflammatory; Anti-ulcer.

INTRODUCTION

Morus indica Linn. (Urticaceae) is a small plant widely distributed throughout India. Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation [1]. Hence, search for new anti-inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects are justified. There is much hope of finding active anti-rheumatic compounds from indigenous plants as these are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective drugs [2]. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects and they are in line with nature, with no hazardous reactions [3]. The enzyme, phospholipase A₂, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A₂ converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation [4 and 5]. So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory and anti-ulcer activities in rats.





EXPERIMENTAL

Plant material

The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of June 2002 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Salem. A voucher specimen (MIA-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of aerial parts of *M. indica* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform: water [6]. After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals

Wister rats of either sex and of approximately the same age, weighing about 150-175 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Acute toxicity studies [7]

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD₅₀ was calculated.

Carrageenan induced rat paw edema

Anti-inflammatory activity was assessed by the method described by Winter et al [8]. The rats were divided into four groups of six animals each. First group (negative control) received 1 ml of normal saline, second group (positive control) received 10 mg/kg p.o., indomethacin, third group received ethanolic extract (100 mg/kg) and fourth group received aqueous extract (250 mg/kg) of *M. indica*, respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma chemical co, St.Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was that of Butte et al [9] as modified by Singh and Ghosh [10]. The paw volume was measured immediately after injection (0 h) and followed by every hour till the 3 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula, % inhibition = 100 (1-Vt/Vc), where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

Aspirin induced ulcer model

Anti-ulcer activity was assessed by the aspirin-induced ulcer method described by Hegde et al [11]. The rats were divided into four groups each consisting of six animals. The first group served as a control group, the second group served as positive control, the third and fourth groups served as test groups. The second, third and fourth group were treated respectively with ranitidine (20 mg/kg), ethanolic extract (100 mg/kg) and aqueous extract (250 mg/kg) of *M. indica*, orally for 8 days. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of aqueous suspension of aspirin (200 mg/kg orally) on the day of sacrifice. The animals were sacrificed 4 h later and the stomach was opened to calculate the ulcer index by Kunchandy method [12].



**Vijayarangan et al.,****Statistical Analysis**

Statistical analysis was performed using student's t-test. The values are represented as mean±SEM. Level of significance was set at $P < 0.001$.

RESULTS

The plant *M. indica* was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of alcohol extract of *M. indica* was found to be 2.2 and for aqueous 3.7 % w/w. On preliminary phytochemical screening of the aerial parts of *M. indica* revealed the presence of alkaloids, flavanoids, saponins and tannins. The LD₅₀ of the extracts were found to be 1149 and 2497 mg/kg for alcohol and aqueous extracts of *M. indica*.

Carrageenan induced rat paw edema

The effect of extracts of *M. indica* on carrageenan-induced edema in rats is shown in Table 1. The results obtained indicate that the ethanolic and aqueous extracts were found to have significant anti-inflammatory activity in rats. The ethanolic and aqueous extracts of *M. indica* reduced the edema induced by carrageenan by 59.46 and 51.35 % on oral administration of 100 and 250 mg/kg respectively, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 70.27 %.

Aspirin induced ulcer mode

The effect of ethanolic and aqueous extracts of *M. indica* on aspirin induced ulcer model is presented in Table 2. The results of the present study indicate that the ethanolic and aqueous extracts significantly reduce the ulcer score and also have activity against gastric ulcers in rats. The control animals had ulcers and haemorrhagic streaks, whereas in animals administered with the extracts of *M. indica* there was significant reduction in ulcer index.

DISCUSSIONS AND CONCLUSION

Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation without producing ulcer. The traditional medical practitioners of Kolli hills, Tamilnadu, are using this plant to cure inflammation and ulcer. To give a scientific validation to this plant, an attempt was made to study the anti-inflammatory and anti-ulcer activities. Carrageenan induced inflammation is a biphasic phenomenon [13]. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances [14]. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and maintenance of the mucosal integrity through endogenous defence mechanisms [15]. The excess gastric acid formation by prostaglandin (PG) includes both increases in mucosal resistance as well as decrease in aggressive factors mainly acid and pepsin [16]. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage the cell membrane of mucosal, parietal and endothelial cells [17]. Thus it can be concluded that the aerial parts of the plant *M. indica* possess significant anti-inflammatory and anti-ulcer activity in rats. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory and anti-ulcer agent with a low toxicity and better therapeutic index.





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Table 1. Effect of ethanolic and aqueous extracts of *M. indica* on Carrageenan induced rat paw edema

| Treatment | Dose (mg/kg, p.o.) | Mean increase in paw volume (ml) | % Decrease in paw volume |
|--------------------------------------|--------------------|----------------------------------|--------------------------|
| Control (Normal saline) | 1 ml | 0.37±0.0073 | - |
| Indomethacin | 10 | 0.11±0.0057* | 70.27 |
| Ethanolic extract of <i>M.indica</i> | 100 | 0.15±0.0026* | 59.46 |
| Aqueous extract of <i>M.indica</i> | 250 | 0.18±0.0045* | 51.35 |

*P<0.001 when compared with control. Values are expressed as mean±SEM (n=6)

Table 2. Effect of ethanolic and aqueous extracts of *M. indica* on aspirin induced gastric ulcer in rats

| Treatment | Dose (mg/kg, p.o.) | Ulcer score | % Protection |
|--------------------------------------|--------------------|-------------|--------------|
| Control (Normal saline) | 1 ml | 4.1±0.02 | - |
| Ranitidine | 20 | 1.6±0.03* | 58.54 |
| Ethanolic extract of <i>M.indica</i> | 100 | 2.6±0.05** | 36.59 |
| Aqueous extract of <i>M.indica</i> | 250 | 2.1±0.05* | 48.78 |

*P<0.001, **P<0.01 when compared with control. Values are expressed as mean±SEM (n=6)





ORIGINAL ARTICLES. PHYSICAL THERAPY

A study on influence of pranayama on high sensitivity C-reactive protein and creatinine kinase levels in chronic obstructive pulmonary disease patients

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Abstract

Purpose: The present study is aimed to observe the differential response of pranayama in reducing high sensitivity C-reactive protein and creatinine kinase levels among chronic obstructive pulmonary disease patients in comparison to control patients.

Material and Methods: An intervention study was done by enrolling 100 chronic obstructive pulmonary disease patients who were managed by therapeutics. The participants were divided into intervention and non-intervention group with 50 each sample. The intervention was in the form of sequenced yogic practices for 6 weeks. The pre-intervention and post-intervention inflammatory maker levels were estimated along with lung function estimation.

Results: The result was analyzed by descriptive statistics. The mean C-reactive protein, serum creatinine kinase value decreased from 9.53 to 7.85mg/L and from 145.01 to 140.57 U/L respectively following 6 weeks of yogic practices intervention. The observed values were found to be statistically significant ($p < 0.05$).

Conclusion: The statistically significant reduction in inflammatory makers, C-reactive protein and creatinine kinase level following 6-week yogic practices in chronic obstructive pulmonary disease patient suggests including such yogic interventions in the regular management protocols of chronic obstructive pulmonary disease patients.

Keywords: chronic obstructive pulmonary disease, yoga, pranayama, high sensitivity C-reactive protein, creatinine kinase



Анотація

Сурешбаладжи Р.А., Начал Аннамалай, Нивета П. Дослідження впливу пранаями на рівень високочутливого С-реактивного білка та креатинінкінази у пацієнтів з хронічною обструктивною хворобою легень.

Мета: Це дослідження спрямоване на спостереження за диференційованою реакцією пранаями на зниження високочутливого рівня С-реактивного білка та креатинінкінази у пацієнтів з хронічною обструктивною хворобою легень порівняно з пацієнтами контрольної групи.

Матеріали та методи: було проведено інтервенційне дослідження шляхом включення 100 пацієнтів із хронічною обструктивною хворобою легень, які отримували терапевтичне лікування. Учасники були розділені на інтервенційну та групу без втручання по 50 у кожній вибірці. Втручання відбувалося у формі послідовних йогічних практик протягом 6 тижнів. Рівні збудників запалення перед втручанням і після втручання оцінювали разом з оцінкою функції легень.

Результати: Результат проаналізовано за допомогою описової статистики. Середнє значення С-реактивного білка, сироваткової креатинінкінази зменшилося з 9,53 до 7,85 мг/л і з 145,01 до 140,57 ОД/л відповідно після 6 тижнів практики йоги. Виявлено, що спостережувані значення є статистично значущими ($p < 0,05$).

Висновок: статистично значуще зниження рівня збудників запалення, С-реактивного білка і креатинінкінази після 6-тижневих занять йогою у пацієнтів з хронічною обструктивною хворобою легень дозволяє включати такі йогічні втручання в регулярні протоколи лікування пацієнтів з хронічною обструктивною хворобою легень.

Ключові слова: хронічна обструктивна хвороба легень, йога, пранаяма, високочутливий С-реактивний білок, креатинінкіназа

Аннотация

Сурешбаладжи Р.А., Начал Аннамалай, Нивета П. Исследование влияния пранаямы на уровни С-реактивного белка высокой чувствительности и креатининкиназы у пациентов с хронической обструктивной болезнью легких.

Цель: Настоящее исследование направлено на наблюдение дифференциальной реакции пранаямы на снижение уровней высокочувствительного С-реактивного белка и креатининкиназы у пациентов с хронической обструктивной болезнью легких по сравнению с пациентами контрольной группы.

Материалы и методы. Было проведено интервенционное исследование с участием 100 пациентов с хронической обструктивной болезнью легких, которые лечились терапевтическими методами. Участники были разделены на группы вмешательства и группы невмешательства по 50 человек в каждой. Вмешательство проводилось в форме последовательных йогических практик в течение 6 недель. Уровни производителей воспалительного процесса до и после вмешательства оценивались вместе с оценкой функции легких.

Результаты: результат был проанализирован с помощью описательной статистики. Среднее значение С-реактивного белка и креатининкиназы сыворотки снизилось с 9,53 до 7,85 мг / л и с 145,01 до 140,57 Ед / л соответственно после 6 недель занятий йогой. Наблюдаемые значения оказались статистически значимыми ($p < 0,05$).

Заключение: статистически значимое снижение уровня воспалительных факторов, С-реактивного белка и креатининкиназы после 6-недельных занятий йогой у пациента с хронической обструктивной болезнью легких предполагает включение таких йогических вмешательств в протоколы регулярного ведения пациентов с хронической обструктивной болезнью легких.

Ключевые слова: хроническая обструктивная болезнь легких, йога, пранаяма, С-реактивный белок высокой чувствительности, креатининкиназа



Introduction

Chronic Obstructive Pulmonary Diseases (COPD) refers to group of progressive lung diseases causing obstructed airflow from lungs characterized by broncho-constriction, difficulty in breathing, chest discomfort, shortness of breath, chronic cough due to significant exposure to noxious particles or gases [1]. The classical examples of COPD are emphysema and chronic bronchitis. Chronic Obstructive Pulmonary Disease is the third leading cause of death worldwide, causing 3.23 million deaths in 2019 [2]. About 80% of these deaths occurs in low- and middle-income countries. COPD is second common cause of NCD-related deaths in India (age >30 years). The risk factors of COPD are smoking, occupational aerosols, indoor and outdoor pollution, dietary habits, co-morbidity and positive COPD family history. But not all COPD cases are smokers.

Non-invasive breathing test-spirometry is a gold standard test for diagnosis of COPD [3]. There are also biochemical markers that can be measured in respired breath, sputum, broncho-alveolar lavage and plasma of COPD patients. The measurement of biochemical markers in plasma is a more reliable method. The COPD patients can be successfully treated by inhaled bronchodilators, antibiotics, corticosteroids, oxygen, methyl xanthines, mucolytic agents and adjuvant therapy.

Pulmonary rehabilitation is a holistic approach that involves exercise therapy and behavior modification to improve health status in COPD. Exercise training and yoga have shown beneficial effects in COPD patients by reducing discomfort in breathing and fatigability, enhancing the exercise tolerance thereby improving quality of life [4].

Pranayama is a common yogic practice, consciously regulating one's own breath. It improves the resting respiratory rate, vital capacity, maximum voluntary ventilation, breath-holding time, maximal inspiratory, expiratory pressures, reduces dead-space ventilation and aerates the lung. Patients, who were trained to perform pranayama were observed to have increased inspiratory and expiratory muscle functions. Role of Pranayama is newly gaining importance in clinical research. The relationship between effect of yoga as an adjunctive therapy on respiratory function and respiratory muscle strength in COPD patients was evaluated using various spirometric parameters like FEV1%, PEF, FVC and FEV1/FVC ratio [5].

Meta analytic studies [6] have revealed that, respiratory interventions including pranayama would help to reduce inflammatory markers could

contribute to the prevention of various metabolic disorders and future cardiovascular events. COPD patients with systemic manifestations, circulating high levels of IL-6 were found to stimulate CRP synthesis by hepatocytes thus leading to increased levels of both the markers [7]. The study revealed the elevated serum creatine phosphokinase levels in obstructive lung disorders is probably derived from respiratory muscles, owing to the increased work of breathing [8]. The literature on pranayama intervention in COPD patients and its consequential alterations in inflammatory marker levels are scarcely available.

The present study is aimed to observe the differential response of pranayama in reducing hs-CRP and creatinine kinase levels among COPD patients in comparison to control patients.

Material and Methods

Study Area: A tertiary care hospital in rural Tiruchirappalli

Study Duration: From February-December 2020

Study Design: Interventional study

Sampling technique: Simple random sampling method

Sample Size: The sample size was calculated with 95% confidence level and 85% power with a p value of less than 0.05 is considered significant. Fifty subjects as interventional group (COPD + medication + pranayama) and 50 subjects (COPD + medication) as control group were taken. The participants included eligible subjects who have met the inclusion criteria.

Inclusion criteria: clinically confirmed COPD patients, aged 20 to 55 years of both genders; With mild to very severe stable physician-confirmed COPD satisfying Global Initiative for Obstructive Lung Disease (GOLD) criteria, those with forced expiratory volume 1 (FEV1)/forced vital capacity ratio <0.7 and post-bronchodilator FEV1<80% predicted; clinically stable for at least 3 months prior to enrollment, able to walk without aid, willing to complete all study assessments and provide informed consent.

Exclusion criteria: recently diagnosed COPD, epilepsy, unstable angina, respiratory tract infection within 1 month of the start of the study, myocardial infarction, angioplasty, heart surgery in the previous 6 months, Basal blood pressure >180/100 mmHg, resting PR >120 bpm, body mass index (BMI) >35 kg/m², previous participation in yoga rehabilitation programs, mentally retardation and related neuromuscular disorders.



Methodology

The study participants, 50 each were grouped into interventional and non-interventional group (randomized). The participants were stabilized on drugs till no further symptomatic improvement occurred. The participants in the interventional group received pranayama practice along with routine medications and the control group were maintained only on medication.

In this study, a combination of relaxation exercise to calm the mind followed by pranayama and asanas for 6 weeks as intervention is as follows:

- Kapalabhati for five minutes (successive rapid exhalations followed by passive inhalations)
- Anuloma-viloma pranayama for 10 minutes (holding one nostril closed with inhaling then holding other to exhale; then reversed and repeated)
- Bhastrika pranayama for five minutes (inhale through both nostrils maximum for four seconds and then exhale for six seconds)
- Sasangasana, 30 seconds each for five minutes (hands above the head, lean forward with exhalation, the forehead to touch the knees with hands to touch the floor and next get back to starting position with inhalation) and
- Bhramari for five minutes (sustained inhalation and slow exhalation with humming sound)

After 6 weeks of pranayama, base-line and post-intervention values were compared.

Care was taken to recruit subjects who were on standard care for COPD. Optimum therapy was defined as maximum dose of drugs administered without any side effects to produce maximum clinical benefit. Optimum dose was decided by the chest physician. It was assumed that 10% improvement in PFT is clinically significant and atleast 50% of the COPD patients will improve with yogic practice. Two inflammatory makers hs-CRP and serum creatinine kinase (sCK) were estimated in

baseline and post intervention period in both the groups.

All procedures were performed according to the Declaration of Helsinki [9] research ethics. Each participant received detailed information about the study and provided written informed consent prior to the study.

Statistical Analysis

The data was entered and analyzed using SPSS version 23. The descriptive statistical analysis was done by calculating percentages and p value. The results were computed for the variables and tabulated. The level of significance for the results was expressed using p value with $p < 0.05$ being statistically significant.

Results

In the interventional group of 50 participants, the mean age was 44.22, standard deviation was 1554.58 ± 9.78 and coefficient of variance was 12.61. The control groups of 50 participants showed mean age of 45.08, standard deviation was 1353.68 ± 6.92 and coefficient of variance was 11.54. CRP levels among the interventional group showed a mild to moderate reduction after follow up when compared to baseline. The mean CRP value decreased from 9.53 to 7.85mg/L after six weeks of yogic practice whereas the coefficient of variance value increased from 61.51 to 69.18 which were significant.

There was a significant reduction in the mean serum creatinine kinase level from 145.01 to 140.57 U/L, in the interventional group whereas the control group demonstrated an increase in serum creatinine kinase levels from 141.64 to 146.37 U/L. Overall this data showed very clearly that the interventional group improved better than the control group. Paired "t" test confirmed the statistical significance of the observed finding with significant $P < 0.05$ (Table 1).

Table 1

Base-line and post-intervention values

| Outcome measures | Interventional group | | | Control group | | |
|------------------|----------------------|--------|---------|---------------|--------|---------|
| | Base-line | Post | p value | Base-line | Post | p value |
| Mean Age | 44.22 | | 0.002* | 45.08 | | 0.015* |
| Gender | | | | | | |
| Male | 23 | | 0.17 | 22 | | 0.16 |
| Female | 25 | | 0.14 | 28 | | 0.15 |
| Transgender | 2 | | 0.001* | - | | - |
| HsCRP(mg/L) | 9.53 | 7.85 | 0.003* | 9.93 | 8.42 | 0.015* |
| Creatine kinase | 145.01 | 140.57 | 0.000* | 141.64 | 146.37 | 0.003* |

Notes: * statistically significant



Discussion

This study analyzed the hypothesis that pranayama used as an adjunctive therapy in COPD patients showed improvement in serum inflammatory markers like hs-CRP and creatinine kinase levels among COPD patients.

A report on effect of pranayama upon respiratory health detailed the different mechanisms involved in potentiating efficiency of lungs of individuals through promoting abdominal respiration and providing relief to the diaphragm and also the effect on non-functional and closed airways were also described [10].

Subjective positive improvement in health and functional status with reduction in disease severity was observed following 3 months of pranayama practice in COPD patients [11]. Improvement in lung function parameters with reduction in overall symptoms was noted in moderate to severe COPD patients with yogic breathing and pranayama exercise [12].

Studies on effect of yoga on COPD patients documented improved lung functional parameters after yogic practice [13-15]. These beneficial studies on the practice of yoga for the COPD patients underlie the suggestion to use yogic practices as an adjunct therapy in COPD management.

Apart from respiratory functional improvement, yogic practices were also observed to cause reduction of biochemical inflammatory makers like CRP in COPD patients after 12 weeks yogic practice intervention [16]. In comparison the present study found statistically significant reduction in CRP levels ($p=0.003$) in intervention group that was given 6 weeks of yogic practice itself.

An increase in sCK levels due to increased work of breathing following an increase in the airway resistance and alveolar ventilation was described in COPD patients [17]. Our study showed a reduction in sCK levels which can be attributed to reduction in airway resistance among the intervention group following the positive effects of yogic practices.

Even though there are various studies regarding COPD and yogic practices, the current study is an emerging topic of interest because it interconnects the importance of practice of pranayama and biomarker levels in COPD patients. This study postulates a hypothesis that breathing techniques such as pranayama is more beneficial in

having favorable effects on pulmonary functions of COPD patients. The serum inflammatory markers like CRP and serum creatinine kinase levels in patients with COPD are reduced when compared to controls of COPD with medication alone. Yogic practices are considered as best adjunct therapy besides pharmacological practices [8].

Conclusion

The present study explains that among yogic asanas, pranayama is a unique method, as it is easy to understand and performed by patients. It requires less effort and provides drastic long-term comfort for COPD patients. It can be considered as a cost-effective rehabilitative technique in management of chronic obstructive lung diseases. The levels of CRP and CK were statistically reduced in COPD patients following 6 weeks of yogic practice as an adjunct to medication. This study concludes that these yogic practices could possibly bring in symptomatic relief in COPD patients. Thus regular practice of yogic breathing and exercise is advisable for COPD patients. Regular practice of pranayama can bring remarkable positive effects on both physical and mental well-being of an individual.

Therefore the study reveals that intervention of pranayama along with medications has significantly reduced the hsCRP and creatinine kinase levels. It can be concluded that Pranayama is an advisable supportive therapy for COPD management.

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Conflict of interest

None declared

Ethical approval

Institutional Ethics Committee approval obtained from Trichy SRM Medical College Hospital and Research Centre, Trichy. (Ref: No.14/ TSRMMCH&RC/ ME-1/ 2020-IEC No. 021 dated 31.01.2020).



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MDCT EVALUATION OF AORTIC ROOT AND ASCENDING AORTIC PATHOLOGIES: PICTORIAL ESSAY

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ABSTRACT

Objective; The purpose of this pictorial essay is to review the MDCT appearance of aortic root and ascending aortic pathologies. Familiarity with atypical anatomy and pathologies of the aortic root, ascending aorta and their clinical presentation may facilitate appropriate diagnosis and management. This can be of immense help to the clinician planning interventional procedures such as stenting, balloon dilatation, or graft surgery. **Conclusion;** Increasing use of MDCT for cardiac imaging has helped in the detection of many benign aortic root anomalies, but a small number are associated with myocardial ischemia and sudden death. Increasing the use of MDCT in cardiac imaging may yield diagnostic information on pathologies of the aortic root and ascending aorta not obtained with invasive coronary angiography. Axial sections, multiplanar reconstructions, virtual angioscopy, and 3D volume-rendered images should aid in the detection and improve the interpretation of such pathologies.

KEYWORDS : Aortic root aneurysm, Aortic dissection, Aortic stenosis, Ascending aorta, Bicuspid aortic valve, Sinus of Valsalva aneurysm.

INTRODUCTION

Multidetector computed tomography (MDCT) cardiac imaging is an important tool in the assessment of coronary artery anatomy and coronary stenosis and is also helpful in assessing the aortic root pathologies which can mimic coronary artery diseases which are sometimes fatal [1]. The aortic root is the proximal-most segment of the aorta from the aortic annulus to the sinotubular junction. Components of the aortic root include the aortic annulus, aortic leaflets with their attachments and trigones, the sinuses of Valsalva (SOV), and the sinotubular junction (STJ).

The aortic root bulges outwards to form three dilatations, the aortic sinuses or sinuses of Valsalva (SOV). The SOVs lie within the pericardial sac and at the centre of the heart. The superior border of SOV is the sino-tubular junction. The right coronary sinus locates anteriorly and gives origin to the right coronary artery, while the left coronary sinus locates posteriorly to the left, giving origin to the left coronary artery. The right posterior SOV does not give rise to the coronary artery and is called the non-coronary sinus. The aortic annulus is defined as a virtual ring at the aortic root where the nadirs of the basal attachment of the valvular leaflets locate. The ascending aorta rises to a point above the pulmonary arteries where the aortic arch begins with the innominate artery origin.

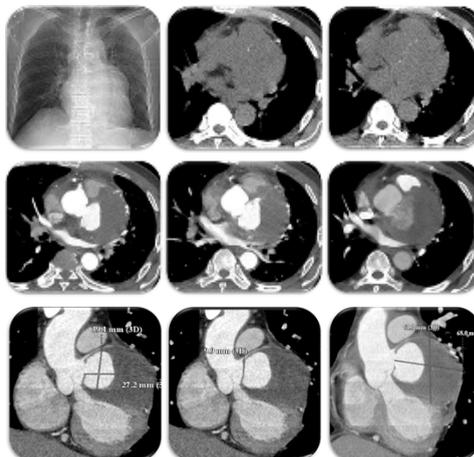
Aortic root and ascending aortic pathologies can be incidentally seen in patients getting a CT for non-aortic indications. Knowledge of the pathologies specific to this part of the aorta and their imaging appearance is useful for diagnosis and early treatment.

ILLUSTRATIVE CASES

Fig.1. (A-O). Chest X-Ray (A) - Shows focal bulge in left cardiac border & evidence of post-thoracotomy. Plain CT (B, C) - Shows an oval iso-density merging with cardia showing medial curvilinear partial rim calcification abutting the aortic

root. Computed Tomography Coronary Angiography (CTCA) axial images (D-F) reveal a saccular aneurysm arising from the left sinus of Valsalva with surrounding large intramural thrombus, posterior-inferiorly compressing on the pulmonary veins & left atrium, anterior-superiorly indenting RVOT. CTCA coronal images (G-I) reveal a saccular aneurysm arising from the left sinus of Valsalva measuring about 39.1 x 27.2 mm & neck diameter of 9.9 mm, surrounded by a large intramural thrombus, superiorly elevating & compressing LMCA, LAD & LCX. Coronary tree images (J-L) reveal a saccular aneurysm arising from the left sinus of Valsalva, antero-superiorly elevating & indenting LMCA, LCX & LAD. MIP Coronary tree (M) & 3D volume-rendered images (N, O) reveals saccular Aneurysm arising from the Left Sinus of Valsalva, antero-superiorly elevating & indenting LMCA, LCX & LAD.

CASE- 1.51 years old male patient with a history of Coronary artery bypass graft (CABG) surgery came with complaints of Chest Pain & discomfort.





CASE-2.2. 4-year-old patient with a history of aortic valve replacement presented with shortness of breath & Chest Pain.

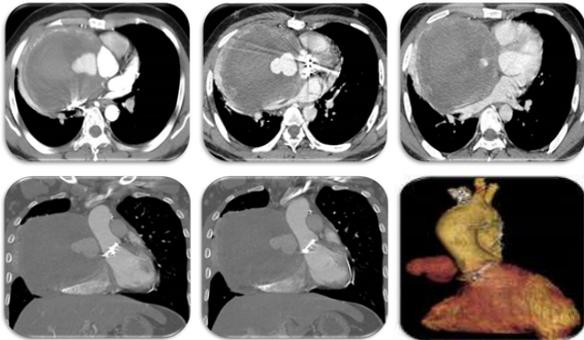


FIG.2 (A-F).CTCA Axial images (A-C) reveal a saccular aneurysm arising from the Right sinus of Valsalva surrounded by a large intramural thrombus, antero-superiorly abutting the chest wall, postero-inferiorly compressing & displacing RCA, pulmonary veins & left Atrium. Coronal (D, E) & 3D volume-rendered images (F) reveal a saccular aneurysm arising from the Right sinus of the Valsalva surrounded by a large intramural thrombus, laterally abutting chest wall & inferiorly compressing on the Right Ventricle

CASE-3.60 years old male referred for CT aortic angiography for abnormalities.

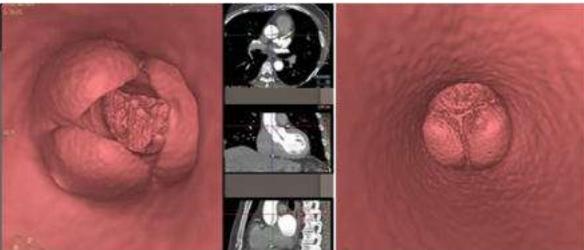


FIG.3. (A, B). CT Aortic angiography and Virtual angiography images show normal open (A) and closed (B) tricuspid aortic valves.

CASE-4. 40 years old woman presenting with nonspecific chest pain.

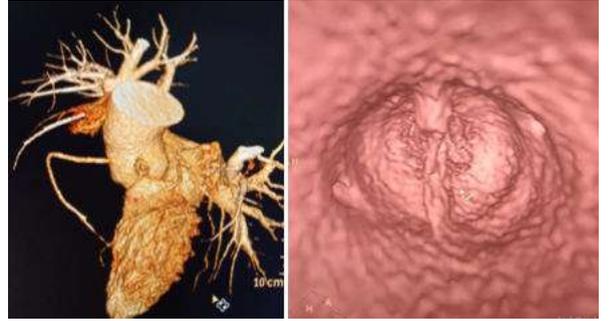
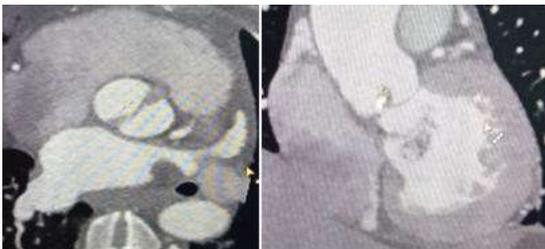


FIG.4 (A-D). CTCA 2 D-Axial (A), reconstructed coronal (B) , 3 D-volume rendered (C) and virtual angiography (D) image shows a closed, thickened, partially calcified bicuspid aortic valve.

CASE-5. 50 Years old female with vague chest pain.

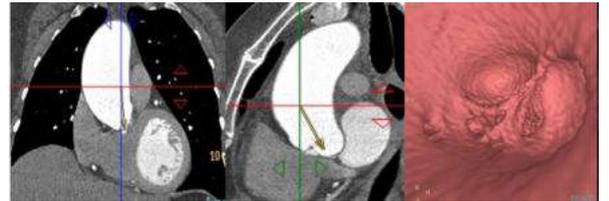


FIG.5 (A-C). CT Aortic angiogram reformatted coronal (A), sagittal (B) and virtual angiography (C) images reveal a thickened aortic valve causing mild aortic stenosis.

CASE-6. 55 years old male with rheumatic fever referred for CT of Thoracic aorta

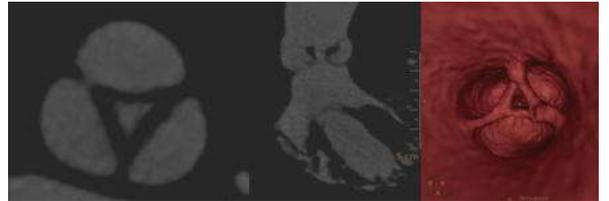


FIG.6 (A-C). CT Aortic angiogram (A, B) and virtual angiography (C) images reveal thickened tricuspid aortic valve leaflets causing mild aortic stenosis.

CASE-7. A 28-year-old woman with Aortic stenosis presented for evaluation of prosthetic aortic valve status after undergoing aortic valve replacement.

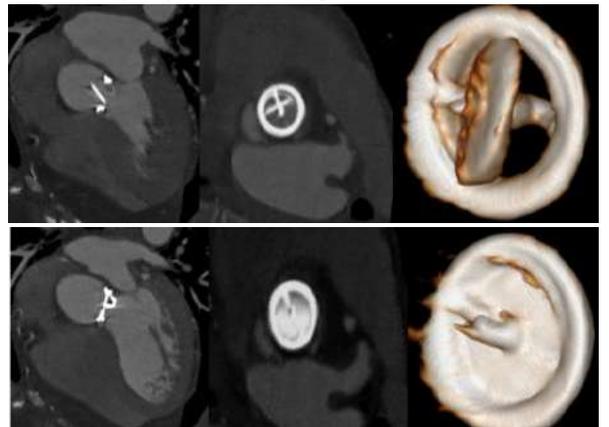
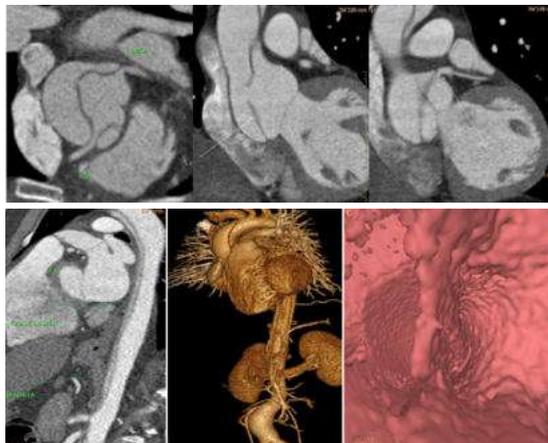


FIG.7. Cardiac CT of aortic valve shows open (A-C) and closed (D-F) prosthetic aortic valve.

FIG.8(A-F). CTCA- 2D reformatted volume-rendered (E) and virtual angiography (F) revealed ostioproximal narrowed main coronary arteries with aortic root dissection involving the right coronary sinus and extending to descending thoracic aorta till

the celiac axis (Debakey Type 1/ Stanford Type A).

CASE-8. 34 years old college lecturer presenting with acute chest pain to the emergency department.



CASE- 9. 52 years old male patient with symptoms of acute coronary syndrome

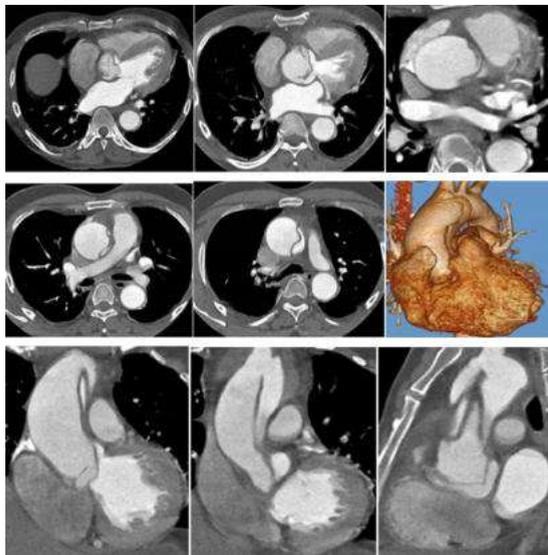


FIG.9 (A-I). CTCA- 2D axial images (A-E), 3-D volume-rendered (F) and reformatted coronal (G-I) images revealed aortic root and ascending aorta dissection(Debakey Type 2/ Stanford Type A). Right coronary artery arising from false lumen with severe reduced blood flow.

CASE.10. 48 years old man presenting with atypical chest pain

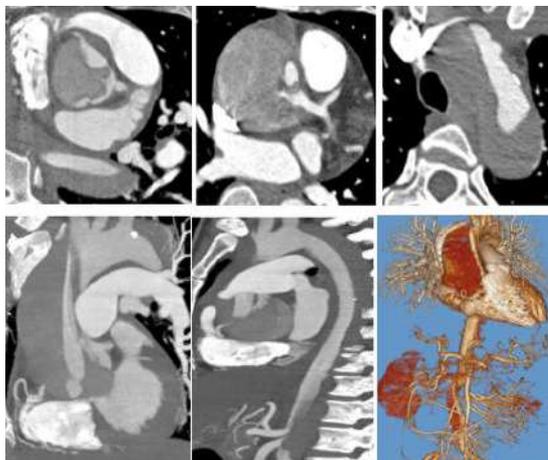


FIG. 10 (A-F). CT Aortic angiogram showing dissection of aortic root, ascending and descending thoracic aorta(Debakey Type 1/ Stanford Type A).Normal origin of right and left coronary arteries from true lumen seen.

CASE.11. 50 Years old male presented with sudden chest pain to emergency medicine.

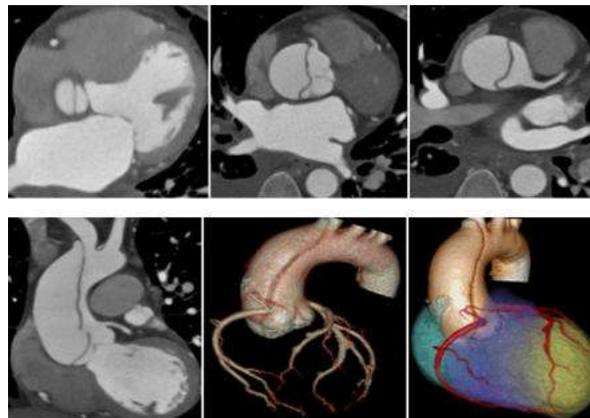


FIG.11(A-F).Computed tomography coronary angiography (CTCA).Axial (A-C), reformatted Coronal (D), coronary tree and 3 D volume rendered images reveal aortic root & ascending aortic dissection (Debakey Type 2 / Stanford Type A). Normal origin of right and left coronary arteries from true lumen seen.

CASE 12. 45 years old male presented with acute chest pain and abdominal pain to the ER

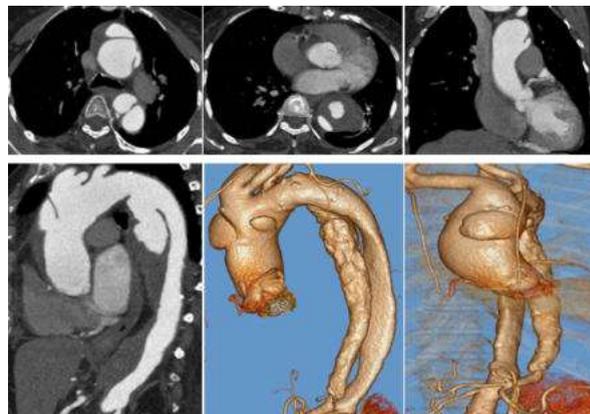


FIG.12(A-F). CT Aortic angiogram Axial (A,B), Coronal (C,D) and 3 D volume rendered images (E,F) showing 2 small focal ascending aortic aneurysmal fissuring, a larger descending thoracic and abdominal aortic fissured aneurysm.

CASE-13. 9 years old boy with vague chest pain.

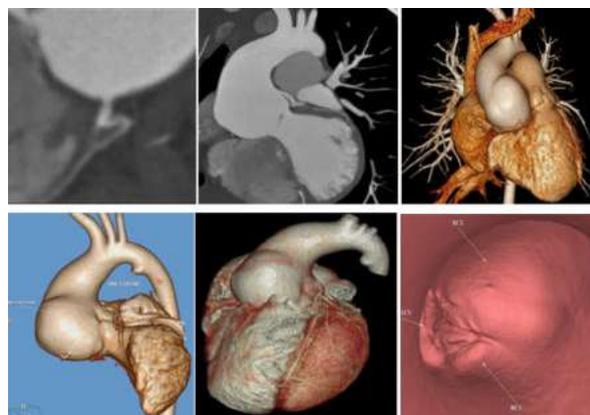


FIG.13(A-F).CTCA 2-dimensional (A, B), 3-dimensional volume-rendered (C-E), and virtual angiography images (F) of thoracic aorta show aortic root saccular aneurysm involving the right coronary sinus and another small aneurysm in the proximal descending thoracic aorta.

CASE .14. 42 years old female with atypical chest pain

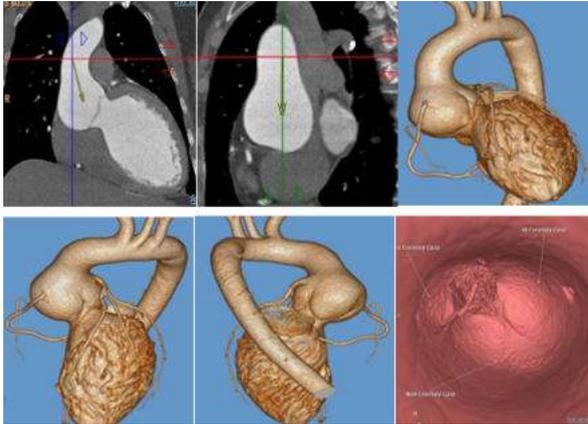


FIG.14 (A-F). CTCA Reformatted coronal (A) and sagittal (B) 2D images, 3-dimensional volume-rendered (C-E), and virtual angiography images (F) of the thoracic aorta show an aortic root saccular aneurysm involving the right coronary sinus.

CASE-15. 48 years male for referred for CTCA.

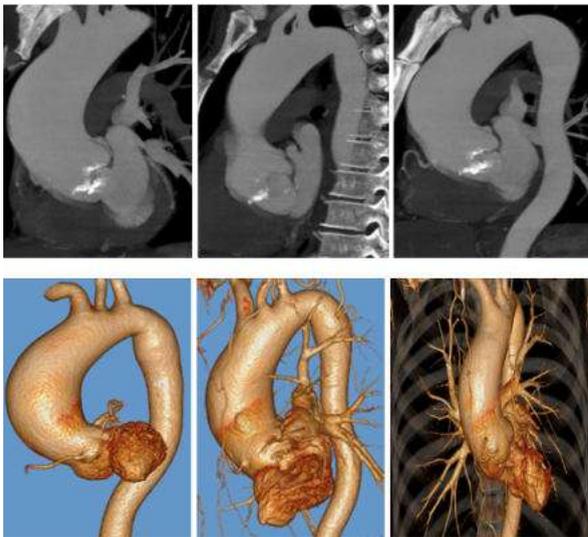


FIG.15 (A-F). CTCA Reformatted sagittal 2D images (A-C), and 3-dimensional volume-rendered (D-F), images of the thoracic aorta show ascending aortic fusiform aneurysm.

CASE-16.38 years old female referred for CT aortic angiogram for thoracic aortic pathologies.

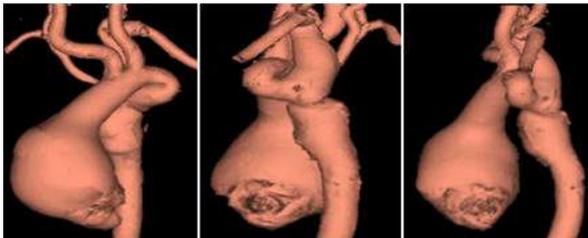


FIG.16(A-C) CT aortic angiography, 3-D volume rendered images show proximal ascending aortic fusiform aneurysm with corctation of descending thoracic aorta.

DISCUSSION;

AORTIC ROOT PATHOLOGY

Sinus of Valsalva Aneurysm (SVA)

SVA was first described by Hopeet al in 1839 as a relatively rare cardiac anomaly & can be congenital or acquired [2,3].More commonly seen in Asian men than in other ethnic groups. Male to Female ratio = 4;1.; Mean age 35.4 years, Age Range; 4 - 96 years.

SVA arises from Right Coronary Sinus (FIG.2) in 65-85%, Non-Coronary Sinus in 10- 30% and Left Coronary Sinus (FIG.1) in < 5%. Congenital causes are due to localized weakness of the elastic lamina or an underlying deficiency of normal elastic tissue (Marfan's, EDS).Acquired causes include infectious diseases (bacterial endocarditis, syphilis, TB), degenerative (atherosclerosis , cystic medial necrosis), deceleration Trauma & 0.15% - 3.5% of all open-heart surgical procedures [3,4,5].

Although both ruptured & non-ruptured SVA may have potentially fatal complications, after treatment the prognosis is excellent. Thus, prompt & accurate diagnosis is critical [3]. Most SVA is diagnosed on the basis of Echocardiography with or without angiography. However, both ECG gated CT & MR imaging can provide excellent anatomic depiction [3].

Iatrogenic pseudo-Aneurysms of the sinus of Valsalva can occur due to hematoma formation after aortic valve replacement or removal of aortic valve calcifications [6].

Unruptured SVA is asymptomatic & incidentally discovered or symptomatic & manifests acutely with mass effect on adjacent cardiac structures [6]. Unruptured SVA may cause impaired mitral valve/tricuspid valve insufficiency, myocardial infarction, or ischemia due to coronary artery compression [3]. SVA may rupture into the right ventricle, right atrium, right ventricular outflow tract, left ventricle, interventricular septum & left atrium. Ruptured SVA results in Aorto-Cardiac shunt & manifests as insidiously progressive CCF, severe acute chest pain with dyspnea or cardiac arrest [3] & rupture into extracardiac space leads to cardiac tamponade [7]. Aortic regurgitation (30-50%) is a common complication of both ruptured & non-ruptured SVA [8].

At imaging, the criteria for diagnosing an SVA include an origin above the aortic annulus, a saccular shape & normal dimensions of the adjacent aortic root & ascending aorta [9].

Bicuspid Aortic Valve and Aortic stenosis

The aortic valvular disease may be congenital in origin or secondary to another disease process (FIG.6). Bicuspid aortic valve (BAV) is the most common congenital anomaly of the aortic valve (FIG.4 & FIG.5), resulting from complex abnormal cusp formation during vasculogenesis [10,11]. Since BAV causes premature fibrosis and calcification of the aortic valves (FIG.4), aortic stenosis is the most common complication [12].Aortic stenosis of the bicuspid valve presents at an age range of 30 to 50 years, earlier than those caused by degeneration (FIG.5). The bicuspid aortic valve is associated with an increased incidence of stenosis, regurgitation, endocarditis, and aneurysmal dilatation of the aorta. Surgery is generally recommended for patients with severe stenosis who are symptomatic or who have significant ventricular dysfunction (FIG.7). Transcatheter aortic valve implantation (TAVI) is an emerging therapeutic option for patients who are not eligible for surgical treatment.

Aortic dissection (AD)

An AD is characterized by an intimo-medial tear of the aortic wall with subsequent separation of the layers (FIG.8). Dissections most commonly arise in the ascending aorta (FIG. 10 & FIG.11) 1 cm distal to the sino-tubular junction or in the descending thoracic aorta at or just beyond the isthmus of the thoracic aorta because of maximum wall shear stress [13].

Most AD with aortic root involvement (FIG.8-11) is due to retrograde dissection from the ascending aorta which increases the chances of rupture into the pericardial space causing cardiac tamponade, dissecting into coronary artery origin (FIG.9), or creating aortic valvular regurgitation [14]. These complications are life-threatening and therefore warrant urgent surgical repair. The involvement of the origin of coronary arteries can lead to ischemia from extension of the dissection into the Ostia or by narrowing from the intimo-medial flap within the aorta without extension into the coronary artery. The right coronary artery (FIG.9), is most commonly affected [13]. The term 'acute aortic syndrome' is used to indicate the triad of acute aortic dissection, intramural hematoma, and penetrating atherosclerotic ulceration as aortic-based causes of acute chest pain.

Aortic Aneurysm

Of all thoracic aortic aneurysms, 60% involve the aortic root (FIG.13 & FIG.14), ascending aorta (FIG.15 & FIG.16), or both [15], and there is a cumulative yearly risk of rupture or dissection of up to 6.9% per year with a maximal diameter of larger than 60 mm [16-18]. True aneurysms involve all the layers of vessel wall, while a false aneurysm / Pseudoaneurysm represent disruption of layers of wall of aorta with containment of extravasated blood by surrounding tissues forming a pseudo-capsule [19]. Based on the normal variation in the size of the proximal aorta, the American college of radiology (ACR) white paper on management of incidentals on thoracic CT suggest a size of 5 centimeter as the cut off for a proximal aortic aneurysm [20]. Blunt thoracic trauma, post surgical & infection are the most common cause of the pseudoaneurysms of heart or the thoracic aorta [21]. Pseudoaneurysms can be complicated with fatal rupture, fistula formation, and compression of surrounding structures. Aortic dilatation may be related to connective tissue disorders like Marfan syndrome, Loeys-Dietz syndrome, Ehlers-Danlos syndromes and Turner syndrome. Many genetic mutations are known to predispose individuals to aortic aneurysms, aortic dissection, or both [22]. A classic appearance of Marfan syndrome is annulo-aortic ectasia with dilatation of the annulus, uniform dilatation of the sinuses, and effaced sinotubular junction [23].

Aortic aneurysmal Thrombus fissuration (FIG.11) is a sign of impending rupture of an aortic aneurysm. It reflects blood dissecting into the intramural thrombus. This sign is observed on contrast-enhanced CT as a linear contrast infiltration from the aneurysm lumen through the intramural thrombus [24].

CONCLUSION

Increasing use of MDCT for cardiac imaging has helped in the detection of many benign aortic root anomalies, but a small number are associated with myocardial ischemia and sudden death. Increasing the use of MDCT in cardiac imaging may yield diagnostic information on pathologies of the aortic root and ascending aorta not obtained with invasive coronary angiography. Axial sections, multiplanar reconstructions, virtual angiography, and 3D volume-rendered images should aid in the detection and improve the interpretation of such pathologies.

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Primary Endobronchial and Mediastinal Synovial Sarcoma in a Young Female

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Abstract

Primary thoracic synovial sarcomas (SS) typically occur as chest wall masses, although they rarely arise in the lung and pleura. Primary pulmonary and mediastinal SS is very uncommon in comparison with metastatic sarcoma and might arise in the tracheobronchial tree manifesting as an endoluminal mass, generally in adults. Signs and symptoms may additionally consist of wheezing, persistent pneumonia, bronchial asthma, chest ache, recurrent cough, atelectasis, haemoptysis, and weight loss. Because of the heterogeneity of signs and symptoms, clinical diagnosis may be tough. Herein, we present a case of primary endobronchial and mediastinal SS in a young female presenting with cough and expectoration associated with haemoptysis and breathlessness on exertion for 1 week with particular emphasis on the radiologic and pathologic findings of this rare lesion.

Keywords: Endobronchial Tumour; Mediastinal Tumour; Pulmonary Synovial Sarcoma; Primary Pulmonary and Mediastinal Synovial Sarcoma; Small Round Cell Tumour; Tracheobronchial Tumour

Introduction

Primary tracheobronchial tumors are rare lesions which may be benign or malignant with different positions along the airway tree. Primary malignant tumors in the tracheobronchial tree are uncommon, accounting for lesser than 1% of all thoracic malignancies [1]. Primary pulmonary SS is very rare compared with metastatic sarcoma, accounting for less than 0.5% [2]. Primary pulmonary and mediastinal SS is an aggressive tumor sharing not unusual histological features with soft tissue synovial sarcoma [3,4]. Despite the fact that, the ideal histogenesis of primary pulmonary SS is uncertain and could origin in pleuripotent mesenchymal cells of bronchial submucosal stromal tissue [5]. Molecular testing for the pathognomonic t(x;18) chromosomal translocation has enabled diagnostic confirmation in approximately ninety percent of instances. In t(x;18)-negative cases, diagnosis must rely on histological and immunophenotypic capabilities [6].

Endobronchial and endotracheal tumors within the pediatric population are more likely to be malignant instead of benign [7]. Primary malignant tumors within the tracheobronchial tree

can produce signs and symptoms of airway obstruction (dyspnea, wheezing, stridor), mucosal infection and ulceration (cough, haemoptysis), or involvement of adjacent structures (recurrent laryngeal nerve palsy & dysphagia) by direct invasion [8,9].

We evaluated the clinical, radiological and pathological findings in young female of primary endobronchial and mediastinal synovial sarcoma.

Case Report

15 years old young girl presented to the respiratory medicine department a tertiary care hospital with complaints of Cough and expectoration associated with haemoptysis and breathlessness on exertion for 1 week. The blood examination showed mildly elevated lymphocytes (45.3%) and monocytes (13.1%). Blood haemoglobin was within normal limits. A provisional diagnosis of Adenocystic carcinoma / Carcinoid was made based on the clinical findings.

Bedside Chest X-ray Antero-posterior view taken shows right lung partial Consolidation collapse and mediastinal shift to the right side (Figure 1).

Patient referred for Multidetector Computed Tomography (MDCT) Chest revealed a well defined multi-lobulated heterogeneously enhancing lesion in the carina measuring ~ 23 x26 mm, extending to the right lower lobe bronchus with significant extraluminal component causing abrupt cut off of the bronchus intermedius causing lower lobe collapse (Figure 2-4). The extraluminal component measuring 44 x 38mm is abutting the 5th costovertebral junction with no obvious erosion or scalloping (Figure 2-4) - features are likely of neoplastic etiology. Differential diagnosis include neuroendocrinal tumor versus germ cell tumour.

Computed Tomography Virtual Bronchoscopy images (Figure 5) exhibit lobulated tumor arising from the right main bronchus, occluding lumen, projecting into the carina & lower trachea. Metastatic SS to the lung (Figure 6) became excluded with an intensive clinical and radiological examination to exclude primary in the body.

The Patient underwent Rigid Bronchoscopy which showed broad based lobulated mass arising from the posterior wall of

trachea extending to the right main bronchus causing a complete luminal obstruction. Endobronchial mass debulking become accomplished with electrocautery snare and specimen sent for Histopathological exam (HPE).

HPE demonstrated fragments of bronchial mucosa with sub-epithelial diffuse infiltration by small round cells having a scanty amount of cytoplasm and hyperchromatic nuclei. Many interspersed thin walled blood vessels are present. Findings were suggestive of Malignant Small Round cell tumour. Immuno-histochemistry (IHC) was done for definite cell typing. The neoplastic cells were negative for LCA, Pancytokeratin, Synaptophysin, S100, Desmin and positive for CD 99, BCL2, Vimentin, EMA and CD 56 with an MIBI index of 80%. The findings favoured the diagnosis of poorly differentiated synovial sarcoma (SS). The patient was advised Translocation studies for confirmation and further management at a better centre of excellence.

Patient was advised Translocation studies for confirmation, but



Figure 1: Chest X-ray Antero-posterior view depict Right lung partial Consolidation collapse and mediastinal shift to the right side.

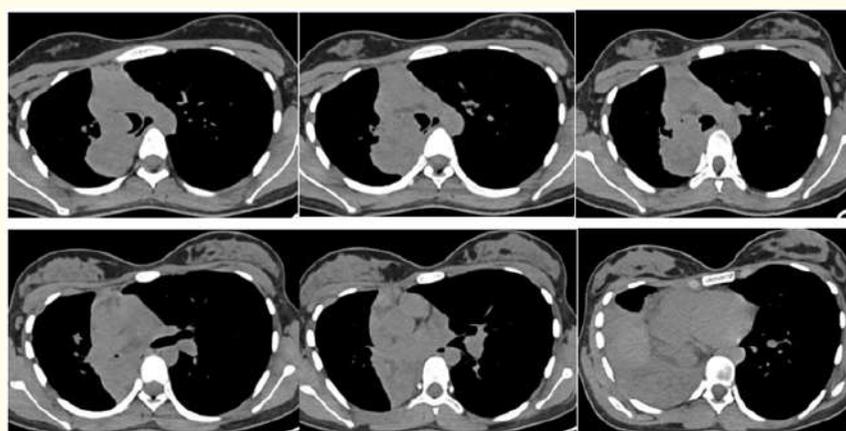


Figure 2: Axial (A-F) Non-contrast Computed Tomography images show a multilobulated isodense mass in the distal trachea extending to the right lower lobe bronchus with significant extraluminal component causing abrupt cut off of the bronchus intermedius causing lower lobe collapse.

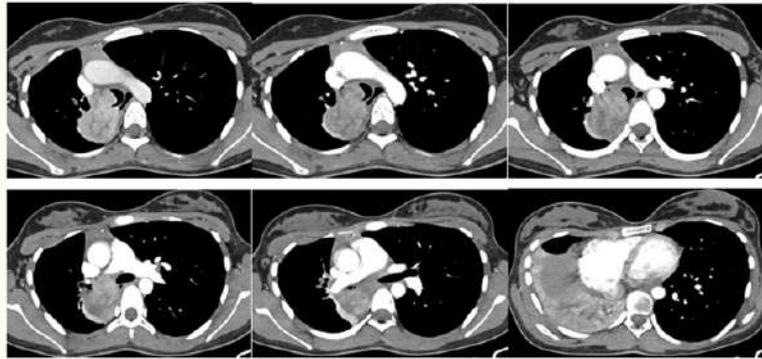


Figure 3: Axial (A-F) Contrast enhanced Computed Tomography images show well defined multi-lobulated heterogeneously enhancing lesion in the distal trachea, carina extending to the right lower lobe bronchus with significant extraluminal component causing abrupt cut off of the bronchus intermedius, causing middle lobe & lower lobe consolidation collapse.

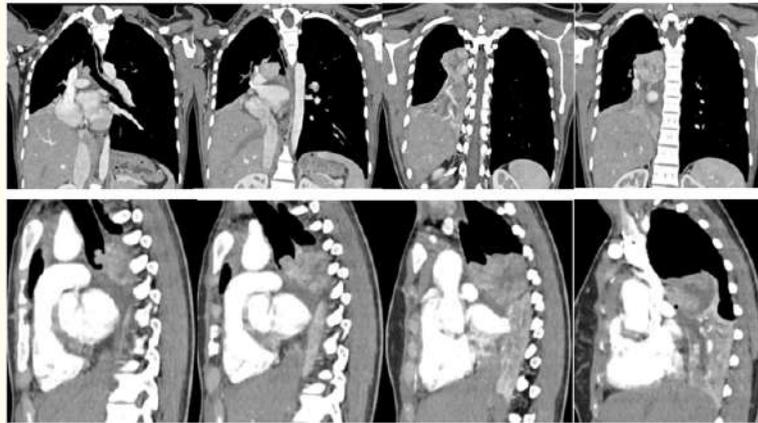


Figure 4: Reconstructed Coronal (A-D) and Sagittal (E-H) Contrast enhanced Computed Tomography images reveal multilobulated endotracheal and right endobronchial tumour occluding the right main bronchus.

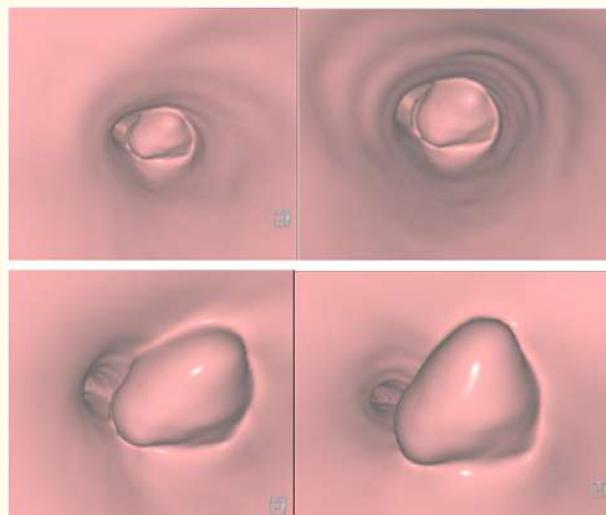


Figure 5: Computed Tomography Virtual Bronchoscopy (A-D) images exhibit lobulated tumor arising from the right main bronchus, occluding lumen, projecting into carina & lower trachea.

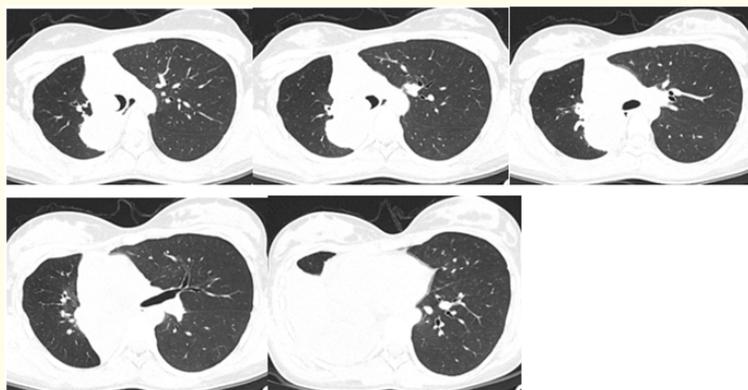


Figure 6: Axial (A-E) Computed Tomography lung window images reveal endobronchial and mediastinal mass with no obvious metastasis.

patient was lost for follow-up.

Discussion

Maximum primary pulmonary and mediastinal synovial sarcomas are located within the lung parenchyma [3, 4] and rarely extend into the bronchial tree [10] or occur inside the heart or pericardium [4]. Findings from 5 earlier series [3, 4, 10] suggest that, in contrast to soft tissue synovial sarcoma, primary pulmonary and mediastinal synovial sarcoma happens in older patients without gender bias. Results from five prior series [3, 4, 10] indicate that, in contrast to soft tissue synovial sarcoma, primary pulmonary and mediastinal synovial sarcoma occurs in older patients without gender bias. Clinical symptoms are site specific with few asymptomatic instances [4]. In contrast our case of primary endobronchial and mediastinal SS occurred in young female, causing cough, hemoptysis and breathlessness on exertion.

Primary pulmonary SS shares similar histomorphological and chromosomal translocations $t(X; 18)$ as its soft-tissue counterpart [10, 11]. Histologically, primary pulmonary and mediastinal synovial sarcoma reportedly shares similar findings with soft tissue synovial sarcoma including dense cellularity, interlacing fascicles, hyalinized stroma, hemangiopericytoma like vasculature, focal myxoid exchange, and mast cellular influx [10]. In our study, the HPE was suggestive of malignant small round cell tumour and immunohistochemistry (IHC) features consistent with poorly differentiated synovial sarcoma (SS). Begueret et al [4] stated that almost forty percent of primary pulmonary and mediastinal synovial

sarcomas have been poorly differentiated, which is similar to our case study.

The term “Small Round Cell Tumours” (SRCT) applies to a cluster of extraordinarily aggressive malignant neoplasms which feature the predominantly small and monotonous undifferentiated cells with extensive nucleocytoplasmic ratios on histology. This group includes Ewing’s Sarcoma (ES), Primitive Neuroectodermal Tumour (PNET) or extraskelatal Ewing’s sarcoma, neuroblastoma, rhabdomyosarcoma, desmoplastic small round cell tumour, non Hodgkin’s lymphoma, small cell osteosarcoma, small cell carcinoma (either undifferentiated or neuroendocrine), olfactory neuroblastoma and mesenchymal chondrosarcoma. Their clinical presentations frequently overlap, for that reason making the diagnosis difficult in some instances. Within the recent past, more and more sophisticated array of immunohistochemical and chromosomal markers have confirmed to be beneficial in classifying these aggressive lesions [12].

Although immunohistochemistry (IHC) may be a useful adjunct, it lacks specificity. Amongst several histological variants of SS, monophasic fibrous subtype is difficult to diagnose and desires to be differentiated from sarcomatoid carcinoma, sarcomatoid variation of mesothelioma, fibrosarcoma, leiomyosarcoma, Ewing’s sarcoma, spindle cell thymoma, and solitary fibrous tumor (SFT) in addition to metastatic sarcomas [2-5]. Therefore the utility of fluorescent in situ hybridization (FISH) strategies to detect particular

chromosomal translocation for diagnosing SS, especially in unusual places similar to the lung [2,4].

SSs are characterized by way of the particular chromosomal translocation t(X;18) (p11.2;q11.2), which has been detected in more than ninety percent of SS [13]. It finally ends up in the fusion of the SYT gene from chromosome 18 to 1 of three exceptionally homologous genes at Xp11, namely, SSX1, SSX2, and in rare instances, SSX4 [13,14].

On chest roentgenogram, the lesion is often uniform with well-circumscribed rounded or lobulated borders [15–17] with a mediastinal shift in some patients [18].

CT shows a well-defined homogeneous or heterogeneously enhancing mass containing necrotic areas and soft tissue components. Ipsilateral pleural effusion is common [15–18], while mediastinal lymphadenopathy is rare [15].

Totally based on 2-year local recurrence quotes [20, 21] and poor five-year disease-specific survival rates [4], primary pulmonary and mediastinal SS is greater aggressive than soft tissue synovial sarcoma. Primary pulmonary SS behaves more aggressively due to the late presentation, massive tumor size and trouble in reaching free surgical margins.

Free surgical margins and adjuvant chemotherapy increases the time for nearby recurrence and sickness-free survival. Although, the prognosis of primary pulmonary SS stays poor with overall survival of 50% [19], the prognosis of primary endobronchial SS subset isn't always defined. The prognosis in our case study couldn't be predicted because the patient was lost for follow up. .

Conclusion

The Majority of primary pulmonary and mediastinal SS are located within the lung parenchyma and barely extend into the bronchial tree. Because of the heterogeneity of symptoms, clinical diagnosis is difficult. Cross sectional CT imaging is crucial to grasp the precise location & extent of tumour. Histologically, primary pulmonary and mediastinal SS reportedly stocks similar features with soft tissue SS. Within the latest past, more and more sophisticated array of immunohistochemical and chromosomal markers have proven to be useful in classifying those aggressive lesions.

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Role of MRI in detecting the recurrence of carcinoma cervix in patients treated with radiotherapy

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Abstract

Aim: To study the role of MRI in detecting the recurrence of carcinoma cervix in patients treated with radiotherapy.

Materials and methods: A Prospective study done in Department of Radio diagnosis, Trichy SRM medical college hospital and Research Centre, Trichy during June 2019 to June 2021.

Methodology: Carcinoma cervix patients who were referred to our department for MR imaging diagnosed post radiotherapy treatment follow up.

Result: From our study, it is evident that post radiation complications are more common to develop after 2-3 years of radiotherapy which also corresponds with the average time of recurrence.

Conclusion: The most common post radiotherapy changes were fatty replacement of bone marrow followed by cystitis and proctitis.

Keywords: Carcinoma cervix, post radiotherapy complications, MRI

Introduction

MR imaging of cervix has evolved over the past two decades as the most useful imaging. It is not only useful for preoperative staging but it also helps in identification of recurrent / residual tumors in treated patients. The prevalence of cervical carcinoma has increased in recent years due to early screening programs. The screening programs prompt the patients to undergo further evaluation. MR imaging proves to be the next best level of modality in cervical carcinoma. MRI accurately stages the carcinoma which is better than clinical staging.

Objectives of the study

- To study the role of MRI in detecting the recurrence of carcinoma cervix in patients treated with radiotherapy.
- To evaluate the validity of MRI in detection of treatment response and recurrence in treated patients of carcinoma cervix.

Materials

From June 2019 to June 2021, around 70 patients who were histopathological proven cases of carcinoma cervix and referred for MRI pelvis to the Department of Radio diagnosis, Trichy SRM medical college hospital and Research centre, Trichy were included in the study.

Study methodology

Histopathologically diagnosed cases of carcinoma cervix who were referred to the Department of Radio diagnosis were included. In this study, two groups of patients were included– newly diagnosed and post treatment cases. After obtaining permission from the institutional medical research ethics committee and taking informed consent from the patients, they were subjected to MRI pelvis using a 1.5 – Tesla system.

Different MRI sequences like TRUF1 coronal, axial and sagittal T2WTSE, axial and sagittal T1WTSE, STIR axial, DWI – MRI with ADC and contrast enhanced oblique axial, coronal and sagittal FST1 WTSE were used. The contrast gadolinium DTPA was given at a dose of 0.1 mmol / kg at a rate of 1 ml / second.

Table 1: Show the MRI non contrast scans

| MRI sequences | Non contrast scans | | Fat suppressed scans | |
|------------------------|--------------------|--------------------|----------------------|------------------------|
| | T1 w FSE | T2 w TSE | Stir | FS t1 W TSE (contrast) |
| Imaging plane | Axial and Sagittal | Axial and Sagittal | Axial | Sagittal axial coronal |
| TR/ TE (m sec) | 633/ 11 | 6320/ 116 | 9060/14 | 500/ 11 |
| FOV | 1785*847 | 1785*847 | 1785*847 | 1785*847 |
| Section thickness (mm) | 4 mm | 4 mm | 4 mm | 5 mm |
| Matrix | 768*768 | 512*512 | 512*360 | 768*768 |

Inclusion criteria

- Carcinoma cervix patients who were referred to our department for MR imaging both newly diagnosed and those who were on post treatment follow up.

Exclusion criteria

- Patients with cardiac pacemakers, new implants, clips within the body and other contraindications of MR imaging like claustrophobia were excluded.

Results

Table 2: Distribution of cases

| Category | No of patients | Percentage |
|-----------------|----------------|------------|
| Newly diagnosed | 26 | 37% |
| Recurrent | 44 | 63% |

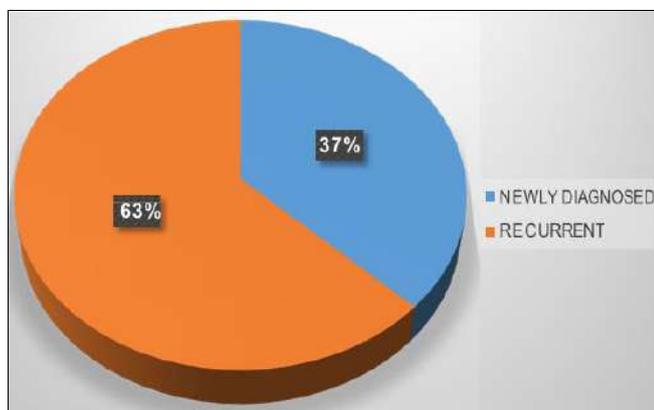


Fig 1: Category of patients

Table 3: Interval between RT and imaging

| Duration post RT (N=39) | No of patients | Percentage |
|-------------------------|----------------|------------|
| < 6 months | 11 | 28% |
| 6-12 months | 10 | 26% |
| 1-5 yrs | 13 | 33% |
| > 5 yrs | 5 | 13% |

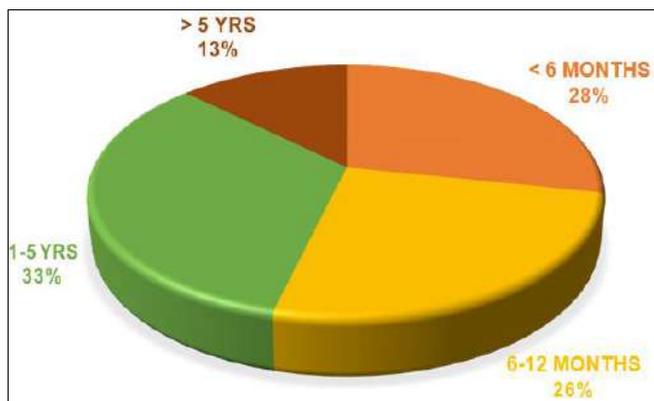


Fig 2: Duration Post RT

Table 4: Correlation between post RT complication and mean duration after RT

| Post radiation complications | Mean duration after RT |
|------------------------------|------------------------|
| Present | 3.11 |
| Absent | 2.13 |

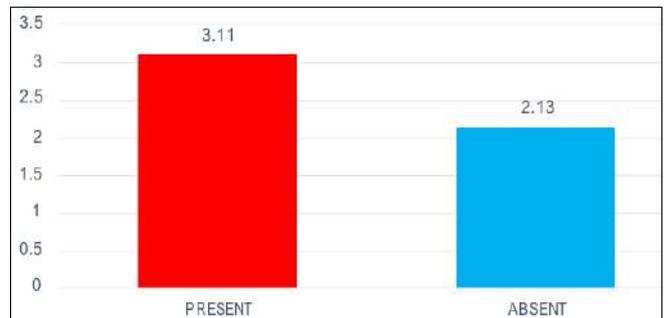


Fig 3: Mean Duration after RT VS Complication

Table 5: Post Radiation Complications

| Post radiation Complication | No of cases | Percentage |
|-----------------------------|-------------|------------|
| Bone marrow changes | 8 | 20.5% |
| Cystitis | 8 | 20.5% |
| Proctitis | 8 | 20.5% |
| Pelvic lipomatosis | 2 | 5% |
| Free fluid | 2 | 5% |
| Vesico vaginal fistula | 1 | 2.5% |
| Ureteric stricture | 1 | 2.5% |
| Pyosalpinx | 1 | 2.5% |

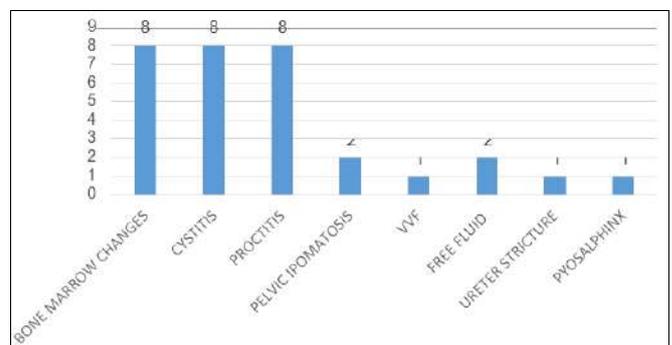


Fig 4: Post radiation complication

Table 6: Comparison between clinical assessment and MRI

| Clinical Assesment vs MRI | New | Recurrent |
|---------------------------|--------|-----------|
| Sensitivity | 88% | 88.89% |
| Specificity | 0% | 30% |
| Positive predictive value | 92.59% | 53.33% |
| Negative predictive value | 95.65% | 75% |
| Accuracy | 88.19% | 57.89% |

A total of 70 patients who are biopsy proven cases of carcinoma cervix are included in the study. Both newly diagnosed and patients treated with chemo radiotherapy are

included. FIGO stage is assigned both clinically and with MRI and the parameters are compared.

Categories of patients

- Two categories of patients are considered for study- 26 newly diagnosed patients (37% of study population) and 44 treated cases (63% of study population). (Table 2, Figure 1)

Timing of imaging after radiotherapy

- Radiotherapy was given for 39 patients totally and most of them (13 patients, 33%) were symptomatic and referred for MRI during the period of 1- 5 years followed by less than 6 months and during the period of 6- 12 months. (Table 3, Figure 2)
- From our study, it is evident that post radiation complications are more common to develop after 2-3 years of radiotherapy which also corresponds with the average time of recurrence. Since most of the patients are referred during this time to look for recurrence of tumor, more complications are also diagnosed during this time. The most common post radiotherapy changes are fatty replacement of bone marrow followed by cystitis and proctitis.

Conclusion

Usefulness of MRI staging in 70 patients of biopsy proven cases of carcinoma cervix were assessed in the study. Both newly diagnosed and patients treated with chemo radiotherapy were included. FIGO stage was assigned both clinically and with MRI and the parameters were compared. 26 newly diagnosed patients and 44 treated cases were included.

From our study, it was evident that post radiation complications were more common to develop after 2-3 years of radiotherapy which also corresponded with the average time of recurrence. The most common post radiotherapy changes were fatty replacement of bone marrow followed by cystitis and proctitis.

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Innovations

Proximal Fibular Osteotomy in medial compartment Osteoarthritis Knee

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Abstract

Problem: Knee osteoarthritis is a common chronic, progressive, degenerative disease in older individuals with accompanying joint pain, stiffness and deformity. Although Conservative managements like toe-out gait modification, valgus knee braces, lateral wedge shoe insoles aim to alter abnormal loading patterns when associated with genu varum deformity the results are not promising. The objective of this study was to evaluate the functional outcome, changes in pain intensity, changes in mechanical knee alignment and radiological changes after performing Proxiaml Fibular Osteotomy. **Design:** The prospective hospital based study titled “Proximal fibular osteotomy in medial compartment osteoarthritis knee” was conducted in tertiary care hospital for the period of 2 years. A total of 15 patients were included in the study and all the relevant information was recorded in a proforma. The patients were followed up at 2nd, 6th and 12th months post-operatively. The outcome variables TFA, Joint space ratios, modified Oxford knee and visual analogue scale scores was recorded at each visit. **Findings:** The mean age of the study subjects was 56.1 years with the range of 50 to 65 years where the gender ratio was 1:1.5, among the male population, 33.3% werewere Drivers by their occupation. Most of the patients affected knee was right side contributing 67% and the mean BMI of our patients was 27.5 ± 3.1 belonging to the overweight category. The mean duration of symptoms in our patients was 5.5 ± 2.2 years with a range from 2 to 8 years. The mean varus angle in our patients was 8 degrees with the range of 5 to 10 degrees. Nearly 60% of our cases had varus deformity between 5 to 10 degrees, 20 % cases had varus deformity less than 5 degree and 20% had varus more than 10 degree. The mean duration of hospital stay was 8 days with the range of 5 to 11 days. Around 53% of the patients were discharged within a week and the remaining 47% were discharged at the second week post-operatively. The mean TFA measured pre-operatively in our study was 182.6 ± 1.67 . At follow ups at 2nd, 6th and 12th months, the mean TFA were 181.1 ± 1.72 ,

179.8±1.61 and 180.07±1.53 which were statistically significant. We found that the mean medial to lateral joint space ratio preoperatively was 0.17±0.03 which was improved to 0.19±0.03, 0.20±0.04, 0.22±0.04 at follow ups of 2nd, 6th and 12th months which were statistically significant. The mean pre-operative modified Oxford knee score was 50.5±3.8 and post-operatively improved to 58.0±3.41, 62.4±4.1, 65.7±3.5 at 2nd, 6th and 12th months which was statistically significant. The mean visual analogue scale score for pain was 6.8±0.9. Post-operatively at 2nd, 6th and 12th months, the mean VAS were 5.0±0.8, 3.9±0.8 and 3.5±0.7 which were statistically significant. **Conclusion:** Early functional and radiological results were promising in terms of pain relief, better functional scores, improvement in tibio-femoral angle and mild opening up of medial joint space.

Keywords: 1. Osteoarthritis knee, 2. proximal fibular osteomy

Introduction

Knee osteoarthrosis is a common chronic, progressive, degenerative disease in older individuals with accompanying joint pain, stiffness and deformity. It has an incidence of 30% in the population elder to 60 years (Shiozaki *et al.*, 1999). The disease involves mechanical, osseous, genetic and environmental factors (Ahlback, 1968). The rate of articular degeneration at weight bearing areas along with hypertrophy of articular cartilage at non-weight bearing areas of the joint which later calcify to form the osteophytes and is accompanied by increasing thickness and eburnation of the subchondral bony plate, outgrowth of osteophytes at the joint margin, stretching of the articular capsule, synovitis, weakness of muscles bridging the joint and narrowing of the joint space (Felson *et al.*, 1987).

The lesions of osteoarthritis stem from degeneration of the articular cartilage and disordered repair. Biomechanical stress is the principle pathogenic mechanism but genetic factors including polymorphisms in genes encoding components of the matrix and signalling molecules may predispose to chondrocyte injury that causes matrix alteration. Chondrocytes proliferate and continuously synthesize proteoglycans but disease develops when degradation exceeds synthesis. This leads to changes in proteoglycan composition as the disease progresses.

Chondrocytes also secrete matrix metalloproteases (MMP) that degrades the type II collagen network. Cytokines and diffusible factors from chondrocytes and synovial cells such as TNF- β , Prostaglandins, and Nitric oxide are also implicated in osteoarthritis (OA) and chronic low-level inflammation contributes to disease progression.

Advanced disease is characterised by chondrocyte loss and severe matrix degradation (3). Chondrocytes proliferate and form clusters in early stages of OA. Concurrently, matrix water content increases, proteoglycan concentration decreases and horizontally arranged collagen type II fibres in the superficial zone are cleaved. These processes result in fibrillation of articular cartilage and full thickness portions of cartilage are sloughed into the joint, forming loose bodies (Wu *et al.*, 2004).

Exposed subchondral bone becomes the new articular surface which is burnished by friction with the opposing surface giving it the appearance of polished ivory – bone

eburnation. Underlying articular bone undergoes rebuttoning, sclerosis and develops small micro fractures creating gaps that allow the synovial fluid to be forced into the subchondral region (Sprenger and Doerzbacher, 2003).

As the loculated fluid collection increase in size, fibrous walled cysts form. Mushroom shaped bony outgrowths- osteophytes develop at the margins of the articular surface and are capped by fibrocartilage and hyaline cartilage that gradually ossify. The synovium is usually congested and becomes fibrotic (Sprenger and Doerzbacher, 2003).

Conservative management like toe-out gait modification, valgus knee braces, lateral wedge shoe insoles aim to alter abnormal loading patterns but has not shown significant results. High tibial osteotomy (HTO) and total knee arthroplasty (TKA) are the two common methods used for treating osteoarthritis. High tibial osteotomy is a relatively extensive procedure and has complications such as neurovascular injury, iatrogenic fracture and non-union (Aglietti *et al.*, 2003). Though total knee arthroplasty corrects alignment, improves function and relieves pain, it is not the treatment of choice in patients of younger age and moderate osteoarthritis (Schnurr *et al.*, 2013)

While comparing High tibial osteotomy and Proximal fibular osteotomy, PFO also provides excellent result in these cases (Yang *et al.*, 2015; Wang *et al.*, 2017). It is hypothesized that Proximal fibular osteotomy releases the taut posterolateral soft tissues and shifts the mechanical axis from medial to lateral compartment thus relieving the medial compartmental pressure and improving the pain and functional status of the affected knee (Liu *et al.*, 2018).

Materials and Methods

Inclusion criteria: The patients with moderate to severe symptomatic medial compartment osteoarthritis knee medically fit for surgery during the study period not responding to adequate conservative treatment with radiological evidence of medial joint space reduction

Exclusion criteria: Secondary osteoarthritis, rheumatoid arthritis, post-traumatic osteoarthritis; those not willing to participate and give written consent; those with previous knee surgery and fractures of femur and tibia; those with Genu valgus deformity and patient unfit for surgery and anaesthesia.

Pre-OP workup: Informed written consent was taken from all the patients participating in the study. The patients after admission were thoroughly examined to evaluate their general physical condition.

The tibio-femoral angle, joint space was assessed by antero-posterior and lateral X-ray of the knee in standing position. All the patients were subjected to routine investigations like routine blood, urine examinations, serum urea, creatinine and electrolytes, blood sugar fasting and post prandial, ECG and chest X-ray. Prophylactic antibiotic in the form of parenteral third generation cephalosporins were given along with the induction of anaesthesia and was continued for 2 days post operatively.

Surgical technique

The surgery was performed with the patient in supine position under spinal or epidural anaesthesia, with a tourniquet. The tip of fibular head was marked with a skin marking pen and the appropriate downward distance measured. Skin and subcutaneous tissue were cut. The incision should be a little more than twice the length of the resected segment (Figure 1). The fibular periosteum was now exposed by separating the peroneus and soleus. The periosteum was incised in line of skin incision and a 1.5 to 2 cm piece of fibula resected with a narrow blade oscillating saw (Figure 2).

The length of fibular segment resected was 1.5 to 2 cm and the distance from fibular head was 6 to 9 cm. The idea here was to cut the two fibular cortices, converting the knee to a more balanced joint, with unicortical support on either side, allowing correction of mechanical axis. The resection was high enough to cause a mechanical axis shift, but yet not high enough to damage the lateral popliteal nerve. The size of the resected segment and its distance from fibular head depends on the patient's height and while shorter patient had a 1.5 cm segment resected 6 cm below fibular head, tall patients had a 2 cm resection, some 8 to 9 cm below (Figure 3 and 4).

Wound was washed, closed in layers (Figure 5) and a light compression bandage



Figure 1: Skin Incision over the proximal part of the fibula over the lateral aspect



Figure 2: Marking confirmed with the help of measuring scale for fibula resection



Figure 3: 1.5-2 cm piece of fibula resection done



Figure 4: Fibular piece of bone resected and confirmed with the measuring scale



Figure 5: Final wound closure

given. The patient was mobilised as soon as tolerated, in most cases within a few days.

Post-OP protocol

Parenteral antibiotics and anti-inflammatory medications were administered regularly for the first 48 hours of surgery. The operated limb was kept elevated and active toe movement exercise was started once the patient recovered from anaesthesia. Check X-rays were taken on the 2nd or 3rd post-operative day. Physical therapy, patient mobilization and weight bearing as tolerated were initiated within 24 to 48 hours of surgery. The skin sutures were removed on post-operative day 10.

Patients were followed up once weekly up to first 2 months, once monthly up to 6th month then two monthly once up to 12th month and thereafter three monthly once at outpatient department. Clinical and functional assessment was done during the follow up studies using the tibio-femoral angle, joint space ratio, modified Oxford knee Score and VAS score.

Results

A total of 15 patients underwent Proximal fibular osteotomy during the study period met the inclusion criteria. In our study, the peak incidence of osteoarthritis was in the age group between 51-60 years comprising of 53.3%. In this study, both male and female cases comprising of 40 and 60% with the ratio of 1:1.5.

In our study, there were 67% cases with right side predominantly and 33% cases with left side knee-affected unilaterally in all the 15 cases. Around 60% of the cases were belonging to the overweight category, 27% were obese and 13 % cases were within the normal BMI range.

In our study, 60% of cases had varus deformity between 6-10 degrees, 20% cases had less than 5 degrees and the remaining 20% cases had 11-15 degrees. Nearly 53% of the cases were discharged within a week and 47% cases discharged after a week post-operatively. In our study, 3 patients got extensor hallucis longus weakness (EHL) and 1 patient got tingling, numbness over dorsum of foot post-operatively which was gradually recovered within 4-6 months.

The mean tibio-femoral (TFA) angle measured pre-operatively was 182.6 (± 1.67) degrees whereas the mean tibio-femoral angle measured post-operatively was 181.1 (± 1.72) at 2nd month, at 6th month follow-up, the mean tibio-femoral angle was 179.8 (± 1.61) and at 12th month, the mean tibio-femoral angle was 180.1 (± 1.53) which was statistically significant.

The mean joint space ratio measured pre-operatively was 0.17 \pm 0.03. The mean joint space ratio measured at 2nd month, 6th month and 12th months were 0.19 \pm 0.03, 0.20 \pm 0.04 and 0.22 \pm 0.04 which were statistically significant (p value < 0.05). The mean Oxford knee score was recorded 50.5 \pm 3.8 pre-operatively and the mean Oxford knee score recorded post-operatively at 2nd, 6th and 12th months were 58.0 \pm 3.9, 62.4 \pm 4.1, 65.7 \pm 3.5 respectively which was statistically significant. The mean VAS score was recorded 6.8 \pm 0.9 pre-operatively and the mean VAS score recorded at 2nd, 6th and 12th months were 5.0 \pm 0.8, 3.9 \pm 0.8 and 3.5 \pm 0.7 respectively which was statistically significant (Figure 6 and 7).

Figure 6: Pre-operative AP radiograph showing medial compartment OA knee- Left side



Figure 7: Post-operative AP radiograph showing resection of proximal fibular segment



Discussion

This study was conducted in a tertiary care teaching hospital in India from August 2018 to July 2020. We compared our results with the earlier studies. The following variables like age of the patient, sex, occupation, BMI, duration of symptoms, side of the knee affected were assessed. Clinical and radiological parameters like degree of varus deformity, tibio-femoral angle, joint space ratio were assessed. Functional assessment was done with Modified Oxford knee score, Visual analogue scale scores for pain. We also tried to assess the post-operative complication like oedema of limb, EHL weakness, paresthesia over the dorsum of foot and infection rate.

The mean age of presentation in our study was 56.1 ± 5.1 years with the range of 50 to 65 years. Our patients were younger than the patients in the studies by other authors. In the study described by Yang *et al.*, 2015, it was 63.5 years, Wang *et al.*, 2017 with mean age of 63.96 years, Liu *et al.*, 2018 with mean age of 59.45 years, Prakash, 2019 with mean age of 56.3 years, Nie *et al.*, 2018 with mean age of 60.34 years.

In this study, the gender ratio of male and female was 1:1.5 and the same gender variance was expressed in various studies with 1:2.2, 1:2.9, 1.5.5 and 1:1.4 respectively (Yang *et al.*, 2015, Wang *et al.*, 2017, Liu *et al.*, 2018 and Prakash, 2019).

The mean duration of symptoms in our patients was 5.5 ± 2.2 years with a range from 2 to 8 years whereas the studies of Zou *et al.*, 2017 whose patients suffered average duration of 1.5 ± 0.4 years and Yang *et al.*, 2015 with symptoms duration ranging from 1.5 to 7 years. This is because most of the patients in this study were not responding to conservative treatment for more than a year before they consented to surgical procedure.

In this study, right knee was found to be commonly involved with incidence of 67% similar to that of other studies which had predominant right knee involvement (Prakash, 2019; Subash *et al.*, 2018).

The mean BMI of our patients was 27.5 ± 3.2 which was comparable to the study by Subash *et al.*, 2018 who included patients only with BMI < 30 . High BMI with increased loading may be detrimental for articular cartilage healing. The mean varus angle in our patients was 8 ± 2 degrees with the range of 5 to 10 degrees. Most (60%) of our cases had varus deformity between 5 to 10 degrees, 20% cases had varus deformity less than 5 degree and 20% had varus more than 10 degree. The study population of Subash *et al.*, 2018 were with a varus deformity only less than 10 degree. Other studies also included knee OA with

varus deformities but the exact degree of deformity was not specified (Yang *et al.*, 2015, Wang *et al.*, 2017, Prakash, 2019; Zou *et al.*, 2017).

The mean duration of hospital stay was 8 days with the range of 5 to 11 days. In this study, 53% of the patients were discharged within a week and other 47% were discharged at the second week post-operatively. The mean TFA measured pre-operatively was 182.6 ± 1.67 . At follow ups at 2nd, 6th and 12th months, the mean TFA were 181.1 ± 1.72 , 179.8 ± 1.61 and 180.07 ± 1.53 which were statistically significant. On comparison with other studies using TFA as a measure for correction of mechanical alignment found the mean TFA improved from 182.7 ± 2.0 pre-operatively to 179.4 ± 1.8 (Yang *et al.*, 2015). A study found that TFA improved significantly from 183.4 ± 2.5 pre-operatively to 168.9 ± 1.3 post-operatively (Zou *et al.*, 2017). Another study found that mean TFA improved from 182 ± 1.8 to 179 ± 1.9 (Subash *et al.*, 2018).

The mean medial to lateral joint space ratio pre-operatively was 0.17 ± 0.03 which improved to 0.19 ± 0.03 , 0.20 ± 0.04 , 0.22 ± 0.04 at follow up of 2nd, 6th and 12th months which were statistically significant (p value <0.05). The results of Wang *et al.*, 2017 described that the joint space ratio improved significantly from 0.40 ± 0.28 to 0.58 ± 0.30 at 12 month follow up. Another study showed that the mean medial joint space change was from 1.3 ± 0.8 to 4.2 ± 2.7 at final follow up of 24 months (Subash *et al.*, 2018). Similarly there was a corresponding reduction in lateral joint space from 7.6 ± 1.2 to 5.4 ± 1.3 which were statistically significant who analysed only the width of lateral joint space and found that the lateral joint space was decreased from 12.2 ± 1.1 preoperatively to 6.9 ± 0.7 at final follow up of 24 months which was again statistically significant (Yang *et al.*, 2015).

In this study, the mean pre-operative knee score was 50.46 ± 3.8 and post-operatively improved to 58.0 ± 3.9 , 62.4 ± 4.1 , 65.7 ± 3.5 at 2nd, 6th and 12th months which was statistically significant with p-value <0.05. Comparing to other studies, the mean functional knee score was 54.4 pre-operatively which was increased to 77.0 post-operatively (p-value <0.05) and the mean score was 52.2 pre-operatively which was increased to 79.0 post-operatively (p-value <0.05) which was statistically significant (Prakash, 2019; Subash *et al.*, 2018).

All the patients in this study had pain as a major clinical symptom which was not responding to conservative treatment. Pain due to OA knee contributes to functional impairment, impairment of mobility reduced quality of life in elderly population. The mean visual analogue scale for pain in this study was 6.8 ± 0.9 . Post-operatively at 2nd, 6th and 12th months, the mean VAS were improved to 5.0 ± 0.8 , 3.9 ± 0.8 and 3.5 ± 0.7 which were statistically significant with the p-value of <0.05.

The mean VAS was comparable to other studies with VAS of 7, 8.02, 6.7, 5.64, 4.6 and 6.9 respectively while analyzing pre-operatively; while analysing post-operative observations, the results of the similar studies with VAS of 2, 2.74, 2.2, 0.27, 0.5 and 2.1 respectively which were statistically significant (Yang *et al.*, 2015, Wang *et al.*, 2017 Prakash, 2019; Nie *et al.*, 2018; Zou *et al.*, 2017; Subash *et al.*, 2018).

Injury to the common peroneal nerve can occur if bone levers are inadvertently placed which could have resulted in the post-operative paresthesia over dorsum of foot in one (6.6%) of the cases included in this study. Similar complications were encountered by Yang *et al.*, 2015 who reported 4 (3.6%) of their cases with paresthesia over foot and 2 (1.8%) cases with

confirmed superficial peroneal nerve palsy and Subash *et al.*, 2018 in 3 (10%) of their cases. The paresthesia recovered uneventfully without any intervention during the follow up at 4-6 months which showed that the injury to the nerve could be due to neuropraxia. A study reported with one (2.5%) case with neurovascular injury. In this study also faced 3 (20%) case with EHL weakness in which could have been due to traction injury of the common peroneal nerve itself (Zou *et al.*, 2017). Adequate precautions were taken by making our incision 6cms below fibular head, osteotomy 8cms below fibular head and not using bone levers for retraction in future cases to prevent injury to common peroneal nerve or its fanning of branches. A study identified that common peroneal nerve (CPN) courses superficial to the lateral surface of the fibula and approximately 2cms distal to fibular head passes into the fibular tunnel and as they wrap around the fibular neck demonstrated broad fanning of fibers with three major divisions including anterior recurrent, superficial and deep branches (Baruah *et al.*, 2018).

Conclusion

Knee osteoarthritis is one of the leading causes of morbidity among elderly patients. If associated with genu varum deformity the results of conservative treatment are not promising. Even though high tibial osteotomy (HTO) and unicompartmental knee arthroplasty (UKA) could correct genu varum deformity and prevent disease progression, they are associated with serious complications. Total knee arthroplasty (TKA) was considered too radical surgery for early OA knee. Proximal fibular osteotomy was a reliable surgery for early OA knee with genu varum deformity provided that deformity less than 15 degrees. The beneficial effect of PFO was mainly because of release of taut posterolateral soft tissue structures attached to fibular head and also correction of non-uniform loading on the tibial weight bearing articular surface. Proximal fibular osteotomy was a simple quick surgery which could be performed within 30-40 mins with minimal blood loss of approximately 30-50 ml. Early ambulation with full-weight bearing was allowed in the post-operative period which was an added advantage. Early functional and radiological results were promising in terms of pain relief, better functional scores, improvement in tibio-femoral angle and mild opening up of medial joint space.

The hypothesis proposed in this study showed that PFO improved pain and functional status of the patient by shifting the mechanical axis from medial to lateral compartment and relieving medial compartmental pressure.

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Innovations

An observational study to determine the impact of discordant drinking pattern of alcohol dependence in male on marital satisfaction and their spouse marital satisfaction

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Abstract

Problem: A stronger foundation for a happy marriage can be found in a couple who share similar values, beliefs and ways of behaving. This study aims to determine the discordant drinking pattern's impact on marital satisfaction of patients and their spouse. **Methodology:** This is a type of cross-sectional study on 100 participants (50 husbands and 50 wives) from psychiatric OPD treatment seeking patients. Nonprobability sampling (convenient sampling) was used. Married alcohol dependence patients and their spouse, patient age between 21 to 50 years, educated till 8th standard and above, alcohol dependence for 1 year and years of marriage at least to be 2 years were included. Psychoactive substance uses disorders other than alcohol dependence and nicotine dependence, diagnosed primary psychiatric disorders, patients with intellectual disability, factors other than substance use were excluded. Determination of alcohol dependence was done using ICD 10 Criteria. Determination of marital satisfaction was done using marital adjustment test. **Findings:** Mean marital satisfaction scores of the study participants were 114.36 ± 3.50 and spouse satisfaction scores were about 90.92 ± 8.82 . The correlation between marital satisfaction scores and spouse marital satisfaction scores are about 0.10, $p=0.49$. This study found that spouses had statistically significantly lower marital satisfaction scores (90.92 ± 8.82) compared to study subjects (114.36 ± 3.50), $t(98)=17.46$, $p < 0.001$. Higher scores indicate greater satisfaction. Among study subjects almost all were having higher marital satisfaction scores which above 100. Among their spouses only 10% ($n=5$) had higher marital satisfaction scores and remaining 90% ($n=45$) had lower marital satisfaction scores inferring distress. **Conclusion:** Discordant drinking patterns and alcohol dependence are associated with lower levels of marital satisfaction for partners.

Keywords: 1. Spouse, 2. Marital satisfaction, 3. alcohol, 4. discrepancies, 5. marital stress

Introduction

A stronger foundation for a happy marriage can be found in a couple who share similar values, beliefs, and ways of behaving. There may be shared histories between those with affective disorders and those with antisocial personalities. Assortative mating is a term used to describe the phenomenon of highly compatible couples (Smith *et al.*, 2012). According to compatibility theories, major dissimilarities between partners can put a strain on a relationship (Kurdek, 1981). This is also true for tobacco and alcohol use.

Recent studies have expanded the study of spouse similarity to the domain of substance use. Similarities in substance use have been observed among married couples, as have similarities in a variety of traits and behaviours (Yamaguchi and Kandel, 1993). The study looked at drug use in 545 couples and discovered significant drug use concordance. A high concordance of marijuana use was found among couples from the year before marriage to the couple's second anniversary in another longitudinal study of substance use during the

transition to marriage (Homish and Leonard, 2005; Homish and Leonard, 2007; Leonard and Roberts, 1998) examined spousal similarity for alcohol use in married couples and discovered significant correlations for average daily volume of alcohol, frequency of heavy drinking and frequency of intoxication among couples in the year before and after marriage.

According to compatibility theory, husbands and wives who use similar substances may have better overall marital functioning than couples who use dissimilar substances (Leonard and Roberts, 1998) examined the relationship between different types of "drinking partnerships" and marital functioning. The similarity, or lack thereof, of drinking patterns between husbands and wives was defined as a drinking partnership. Discordant drinking patterns were associated with lower marital functioning (Mudaret *et al.*, 2001) marital functioning was assessed in a community sample of newlyweds to see if the configuration of partners' drinking patterns was related to marital functioning. There were four groups of drinking patterns: one concordant for use, one concordant for nonuse, and two discordant groups (husband or wife only use).

Furthermore, different levels of consumption were considered (any alcohol use, regular drinking, heavier drinking, and frequent intoxication). There were no significant differences in marital quality between the two concordant groups (neither engage in behaviour vs. both do) and no differences between the two discordant groups for heavier drinking and frequent intoxication (husband or wife only). However, discordant couples had significantly lower marital quality than couples in which neither partner used alcohol at these levels or couples in which both partners consumed at these levels. The latter finding implies that mutual patterning of drinking (i.e., concordance of drinking behaviours vs. discordance of drinking behaviours) is a key factor in the relationship between alcohol consumption and marital functioning, and may be more important than either partner's level of drinking (Leadley *et al.*, 2000). Using data from the Ninth National Alcohol Survey, researchers discovered that inconsistent alcohol use was related to relationship distress and the occurrence of violence. Both of these studies, however, relied on cross-sectional analyses and did not investigate longitudinal effects.

According to compatibility theory, husband and wife substance use similarities may be associated with better overall marital functioning. The drinking patterns are divided into four groups: one for use, one for nonuse, and two discordant groups (husband only use, wife only use). An erratic drinking pattern would be associated with lower marital satisfaction. When compared to couples where both partners consumed at equal levels, discordant couples had significantly lower marital quality (Homish and Leonard, 2007). The marital adjustment test is used to determine marital satisfaction (Locke and Wallace, 1959). This study aims to determine the discordant drinking pattern's impact on marital satisfaction of patients and their spouse.

Material and Methods

Type of Study: Cross-sectional study

Study Settings: Department of psychiatry in tertiary care hospital.

Study Population: Alcohol dependence patients and their spouse.

Period of study: The study was conducted for the period of 6 months (March to September 2022)

Sample Size: Hundred 100 participants (50 husbands and 50 wives) from psychiatric OPD treatment seeking alcohol dependence patients and their spouse

Ethical Consideration: The study was approved by the Institutional ethical Committee (Ref.No:211/TSRMMCH&RC/ME-1/2022-IEC No:079 dated 10.03.2022) and details of the alcohol dependence patients and their spouse were collected only after obtaining informed consent.

Procedure: Nonprobability sampling (convenient sampling) was used. Married alcohol dependence patients and their spouse, patient age between 21 to 50 years, educated till 8th standard and above, alcohol dependence for 1 year and years of marriage at least to be 2 years were included. Psychoactive substance uses disorders other than alcohol dependence and nicotine dependence, diagnosed primary psychiatric disorders, patients with intellectual disability, factors other than substance use were excluded.

The methodology adopted for the present study is dealt as the following: selection of patients according to inclusion and exclusion criteria were done, recording of data in preformed and pretested semi-structured proforma for sociodemographic profile, marriage profile and substance use profile were done. Determination of alcohol dependence was done using ICD 10 Criteria. Determination of marital satisfaction was done using marital adjustment test.

The Marital Adjustment Inventory was utilised to analyse couple dynamics (Locke and Wallace, 1959). There are a total of 15 questions on the MAT. The extent to which one is happy can be gauged from just one question (7 response choices from very happy to perfectly happy). In eight more questions, respondents are asked to rate the extent to which they agree with their partners on various issues (e.g., family finances, ways of dealing with in-laws, matters of recreation, etc.). A 6-point scale ranging from 100% agreement to 100% disagreement was used to rate these statements. Clinically significant marital distress is typically defined as a score of less than or equal to 100.

Statistical analysis: For continuous variables, descriptive statistics were reported as mean SD and for categorical variables, frequencies percentage. Statistical significance was determined using Chi-Square at the 5% level. As long as the expected cell count is less than 5, Fischer's exact test applies. Independent t test was used to compare the marital satisfaction scores between patients and their spouses. IBM SPSS Statistics for Windows, Version 26.0., IBM Corp., Chicago, IL was used to statistically analyse the data.

Results

The normality test was applied using Kolmogorov–Smirnov test and test result shows statistically insignificant with test statistics 0.118, $p=0.079$ and 0.091, $p=2.00$ for spouse and patients respectively. Table 1 shows the descriptive statistics of marital satisfaction scores of the patients and their spouses. Mean marital satisfaction scores of the study participants were 114.36 ± 3.50 and spouse satisfaction scores were about 90.92 ± 8.82 .

The correlation between marital satisfaction scores and spouse marital satisfaction scores are about 0.10, $p=0.49$. This study found that spouses had statistically significantly lower marital satisfaction scores (90.92 ± 8.82) compared to study subjects (114.36 ± 3.50), $t(98)=17.46$, $p<0.001$ (Table 2).

Higher scores indicate greater satisfaction. Among study subjects almost all were having higher marital satisfaction scores which above 100 (Figure 1). Among their spouses only 10% ($n=5$) had higher marital satisfaction scores and remaining 90% ($n=45$) had lower marital satisfaction scores inferring distress (Figure 2).

Discussion

Previous cross-sectional research has found that couples reporting a disparate pattern of alcohol use at the time of marriage have lower levels of marital satisfaction compared to couples reporting either both partners using or neither partners using (Mudar *et al.*, 2001). This held true for both chronic heavy drinking and frequent drug use. This study provides longitudinal evidence that drinking patterns that differ from one another are associated with a lower level of marital happiness. Discordant drinking was found to significantly correlate with lower levels of marital satisfaction for both men and women. It's also noteworthy that heavy drinking frequency wasn't a factor in this effect after being accounted for statistically.

Latent growth modelling was also used by (Kearns and Leonard, 2005) to investigate the association between one spouse's drinking and marital satisfaction over time. There was some evidence that changes in partner drinking were related to changes in marital satisfaction, but there was no evidence that partner drinking predicted subsequent changes in marital satisfaction for husbands or wives. Overall, these findings suggest that, within a representative sample of the population at large, marital satisfaction is most strongly and persistently associated with asymmetry in drinking patterns between partners, rather than with heavy drinking by either partner.

There may be a number of causes for the observed effects. Heavy drinking on the part of one spouse but not the other has been linked to lower marital satisfaction, which may be a result of underlying differences between the partners. However, when differences were modelled as a quadratic factor, they were found to be associated with marital satisfaction. Indeed, the disparities in alcohol consumption between husband and wife were found to be the most significant predictor of marital contentment, rather than the heavy drinking habits of either partner (Weisfeld, 1993).

In the current report, the linear term was not significant, but if it had been, it would have suggested that the relationship between alcohol consumption gaps and marital satisfaction varied depending on which spouse consumed more alcohol. According to compatibility theorists, happy marriages are most common amongst people who are very similar to one another (Roach *et al.*, 1981; Weisfeld, 1993) tested the hypothesis that

couples who are more similar to one another will be happier in their marriages and found support for this finding across a wide range of characteristics in both partners. This suggests that husbands' and wives' divergent patterns of heavy drinking may reflect fundamental differences in their worldviews or their conceptions of what constitutes acceptable conduct within the context of marriage.

Disagreements in drinking habits could be a symptom of less communication and affection between spouses, which could explain the link between alcohol differences and marriage health. Couples who have different habits of using substances may spend less time together, which can negatively impact the health of their marriage. Couples who spend time apart on their own tend to be less happy in their marriage, according to studies examining the correlation between marital satisfaction and leisure activities (Holman and Jacquart, 1988).

There are a few caveats that should be taken into account before drawing any conclusions from this report. Although we discovered that a disparity in heavy drinking between the husband and wife was a predictor of lower marital satisfaction, it is possible that other factors were also responsible for the decreases we observed. For example, depression (Fincham et al., 1997), the birth of a first child (Hackel and Ruble, 1992), the number of children (Twenge et al., 2003), and expectations about marriage (McNulty and Karney, 2004) are just a few factors that have been found to be associated with changes in marital satisfaction. Duration of marriage wasn't assessed. Hence our results may not apply to longer-married or subsequent-marriage pairs.

Conclusion

Discordant drinking patterns and alcohol dependence are associated with lower levels of marital satisfaction for partners.

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Table 1: Descriptive statistics of marital satisfaction (MS) scores of patients and their spouses (N=100)

| Description | Mean | N | Standard Deviation | Standard Error Mean |
|-------------|--------|----|--------------------|---------------------|
| MS SCORE | 114.36 | 50 | 3.50 | 0.49 |
| Spouse | 90.92 | 50 | 8.82 | 1.24 |

Table 2: Association of marital satisfaction scores of patients with their spouses (N=100)

| Independent Samples Test | | | | | | | | | |
|---|--------|-------|------------------------------|----|-----------------|-----------------|-----------------------|---|--------|
| Levene's Test for Equality of Variances | | | t-test for Equality of Means | | | | | | |
| Marital satisfaction scores | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| | 45.556 | 0.000 | 17.46 | 98 | 0.000 | 23.440 | 1.342 | 20.776 | 26.104 |

Figure 1: Marital Satisfaction scores of the study participants (n=100)

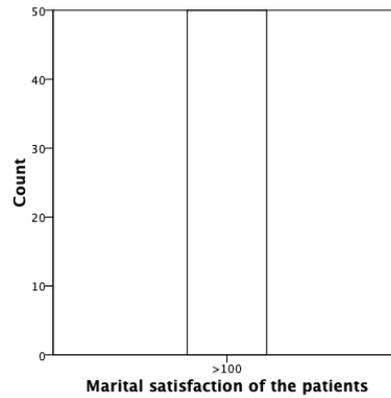
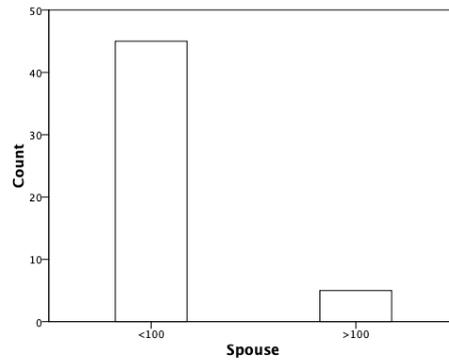


Figure 2: Spouse Marital Satisfaction Score (n=100)



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PROFILE OF AUTO HEMAGGLUTININ IN COVID19 PATIENTS IN A BLOOD BANK AT A TERTIARY CARE HOSPITAL

Pathology

| | |
|----------------------------|---|
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ABSTRACT

Objectives: COVID-19 is associated with a wide range of clinical manifestations, including autoimmune features and autoantibody production. Autohaemagglutinin are anti human globulin [IgG] present in patients plasma/serum that agglutinates own red blood cells and interferes with blood group. Polyagglutinin is the phenomenon in which patients red cells are agglutinated by all group compatible sera. It is caused by alteration of antigenic structure on the red cells membrane, with exposure of previously hidden red cell antigen. This occurs in various bacterial and viral infections. During routine blood grouping both by forward and reverse grouping, these autoagglutinins which can be both IgM or IgG antibodies. The later can be detected by anti human globulin Test or coombs test. The objective was to study the existence of auto hemagglutinins in COVID19 positive patients. **Materials and Methods:** In this study, blood samples of 500 patients who were diagnosed positive for COVID19 were collected and processed for anti human immunoglobulin test in the blood bank at Trichy SRM medical college hospital and research centre. The duration of the study was June 2020 to May 2021. Observation and result: Among 500 COVID 19 positive patients, we observed 112 patients were found to be positive for auto hemagglutinin in blood. **Conclusion:** There has been 22.4% of patients found to be positive for auto haemagglutinin (Cold agglutinin) among COVID 19 positive patients which is a significant level of percentage.

KEYWORDS

Autohaemagglutinin, Cold agglutinin, Coombs, Immunoglobulin

Introduction

Cold agglutination is a type of extravascular autoimmune hemolytic anemia (AIHA). This condition is characterized by the presence of autoantibodies, also known as cold agglutinins, which causes agglutination at a temperature as low as 3-4 °C when the red blood cells circulate in the cooler parts of the body [1]. Primary cold agglutinin disease (CAD) may occur in the absence of an underlying condition. In contrast, secondary cold agglutinin syndrome (CAS) is associated with infections, autoimmune conditions, lymphoproliferative diseases and Waldenstrom macroglobulinemia [2]. Mycoplasma infection and Epstein-Barr virus (EBV) infection are the most common causes observed [3]. Case reports have described cold agglutinins in the setting of other infections such as HIV, rubella virus, influenza viruses, or varicella-zoster virus (chickenpox) as well [4,5]. Not all individuals with these infections who develop cold agglutinins will have clinically significant hemolysis [6]. We evaluate the existence of auto haemagglutinins (cold antibodies) among COVID19 (SARS-CoV-2) positive patients.

Materials and Methods

Setting: Blood Bank, Department of Pathology and transfusion medicine, Trichy SRM Medical College Hospital and Research Centre, Trichy.

Duration: June 2020 to May 2021

Type of study: Prospective study

Sampling Size calculation: Based on previous studies and statistical formula, sample size was determined with alpha error of 0.05 and power of 0.95

Sample size: 500

Inclusion criteria: Patients who were positive for COVID19 infection and got admitted in Trichy SRM Medical college hospital during the time of study period were included in the study.

Exclusion criteria: : Patients who were negative for COVID19 infection.

Data collection procedure: Blood samples from patients who were positive for COVID19 infection were collected and studied for blood grouping test and Coombs test.

Data analysis: Categorical variables was expressed in percentages and pie chart. Student T test was applied for calculating statistical significance when data followed nominal distribution. Mann whitney test applied when data followed non nominal distribution. Nominal categorical data between the group was compared using Chi-square test or fisher's exact test as appropriate. P<0.05 was taken to indicate a statistical significant difference.

Observation and result

Table-1: Percentage of prevalence of auto hemagglutinin among COVID 19 patients

| Coombs test | No. of Covid19 positive patients | Prevalence percentage (%) |
|---|----------------------------------|---------------------------|
| Positive for auto hemagglutinin (Cold antibodies) | 112 | 22.4 |
| Negative for auto hemagglutinin (Cold antibodies) | 388 | 77.6 |
| Total No. of cases during study period | 500 | 100 |

Figure-1: Autohemagglutination noted among Red Blood Cells

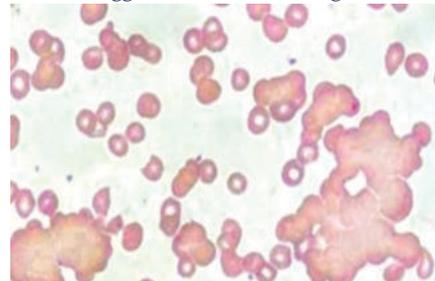
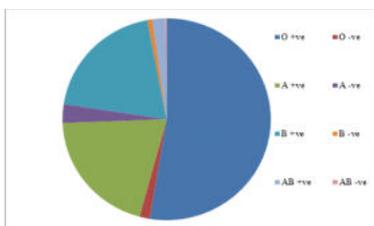


Table-2: Statistical calculation applied in the study

| Statistical calculation | Values |
|-------------------------|----------------|
| Difference | 55.2% |
| 95% Confidence Interval | 45.5% to 62.9% |
| Chi-squared | 116.5 |
| Significant level | P < 0.0001 |

Table-3: Blood group of the study population

| Blood group | No. of patients | Percentage (%) |
|-------------|-----------------|----------------|
| O positive | 263 | 52.6 |
| O negative | 08 | 1.6 |
| A positive | 101 | 20.2 |
| A negative | 15 | 3.0 |
| B positive | 98 | 19.6 |
| B negative | 04 | 0.8 |
| AB positive | 10 | 2.0 |
| AB negative | 1 | 0.2 |

**Figure-2: Prevalence of blood groups among study population****Table-4: Gender distribution among the study population**

| Gender distribution | No. of patients | Percentage (%) |
|---------------------|-----------------|----------------|
| Male | 323 | 64.6 |
| Female | 177 | 35.4 |
| Total | 500 | 100 |

Table-5: Age distribution among the study population

| Age distribution | No. of patients | Percentage (%) |
|------------------|-----------------|----------------|
| <10 years | - | - |
| 10-19 years | - | - |
| 20-39 years | 18 | 3.6 |
| 40 – 59 years | 98 | 19.6 |
| >60 years | 384 | 76.8 |
| Total | 500 | 100 |

Discussion

This research work discusses the presence of transient cold agglutinins in patients diagnosed with SARS-CoV-2 infection. The study population we selected were positive for SARS-CoV-2 infection and got admitted in hospital. Among 500 patients of SARS-CoV-2 infection, 112 patients were found to have autohemagglutinin (Cold antibodies) which were detected by anti human immunoglobulin test or Coombs test [Table-1, Fig. 1]. Followed which antibody titration was done. All 112 patients were found to have cold agglutinins with titres of 1:40 or above. The prevalence percentage of auto haemagglutinin presence in the blood among 500 patients was found to be 22.4%. Chi squared test done which was found to be 116.54. Confidence Interval calculated which was 45.5% to 62.9%. P value we obtained was $P < 0.0001$ which was statistically significant [Table-2]. Finland et al. and Peterson et al. have reported the presence of cold agglutinins in their study on atypical pneumonia patients [7,8]. Among the 200 patients in that study, 137 patients were found to have cold agglutinins with titers of 1:40 or above. They identified the presence of cold agglutinin titers in the atypical pneumonia patients. Subsequently, a study done by Finland et al. demonstrated a maximum of cold agglutinin titers observed during the middle of the second week and the middle of the fourth week [9]. Titers drop rapidly after reaching the peak value. Significantly lower titers are observed in the third and fifth weeks from the onset of symptoms [10-14]. The highest titer has been observed to be anywhere from 1:40 to 1:1280 [15]. The levels of titers have been found to be correlated with the severity of symptoms, duration, the extent of the pulmonary lesion, and fever spike; 11 out of the total of 200 patients were found to have hemolytic anemia [16,17]. A similar trend was noted related to mycoplasma infections in other studies [18].

Blood grouping also done in all 500 patients of our study population which was found to be 263 patients (52.6%) with O positive group, 8 patients (1.6%) with O negative, 101 patients (20.2%) with A positive, 15 patients (3%) with A negative, 98 patients (19.6%) with B positive,

4 patients (0.8%) with B negative, 10 patients (2%) with AB positive, 1 patient (0.2%) with AB negative [Table-3, Fig.2].

Age and gender wise distribution among the study population who were positive for SARS-Cov2 infection and got admitted in hospital also noted. Total patients who were positive for SARS-Cov2 infection and got admitted in hospital noted during the study were 500. Among 500 patients, 323 (64.6%) were male patients and 177 (35.4%) were female patients. Hence it is inferred that male population who got positive for infection more than females. Among the total study population (500) age wise distribution also noted. 18 patients (3.6%) were between 20 – 39 years of age. 98 patients (19.6%) were between 40- 59 years of age and 384 patients (76.8%) were > 60 years of age. It has been inferred that patients who were positive for SARCoV 2 infection belongs to the age between > 60 years of age [Table-4,5].

Summary & Conclusion

We concluded that there has been significant percentage of presence of auto hemagglutinin found among SARS CoV2 infected patients. Transient cold agglutination in COVID-19 might be correlated with disease severity. The exact pathophysiology of cold agglutinins in COVID-19 is not known, but the focus in the management of cold agglutinins should be to treat the underlying cause. There is a need for continuous observation of cold agglutinin disease in COVID-19 patients to fully establish the association. Cold agglutination can be observed in virus infection without significant hemolysis. SARS-CoV-2 infection should be considered as one of the causes behind the development of cold agglutination.

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Role of myocardial performance index (MPI) and cerebro-placental ratio (CPR) in predicting adverse perinatal outcome

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Abstract

Introduction: This study aimed to evaluate the association of the Myocardial Performance Index (MPI) and Cerebro-Placental Ratio (CPR) in predicting adverse perinatal outcomes in fetuses who are appropriately-grown (AGA), small-for-gestational-age (SGA) and growth restricted (FGR).

Methods: Singleton pregnancies were recruited after 24 weeks. The patients were recruited after having been classified as AGA (AC/EFW > 10th centile), SGA (AC/EFW—3rd–10th centile without doppler abnormalities), and FGR (AC/EFW < 3rd centile or 3rd–10th centile with doppler abnormalities). A total of 103 cases comprising 48 AGA, 11 SGA, and 44 FGR fetuses were recruited. The Pulsatility Index of the Umbilical artery, Middle cerebral artery, Ductus Venosus, and Aortic Isthmus was obtained. MPI and CPR were calculated too. The primary outcome was to evaluate the predictive value of MPI and CPR for the composite adverse perinatal outcome.

Results: The mean gestational age of recruitment was 30 weeks. The OR for Composite Adverse Perinatal Outcome in FGR group for MPI > .47 and CPR < 1.67 was 3.48 (95% CI: 1.00–12.24, *p*-value < .05) with sensitivity and specificity of 65% each and 11.08 (95% CI: 2.62–46.83, *p*-value = .001) with the sensitivity of 82% and specificity of 70%, respectively. When combined together, MPI and CPR yielded an OR of 58.5 (95% CI: 4.58–746.57, *p*-value = .002) with a sensitivity of 56.5% and specificity of 95% in the FGR group.

Conclusions: MPI in conjunction with CPR can be used together to predict adverse perinatal outcomes in FGR.

KEYWORDS

fetal echocardiography, fetal growth restriction, myocardial performance index (MPI) and cerebro-placental ratio (CPR), perinatal outcome

1 | INTRODUCTION

Fetal growth restriction is a frequently encountered condition in the field of obstetrics. It encompasses a dynamic set of adaptive responses to placental insufficiency. With more studies exploring the pathophysiology and role of observational modalities that include arterial, venous doppler studies and biophysical profile, a predictable chronological pattern has emerged to monitor this progressive disorder.¹

FGR is associated with adverse outcomes such as iatrogenic prematurity, hyaline membrane disease, intraventricular hemorrhage and necrotizing enterocolitis.¹ Hence, it is imperative to detect at-risk fetuses to reduce overall morbidity and mortality. Since no medical treatment has been demonstrated to benefit the management of fetal growth restriction, the primary treatment includes intensive fetal surveillance and planned delivery.² The central tenet behind the clinical management of FGR is to combine several modalities of fetal surveillance to triage at-risk pregnancies and proceed with termination, all the while weighing the risks of prematurity associated with delivery. There is a wide variation in the clinical management of FGR. However, the stage-based protocol postulated by Figueras et al. provides a systematic approach to triaging at-risk pregnancies.³

The role of Umbilical artery, Middle cerebral artery, and Ductus venosus doppler studies in the management of FGR has been extensively studied and affirmed as crucial factors in its management. There is a gradual but predictable decline noted in these vessels in the stage-based approach, suggesting an overall cardio-vascular compromise. Despite the changes studied in various vessels, a significant number of cases of at-risk fetuses are not detected, which contribute to unexplained stillbirths. Therefore, it is paramount to assess indicators which add to the growing armamentarium of tools for detecting cardio-vascular deterioration. With technological improvements in fetal echocardiography, cardiac alterations in growth-restricted fetuses have been studied and have shown promising results in fetal monitoring.^{2,4}

Myocardial performance index (MPI) aids in evaluating fetal cardiac compromise. MPI is a non-invasive doppler-derived indicator that evaluates cardiac function holistically. Myocardial Performance Index has been studied as a promising method of evaluating fetal cardiac adaptive changes in growth-restricted pregnancies. However, the data reflecting a correlation and definite cut offs to be used for prognostication has been sparsely published.^{2,5}

The purpose of this study was to evaluate the clinical utility of MPI as an adjunct to the standard doppler studies done for FGR monitoring and assess its role in predicting adverse perinatal outcomes.

2 | METHODOLOGY

This prospective cohort observational study was carried out in the department of Obstetrics and Gynaecology at AIIMS, New Delhi, between January 2018 and January 2020. The study commenced after receiving approval of the institutional ethics committee.

The inclusion criteria for recruitment were singleton pregnancies with correct dating by first-trimester scan, a normal anomaly scan and an ultrasound diagnosis of either AGA, FGR or SGA based on biometry and doppler. AGA was defined as AC/EFW > 10th centile, SGA was considered when AC/EFW was between 3rd and 10th centile with no doppler abnormalities and FGR was diagnosed with AC/EFW < 3rd centile or 3rd–10th centile with abnormal dopplers.

Pregnancies complicated with congenital malformations, chromosomal disorders, abnormal fetal heart rates and multiple gestations were excluded.

This study was conducted at a tertiary care teaching hospital. The recruitment of subjects for this study was done from the high-risk pregnancy clinic. Henceforth, the participants had a higher proportion of either pre-existing conditions or obstetric morbidity. The recruitment into AGA and SGA group was strictly done based on the ultrasonographic biometry with AC/EFW > 10th centile and AC/EFW > 3rd and < 10th centile with no doppler abnormalities, respectively.

The AGA and SGA group included patients with pre-existing conditions such as idiopathic immune thrombocytopenia, rheumatic heart disease, pituitary adenoma, epilepsy, etc.

Within the AGA group there were various obstetrical co-morbidities such as pregnancy-induced hypertension/chronic hypertension seen in 7/48, gestational/pregestational diabetes was present in 11/48, and intra-hepatic cholestasis of pregnancy in 3/48 patients.

2.1 | Sample size calculation

Perez et al.⁶ evaluated the role of cardiac function in SGA and FGR against low-risk controls. Cardiac function was studied using global parameters such as MPI and ductus venosus PI. In the study, the left-MPI was found to be $.45 \pm .14$ and $.57 \pm .1$, respectively, and the difference was statistically significant. Presuming similar results for the current study, a sample size of 35 subjects in each arm (control/AGA and FGR) was adequate enough to provide 80% power with a 5% level (one-sided) of significance. ($p < .05$)

This calculation did not take into consideration the difference in CPR among the three groups.

As per the sample size calculated (as given below, a minimum of 35 patients were required in AGA and FGR group. Within the duration of the study, a total of 103 pregnant patients from the antenatal clinic were recruited as per the aforementioned inclusion criteria. This included 48 appropriate for gestational age (AGA) and 44 Fetal Growth Restricted (FGR) fetuses and 11 small for gestational age (SGA) were also recruited. (Figure 1)

Since we followed a strict inclusion for SGA, we could not get an adequate number of subjects among the SGA group.

The baseline characteristics such as age, period of gestation at recruitment, and parity index were documented. All patients underwent a scan at the time of recruitment for growth, liquor, Doppler, and fetal echocardiography using the E8 General Electric Voluson ultrasound system (GE Medical Systems, WI, USA). A detailed study of fetal

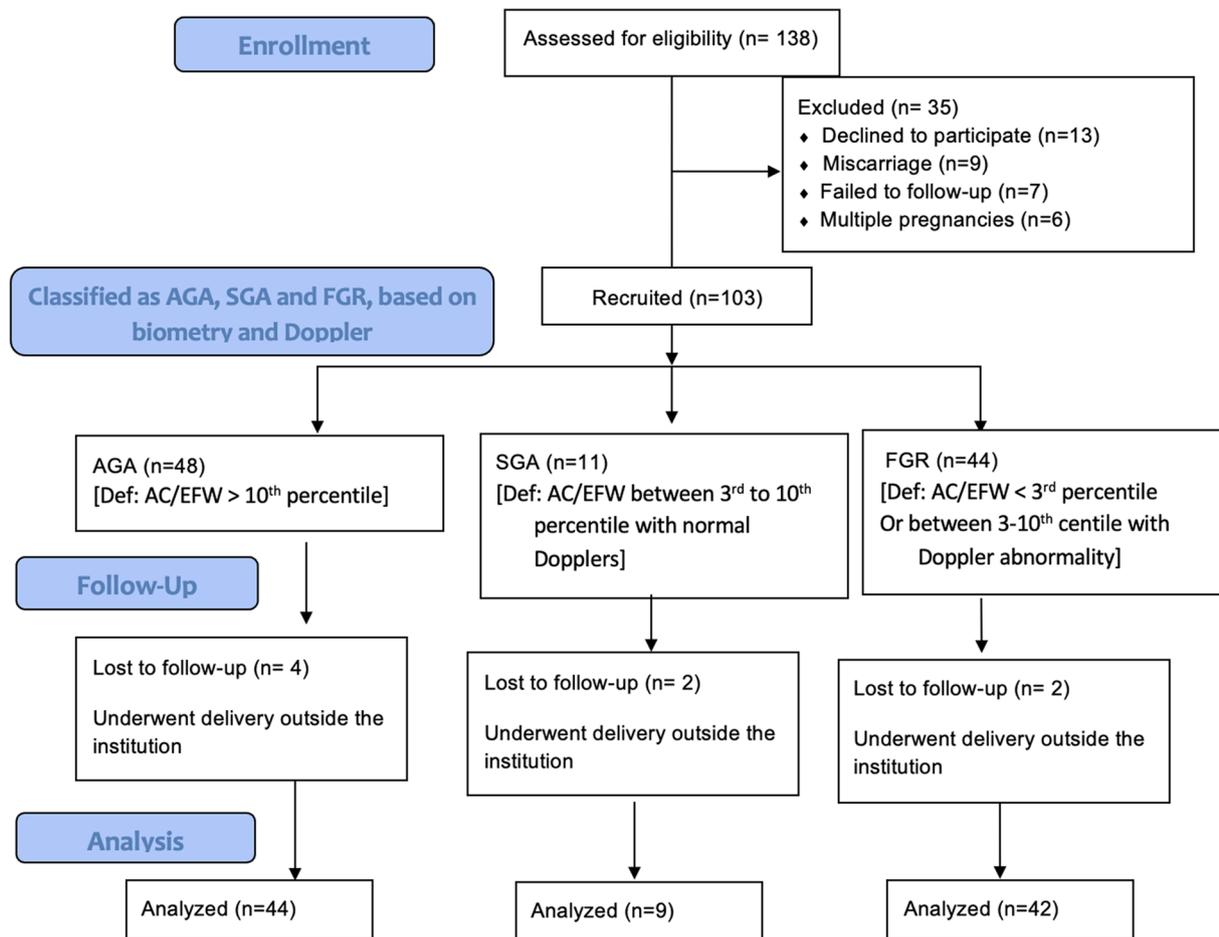


FIGURE 1 Flow chart for methodology

Doppler was done which included an assessment of Pulsatility index (PI) for the Umbilical Artery (UA), Middle Cerebral Artery (MCA), Ductus Venosus (DV), Aortic Isthmus (Ao) as per ISUOG guidelines.⁷ The cerebro-placental ratio was calculated using MCA and UA artery PI.

Doppler studies were considered to be abnormal in the presence of any of the following:

UA PI, Ao PI, and DV PI were more than or equal to 95th centile for gestational age or absent or reversal of end-diastolic flow, MCA PI or CPR was lesser or equal to 5th centile for gestational age.⁵

A detailed fetal echocardiography was done which included serial examination in gray scale and color study of the standard four-chamber view, outflow tracts, three-vessel (3 V), and three-vessel trachea view (3VT), and the longitudinal views of aortic arch and ductus arch for both structural and functional assessment. Modified Myocardial Index was determined using the technique studied by Hernandez et al.⁸ The cardia was examined in the transverse plane to get a four-chamber view in the apical view. The Doppler gate was placed along the internal leaflet or MV to sample flow across aortic and mitral valve and register the opening and closing of AV clicks. The doppler sweep velocity was configured to 5 cm/sec and the wall motion filter at 300 Hz. Three individual time periods were highlighted as isovolumetric contraction time (ICT), from the start of mitral valve closure to the opening of the aortic

valve, ejection time (ET) which included the time interval aortic valve was open and isovolumetric relaxation time (IRT), from the closure of aortic valve to the opening of the mitral valve. The Mod-MPI = (ICT + IRT)/ET.⁸

All pregnancies were followed till delivery, and birth weight, route of delivery, APGAR scores, and need for NICU admission with development of adverse perinatal outcomes as listed below were documented. The adverse perinatal outcome included perinatal mortality, development of respiratory distress, Intra-ventricular hemorrhage, Necrotizing enterocolitis, need for NICU admission. The composite adverse perinatal outcome was considered if any of the above- mentioned outcomes occurred.

3 | STATISTICAL ANALYSIS

The data analysis was done using STATA version 16.1. Continuous variables were assessed for normality assumption using Kolmogorov-Smirnov test. The normally distributed data was subjected to descriptive statistics (mean, SD, and range). The mean values were compared using one-way analysis of variance and Bonferroni post-hoc test subsequently for pair-wise comparison.

TABLE 1 Clinical characteristics and biometry for each group

| Baseline Parameters | AGA (n = 48) Mean (SD) | SGA (n = 11) Mean (SD) | FGR (n = 44) Mean (SD) | p-value for ANOVA Test |
|---|---------------------------|---------------------------|---------------------------|---------------------------|
| Age (yrs) | 26.7 (4.5) | 26.7 (4.1) | 28.1 (4.8) | .497 |
| POG at the time of recruitment (wks) | 28.4 (3.1) | 30.0 (4.7) | 30.9 (5.1) | .003 |
| Parity, n(%) | | | | |
| - Primigravida | 28 (58.3) | 4 (36.4) | 15 (34.1) | .034 |
| - Multigravida | 20 (41.7) | 7 (63.6) | 29 (65.9) | |
| Biometry (mean percentile) | 37.44 | 13.7 | 5.69 | .001 |
| - Abdominal Circumference (percentile) | 1299.01 | 1437.96 | 1334.27 | .001 |
| - Estimated Fetal Weight- (gm) (percentile) | 43.80 | 21.54 | 10.86 | |
| Amniotic Fluid Index | 13.7 (2.2) | 14.8 (2.4) | 10.8 (4.1) | .001 |

The median and interquartile range values were calculated for the skewed data. Kruskal–Wallis test was used to compare median values. The frequency of categorical variables was compared using Chi-square/Fishers exact test as appropriate. Bivariate logistic regression analysis was conducted to detect the significant variables for adverse perinatal outcome data. The corresponding odds ratio with 95% CI for those variables was computed. ROC analysis was done for each variable to establish the cut-off value with acceptable sensitivity and specificity. The relationship between cardiovascular parameters was established using multiple linear regression analysis separately for each group after adjusting for confounding variables. We considered a two-sided probability of $p < .05$ to be statistically significant for all statistical tests.

4 | RESULTS

We recruited 103 singleton pregnancies, with 48 appropriate for gestational age (AGA), 11 small for gestational age (SGA), and 44 Fetal Growth Restricted (FGR).

Data for 95 patients were available at the end of the study, while eight patients were lost on follow-up.

The FGR group was staged at the time of recruitment according to severity as per the stage-based protocol given by Figueras et al.³ There were 35 (79.6%) fetuses with stage 1 FGR, 6 (13.6%) with stage 2 FGR and 3 (6.8%) with stage 3 FGR, respectively.

Three intrauterine deaths were noted among the FGR group. These were three cases of early-onset FGR with reversal of end-diastolic flow (REDF) detected at 24, 26, and 29 weeks of gestation, detected at the time of referrals, shortly before they had IUD. They were all late referrals with no available screening for aneuploidy but had normal anomaly scans. One patient had pre-eclampsia.

In addition, the FGR cohort had 27/44, 61.4% cases of early-onset FGR and 17/44, 38.6% cases of late onset FGR. However, for the purpose of analysis we did not differentiate between early and late-onset FGR.

No statistically significant difference in the mean ages among the groups was noted at the recruitment time (Table 1).

There were more multigravida in the FGR group as opposed to the AGA group. Liquor was found to be reduced in the FGR group with

mean value of 10.8 cm, which was statistically significant ($p < .001$) relative to AGA (13.7 cm) and SGA (14.8 cm).

The PI values for the Umbilical artery and Aortic Isthmus were increased in the FGR group than AGA and SGA groups. MCA PI & CPR was lesser in the FGR group against AGA and SGA groups, which was statistically significant. Raised IRT was noted in the FGR group. No statistically significant result was observed when DV PI, ET and ICT were compared among the three groups (Table 2). MPI was raised in the FGR group and significantly ($p < .05$) higher than in AGA and SGA groups.

Group-wise incidence of Caesarean section varied from 50% in AGA group to 69% in FGR group and was higher than vaginal deliveries that varied from 30.9% in FGR group to 47.8% in AGA group. There was no statistical difference noted among the AGA, SGA, and FGR group when the incidence of LSCS and vaginal delivery was compared. The period of gestation at delivery, birth weight and APGAR 5 was lower in FGR than SGA and AGA alike and was statistically significant. There were 29 cases of composite adverse perinatal outcomes, with FGR group contributing to 20 NICU admissions for 15 cases of respiratory distress and IVH. There were three cases of stillbirths in the FGR group. There were two cases each of respiratory distress from the AGA and SGA group. Table 3 lists the breakdown of perinatal outcomes. The composite adverse perinatal outcome was considered if any of the above-mentioned outcomes occurred. Data for 95 patients were available at the end of the study, while data for eight patients were lost on follow up. The follow up was not available for four cases of AGA and two cases of SGA and FGR respectively as they underwent deliveries outside the institution.

The areas under receiver operating characteristic (ROC) curve for Mod MPI, IRT and CPR for predicting composite adverse perinatal outcome were .715, .727, and .763 respectively (Figure 2 and 3). The sensitivity and specificity of Mod-MPI for predicting composite adverse perinatal outcome in the FGR group, were 65% each when the cut off was .47. The sensitivity and specificity of Iso-volumetric Relaxation Time (>44.5 ms) for predicting composite adverse perinatal outcome was 57% and 85%, respectively. The sensitivity and specificity of CPR (<1.67) for predicting composite adverse perinatal outcome were 82% and 70%, respectively in the FGR group (Figure 3).

Neither CPR nor MPI showed statistically significant value in predicting adverse perinatal outcomes in both AGA and SGA groups.

TABLE 2 Doppler parameters for each group

| Doppler Parameter Mean (SD) | AGA (n = 48) | SGA (n = 11) | FGR (n = 44) | p-value |
|-------------------------------------|----------------|----------------|----------------|---------|
| Umbilical Artery PI | 1.01 (.15) | 0.94 (.13) | 1.23 (.52) | .005 |
| Middle Cerebral Artery PI | 1.99 (.31) | 1.82 (.23) | 1.74 (.38) | .003 |
| Cerebro-Placental Ratio | 2.01 (.44) | 1.96 (.37) | 1.58 (.59) | .001 |
| Ductus Venosus PI | .41 (.13) | .45 (.15) | .50 (.31) | .174 |
| Aortic Isthmus PI | 2.25 (.31) | 2.35 (.33) | 2.46 (.38) | .018 |
| Isovolumetric Contraction Time (ms) | 31.39 (8.50) | 35.45 (10.38) | 36.38 (13.85) | .081 |
| Isovolumetric Relaxation Time (ms) | 33.94 (11.19) | 34.79 (9.98) | 42.90 (14.50) | .003 |
| Ejection Time (ms) | 167.85 (15.21) | 165.45 (15.22) | 164.25 (14.50) | .476 |
| Myocardial Performance Index | .38 (.09) | .40 (.15) | .48 (.11) | .001 |

TABLE 3 Perinatal outcomes presented as mean (SD)/n (%)

| Perinatal Outcomes | AGA (n = 44) | SGA (n = 9) | FGR (n = 42) | p-value |
|--|------------------|------------------|------------------|---------|
| Mode of delivery, n(%) | | | | |
| Vaginal | 21 (47.8) | 4 (44.4) | 13 (30.9) | *a |
| LSCS | 22 (50.0) | 5 (55.5) | 29 (69.) | .598 |
| Instrumental | 1 (2.2) | 0 | 0 | .127 |
| | | | | .561 |
| Period of gestation at the time of delivery (wk) | 37.6 (1.9) | 36.8 (1.6) | 35.5 (3.1) | *b |
| | | | | .004 |
| Birth Weight (gm) | | | | .001 |
| Mean (SD) | 2820.39 (447.26) | 2557.33 (442.27) | 2045.02 (573.87) | |
| APGAR Mean (SD) | | | | |
| 1 Min | 8.57 (.78) | 8.66 (.70) | 8.02 (1.80) | *b |
| 5 Min | 8.93 (.33) | 9.00 (.01) | 8.65 (.69) | .134 |
| | | | | .045 |
| Adverse Perinatal Outcomes n(%) | | | | *a |
| RDS | 2 (4.5) | 2 (22.2) | 15 (35.7) | .001 |
| IVH | 0 | 0 | 1 (2.3) | .512 |
| NICU Admission | 4 (9.0) | 2 (22.2) | 20 (47.5) | .001 |
| Mortality | 0 | 0 | 3 (7.1) | .148 |
| Composite Adverse Perinatal Outcomes | 4 (9.0) | 2 (22.2) | 23 (54.7) | .001 |

*a: Chi-Square Test.

*b: ANOVA Test.

4.1 | Logistic regression analysis for predicting composite adverse perinatal outcome

Mod-MPI > .47 and CPR < 1.67 correlated with composite adverse perinatal outcome in the FGR group (OR = 3.48, 95% CI: 1.00–12.24, p value < .05, sensitivity & specificity ; 65% each and OR = 11.08, 2.62–46.83, p value = .001, sensitivity = 82% & specificity = 70% respectively).

Combined MPI and CPR: In the FGR group, the cut-off values for MPI were taken >.47 and CPR <1.67; there were 13 cases with both doppler findings associated with adverse perinatal outcome. There were 10 cases with no abnormal MPI and CPR values, while 17 cases had one of the two abnormal findings. Using Logistic Regression

Analysis, it was found that the OR (Odds Ratio) was 58.5 (95% CI: 4.58–746.57, p -value = .002) with the sensitivity of 56.5% and specificity of 95.0%.

The OR (Odds Ratio) was 3.6, while either of the two dopplers were considered abnormal. However, this was statistically insignificant. (p -value = .161).

To assess the ability of abnormal MPI level (>.47) and CPR level (<1.67) in predicting composite outcome among FGR group we carried out logistic regression analysis by adjusting birth weight. The analysis indicated that the regression coefficients of both birth weight, and abnormal levels of MPI and CPR were statistically significant (p < .05). Increase in birth weight will reduce the risk of getting composite outcome to .992 (OR) with 95% CI: .986–.998. While in cases with

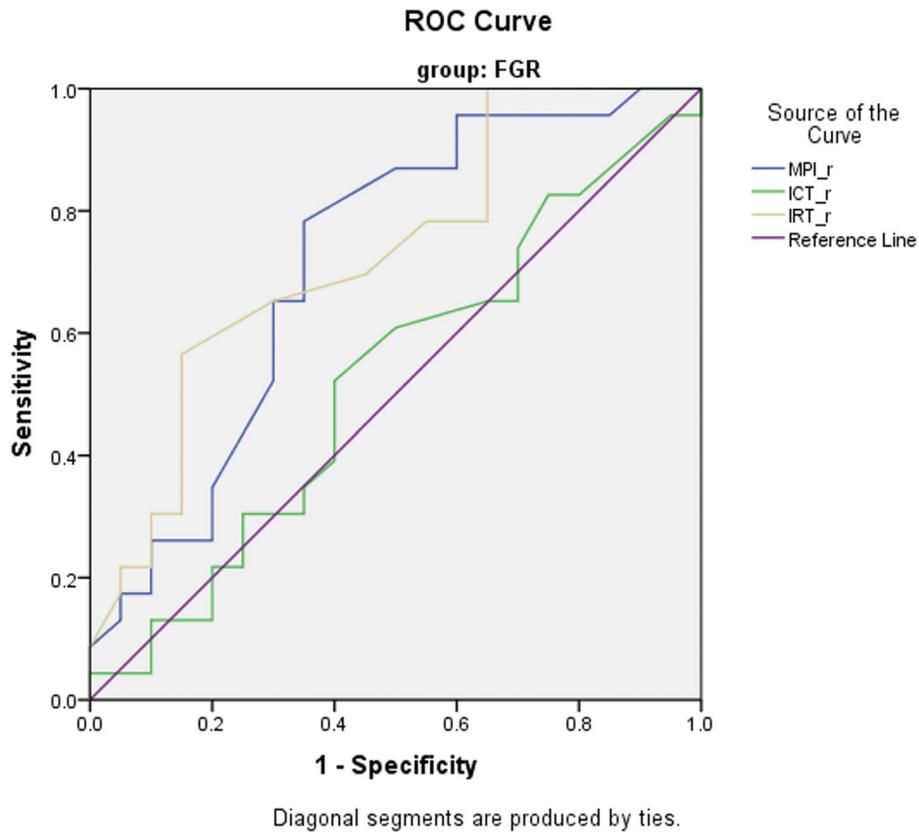


FIGURE 2 ROC for MPI

TABLE 4 Comparison of MPI and CPR in Stage 1 and Stage 2 FGR

| Characteristics | STAGE 1 FGR(n = 35) | STAGE 2 FGR(n = 6) | p-value |
|-----------------|---------------------|--------------------|---------|
| Mean MPI | 0.46 ± .10 | 0.59 ± .09 | .006 |
| Mean ICT | 35.21 ± 3.90 | 43.83 ± 11.21 | .159 |
| Mean IRT | 41.46 ± 14.73 | 52.00 ± 9.27 | .099 |
| Mean ET | 164.43 ± 12.00 | 163.00 ± 18.41 | .859 |
| Mean CPR | 1.69 ± .54 | .84 ± .26 | .001 |

both abnormal MPI and CPR levels the odds ratio of having composite adverse outcome was 152.5 (95% CI: 3.11–7495.2). Both these significant variables in the model the sensitivity and specificity is 90%.

There were 44 patients in the FGR Group. On further subdivision, it was noted that six patients had stage 2 FGR while 35 patients had stage 1 FGR.

The following table (Table 4) represents the data distribution pertaining to the subtypes of FGR. Of the six, three pregnancies progressed to REDF while CS terminated the other three in view of AEDF.

The pregnancies complicated with stage 2 FGR showed higher MPI, ICT, and IRT and lower ET and CPR against stage 1 FGR. However, differences only in MPI and CPR were found to be statistically significant.

Myocardial Performance Index was noted to be **higher** with mean of .59 in stage 2 FGR against .46 in stage 1 FGR. ($p = .006$). Cerebro-

placental Ratio was **lower** with mean of .84 in stage 2 FGR as opposed to a mean of 1.69 in stage 1 FGR. ($p = .001$).

5 | DISCUSSION

This study showed that FGR fetuses have cardiovascular dysfunction that is reflected with raised Mod-MPI values. The degree of dysfunction was more remarkable in the advanced stages of FGR, with mean values of .59 and .46 in stage II and stage I FGR, respectively. These findings have been consistent with other studies reported in the literature.^{10,11} A Mod-MPI cutoff > .47 in the FGR group has a 65% sensitivity and specificity with an odds ratio of 3.48 in predicting adverse perinatal outcome.

In addition, this study reaffirmed the importance of CPR in predicting adverse perinatal outcomes in FGR pregnancies. CPR was found

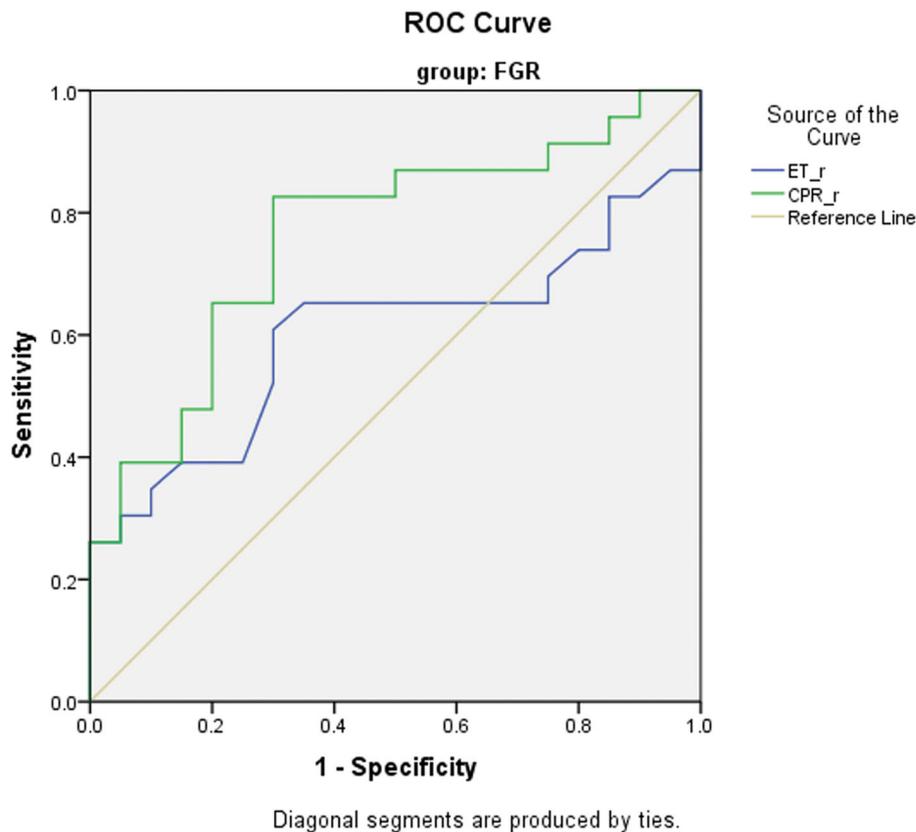


FIGURE 3 ROC for CPR

to be the lowest in the FGR group. A CPR cutoff < 1.67 was found to have 82% sensitivity and 70% specificity with an odds ratio of 11.08 in predicting adverse perinatal outcomes. As per the nomograms given by Ciobanu et al.¹² for cerebroplacental ratio, the cut off taken in our study of 1.67 corresponds with 10th–25th percentile for 30–31 week period of gestation which is the average gestation for recruitment for the study group. The mean gestational age of examination was 30.9 weeks for the FGR group. In their paper, CPR values were noted to peak around 32–34 weeks of pregnancy with a subsequent fall.

In the meta-analysis by Volgraff et al. the mean sensitivity and specificity for composite adverse perinatal outcomes was 59% and 91%, respectively when CPR was taken < 1.1 .¹³ However, in our study the cut-off of CPR < 1.67 , yielded higher sensitivity of 82% achieved with comparable specificity.

MPI, CPR, and IRT were independent predictors of composite adverse perinatal outcomes in the FGR Group. Also, there was no association between Mod-MPI and CPR in predicting adverse perinatal outcomes SGA and AGA pregnancies.

These findings can be explained by the cardiovascular changes that occur in response to chronic hypoxia and undernourishment due to uteroplacental insufficiency. The heart undergoes remodeling to sustain adequate cardiac output for fetal growth.

These changes are both structural and functional. Due to chronic hypoxia, the heart becomes more globular secondary to myocardial hypertrophy in fetal growth restriction. These structural changes ren-

der the ventricles less compliant, which lead to both systolic and diastolic dysfunction.⁶

Mod-MPI is conveniently sampled and a reproducible marker for cardiac function that can be combined with routine doppler assessment in the management of FGR. This addition brings forth the value of cardiac evaluation in FGR fetuses known to exhibit intra-uterine cardiac remodeling in response to placental dysfunction. The presence of anatomical proximity between the mitral valve to the left outflow tract and aortic valve helps in sampling MPI in a single doppler view. Raised Mod-MPI values were noted in left ventricular dysfunction. Thus, Mod-MPI serves as a promising indicator for detecting fetal cardiac compromise to uteroplacental insufficiency.

This study's central premise was to study the predictive value of MPI with adverse perinatal outcomes in both the SGA and FGR Group. The utility of Mod-MPI has been contentious, with opposing results reported in the literature. Perez et al.⁸ showed the presence of elevated MPI in both FGR and SGA groups. Kaya et al.⁹ noted that ICT, IRT, and MPI were elevated in the late-onset FGR and SGA groups. ET was lowered in both groups. There was no statistically significant result pertaining to the usage of MPI or its components in predicting adverse perinatal outcomes. Nassr et al.¹⁰ and Borhat et al.¹¹ reported the benefits of MPI in predicting adverse perinatal outcomes, namely NICU admissions in the FGR group. Zhang et al.¹⁴ reported that increased MPI was seen in both late and early-onset FGR.

In our study, there was no association of MPI in predicting adverse perinatal outcomes in SGA pregnancies. It was shown to be elevated

in the FGR group and useful in predicting composite adverse perinatal outcomes. To further investigate the clinical utility of MPI, we used logistic regression to analyze various parameters making an impact on perinatal outcomes. CPR and MPI were studied to be independent variables for the prediction of composite adverse perinatal outcomes, despite the adjustment for birth weight.

To the best of our knowledge, no study had evaluated CPR in conjunction with MPI as markers for adverse perinatal outcome.

The data suggested that a combination of CPR and MPI can better the predictive accuracy for the adverse perinatal outcome as opposed to when they are assessed individually. These findings suggest that a combination of various cardio-vascular parameters may help with improved risk stratification for identifying at-risk fetuses in clinical management for FGR.

6 | LIMITATIONS

There were very few SGA cases to provide meaningful results. There is a lack of longitudinal data over the course of pregnancy in all groups. In addition, the various biometric/functional parameters such as global sphericity index (GSI)¹⁵ and umbilical vein flow were not evaluated in this study.^{16,17}

In our study, we did not differentiate between early onset and late onset FGR. Thereby, no distinct analysis has been conducted for these subtypes.

The recruitment of AGA pregnancies was done from high-risk antenatal clinic where obstetric comorbidities such as preeclampsia and diabetes could have potential effect on cardiac Doppler. Henceforth, the AGA group did not truly represent low risk controls.

7 | CONCLUSION

Fetal growth restriction is associated with raised Mod-MPI, IRT, and lowered CPR. Mod-MPI in conjunction with CPR could be used together to predict adverse perinatal outcome in fetal growth restriction. There is no role of CPR and Mod-MPI in AGA and SGA fetuses in detecting adverse perinatal outcomes.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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Evaluation of the cell-free DNA integrity index as a liquid biopsy marker to differentiate hepatocellular carcinoma from chronic liver disease

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Background: Hepatocellular carcinoma (HCC) occurs in the majority of patients with underlying chronic liver disease (CLD) of viral and non-viral etiologies, which requires screening for early HCC diagnosis. Liquid biopsy holds great promise now for early detection, prognosis, and assessment of response to cancer therapy. Cell-free DNA (cfDNA) as a liquid biopsy marker can be easily detected by a real-time quantitative PCR (RT-qPCR) assay for a change in its concentration, integrity, and fragmentation in cancer.

Methods: Patients with HCC (n = 100), CLD (n = 100), and healthy (n = 30) controls were included in the study. The cfDNA was isolated from serum and real-time quantitative PCR (RT-qPCR) was carried out using primer pairs for large (>205 bp) and small (110 bp) fragments of repetitive elements (ALU and LINE1) and housekeeping genes (β -Actin and GAPDH). Total cfDNA concentrations and integrity index were determined by the absolute quantitation method (L/S ratio or cfDII-integrity). The cfDII as a measure of fragmentation was determined by comparative Ct ($2^{-\Delta\Delta Ct}$) method of relative quantification (cfDII-fragmentation). Using a receiver operating characteristic (ROC) curve, cfDII-integrity and cfDII-fragmentation were used to differentiate HCC from CLD patients or healthy controls.

Results: The total cfDNA concentrations in the sera of HCC (244 ng/ml) patients were significantly higher than those of CLD (33 ng/ml) patients and healthy (16.88 ng/ml) controls. HCC patients have shown poor DNA integrity or excess cfDNA fragmentation than CLD patients and healthy controls. The cfDII-integrity of GAPDH and ALU fragment significantly differentiate HCC from CLD at AUROC 0.72 and 0.67, respectively. The cfDII-fragmentation following normalization with cfDNA of healthy control has shown significant differential capabilities of HCC from CLD at AUROC 0.67 using GAPDH and

0.68 using the ALU element. The ROC curve of LINE1 and β -actin cfDII was not found significant for any of the above methods. The cfDII-fragmentation trend in HCC patients of different etiologies was similar indicating increased cfDNA fragmentation irrespective of its etiology.

Conclusion: The cfDII measuring both DNA integrity (L/S ratio) and fragmentation of the Alu and GAPDH genes can differentiate HCC from CLD patients and healthy individuals.

KEYWORDS

liquid biopsy, circulating free DNA, circulating tumor DNA, hepatocellular carcinoma, chronic liver disease, DNA integrity index

1 Introduction

Hepatocellular carcinoma (HCC) accounts for approximately 90% of primary liver cancers (Llovet et al., 2021). As per Global Cancer Observatory (GLOBOCAN 2020) data, the liver cancer incidence rate in males is fifth, and in females, it is ninth among all forms of cancer. Of all the cancer-related deaths, the liver cancer-related mortality rate was second for males and sixth for females worldwide in 2020 (Sung et al., 2021). Primary liver cancer and liver metastasis account for 6.8 deaths per 100,000 people in India (Dikshit et al., 2012). HCC predominantly develops in patients with underlying chronic liver disease or cirrhosis (Valery et al., 2018). Chronic liver disease (CLD) is a progressive deterioration of liver functions for more than 6 months and can progress from liver inflammation to fibrosis, cirrhosis, HCC, and end-stage liver failure (Sharma and Nagalli, 2021). Chronic hepatitis B and C (CHB and CHC) are the major causes of HCC in the developing world, whereas non-alcoholic steatohepatitis (NASH) is the growing cause of HCC worldwide. The majority of HCC patients are diagnosed at an advanced stage and found unsuitable for curative treatments such as liver resection and liver transplantation (Yuen et al., 2000; Bruix and Llovet, 2002). Currently, imaging-based techniques (CT/MRI) are used for diagnosis, requiring infrastructure and a long wait period. Imaging-based techniques also do not give much insight into the molecular basis of HCC. Liquid biopsy-based biomarkers such as cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), cancer stem cells (CSC), microRNA, and exosomes have recently been shown to be of importance in detection, prognosis, and prediction of response to cancer treatment. Recently, we have shown the role of microRNA as a liquid biopsy marker predicting response to locoregional therapy in HCC (Nadda et al., 2020). The advantages of liquid biopsies are the ease of detection and the non-invasive technique. This may have the potential to be a diagnostic and screening modality in the future (Qu et al., 2019).

Several Liquid biopsy-based tests are now approved by U.S. Food and Drug Administration (FDA) in clinical practice for non-small cell lung cancer (NSCLC) and colorectal cancer

(CRC). The ctDNA-based test first to be approved by FDA is for the detection of EGFR mutations in NSCLC patients to start treatment with EGFR-TKIs (Rijavec et al., 2019; Abdayem and Planchard, 2021). Later, FDA also approved other broad NGS-based ctDNA tests such as Guardant360 and FoundationOne Liquid CDx to determine targeted therapies or chemoresistance for solid tumors (Rolfo et al., 2021). Detection of SEPT9 gene aberrant methylation (EpiProColon or mSEPT9 methylation test) for CRC screening is also another FDA-approved single gene related cfDNA-based test (Song et al., 2017). Now multimodal liquid biopsy-based test (LUNAR-2 or Shield Test) is on trial and showing promising results for early detection of CRC. This test includes ctDNA assessment of somatic mutations, tumor-derived methylation, and cfDNA fragmentations (Broccard et al., 2022). The ctDNAs are small fraction of cfDNA that mainly derived from primary tumors, metastatic tumors, and CTCs. The ctDNA carries entire tumor genetic information whereas there is spatial genetic heterogeneity in tumor tissue biopsy, Analysis of ctDNA gives a detail overview of the genomic landscape of tumour (Zhao et al., 2021).

The cfDNAs are originated both from tumor and extra-tumoral normal cells. The proportions of ctDNA in the total cfDNA greatly vary between <1% and >40%, depending upon clinical-pathologic features of tumor, microenvironment, location, and metastasis (Diaz and Bardelli, 2014; Chen et al., 2019). These cfDNAs enter to the bloodstream *via* processes of apoptosis, necrosis, secretion, autophagy, and necroptosis (Thierry et al., 2016). Pathological cell death conditions such as necrosis, autophagy, or mitotic catastrophe in cancer conditions result in the release of smaller cfDNA fragments into the circulation as opposed to large cfDNA fragments in physiological apoptotic cell death (Giacona et al., 1998; Jin and El-Deiry, 2005). More fragmented cfDNA and its higher concentration have been reported in cancer patients of various aetiologies (Stroun et al., 1989; Fleischhacker and Schmidt, 2007). The cfDNA integrity index (cfDII) is the calculated as the ratio of large to small DNA fragment concentrations of a known gene to measures cfDNA fragmentation. The smaller fragments are <180bp size which corresponds to apoptotic DNA fragmentation size. The selection of genes for cfDII are mostly

repetitive DNA elements as there is high probability of it to be released into circulation or specific housekeeping genes to be used as cancer biomarker (Stroun et al., 2001). Increased cfDII of ALU (*Arthrobacter luteus*) elements was reported in endometrial cancer (Vizza et al., 2018), colorectal (Umetani et al., 2006a), breast (Tang et al., 2018), and prostate cancer (Khani et al., 2019). The cfDII of other repeat elements LINE-1 and housekeeping genes such as β -actin and GAPDH are also explored as liquid biopsy marker in different cancers including HCC (Chen et al., 2012), pancreatic cancer (Tuchalska-Czuron et al., 2020), breast cancer (Cheng et al., 2018), and renal cell carcinoma (Gang et al., 2010). The cfDII was also studied to differentiate breast cancer (Umetani et al., 2006b) or HCC (Wu et al., 2020) from healthy subjects. However, both increased and decreased cfDII levels are reported in cancer, depending upon the absolute and relative quantification methods of real-time quantitative PCR (RT-qPCR) (Madhavan et al., 2014; Cheng et al., 2017). To avoid confusion of cfDII interpretations and to use as a biomarker, we renamed it as cfDII-integrity and cfDII-fragmentation. The cfDII-integrity is the ratio of large to small DNA fragment (L/S ratio) concentration, which is determined by absolute quantification method of RT-qPCR that determine mainly the integrity of cfDNA. Higher integrity is expected in healthy individuals as the L/S ratio is closer to 1, whereas, decreased cfDII-integrity is expected in cancer. In the relative quantification method, normalization with a calibrator using either healthy control cfDNA or genomic DNA is used. The cfDII determined by this method estimates smaller fragment concentrations with reference to larger fragment concentrations in the same individual. It takes care of variations of large to small fragment concentrations in the same individuals and accurately measure of cfDNA fragmentation hence renamed as cfDII-fragmentation. This is expected to rise in log fold in cancer patients; hence, it can be used as a better liquid biopsy marker for cancer or HCC progression from CLD.

In this study, we used RT-qPCR to determine the cfDII by absolute and relative quantification methods. Both cfDII-integrity and cfDII-fragmentation was evaluated for four genes including repetitive elements (ALU and LINE-1) and housekeeping (GAPDH and β -actin) genes to differentiate HCC from CLD patients by plotting receiver operating characteristic (ROC) curve.

2 Materials and methods

2.1 Patients

This prospective observational study was carried out at the All India Institute of Medical Sciences, New Delhi, India, a tertiary care hospital, from February 2019 to July 2021. The study protocol was approved by the institute's ethical committee (Reference number: IECPG-38/23.01.2019, RT-13/28.02.2019). A total of 200 consecutive patients (HCC, $n = 100$ and CLD, $n = 100$)

attending the liver clinics were included in this study. The male and female proportions in HCC were 84% and 16%, and in the CLD patient population, the proportions were 67% and 33% respectively. HCC was diagnosed as per the European Association for the Study of the Liver (EASL) criteria (EASL, 2012; Galle et al., 2018), and both viral (HBV, HCV) and non-viral (alcoholic and non-alcoholic) etiologies were included. All HCC patients were staged as per the BCLC classification (Llovet et al., 1999; Bruix and Sherman, 2011), and all BCLC stage (A–D) patients were included. Similarly, all consecutive chronic liver disease (CLD) patients, including cirrhotic and non-cirrhotic patients of viral (CHB and CHC) and non-viral etiologies, were included. All participants were more than 18 years old and gave written consent for this study. Those with HIV, pregnant women, renal failure, and sepsis were excluded from the study. Healthy volunteers ($n = 30$) negative for HBV and HCV were included as controls. The demographic profile and clinical and biochemical parameters of all patients were recorded.

2.2 Blood sample collection and cell-free DNA isolation

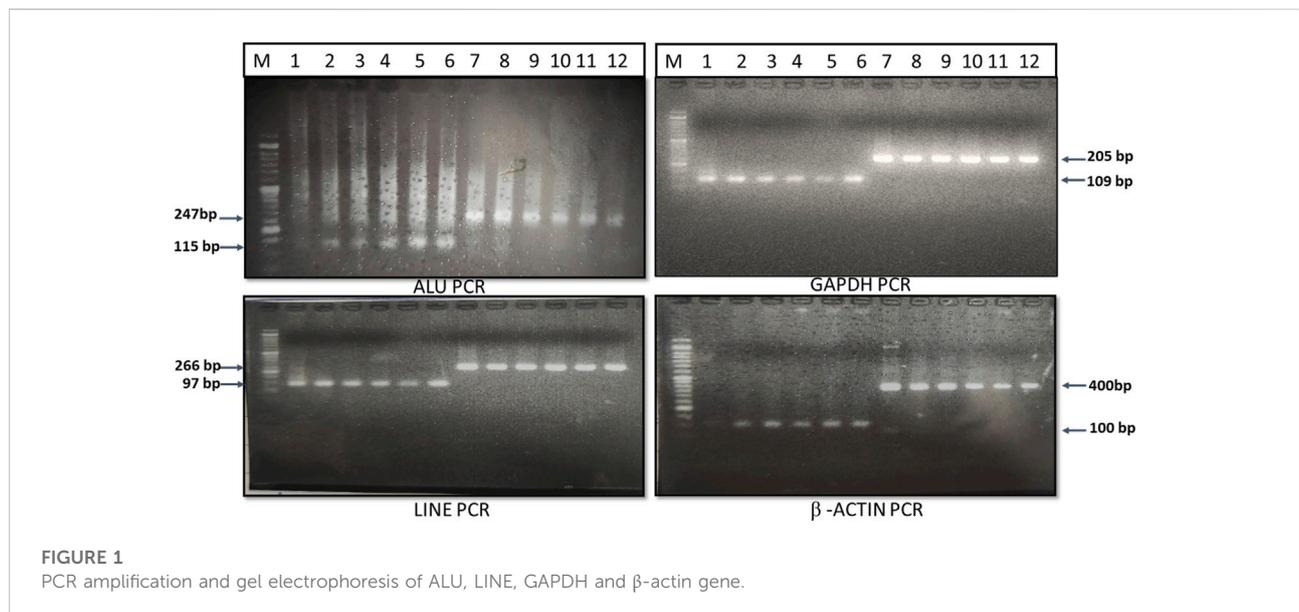
The blood samples were collected from patients at one time point before start of anticancer treatment. The peripheral blood samples were collected in a vacutainer with a gel clot activator and quickly serum was separated to avoid damage to the genomic DNA. The cfDNAs were isolated from serum samples by the QIAasymphony automated nucleic acid extraction system (Qiagen) using the QIAamp DSP (Diagnostic Sample Preparation) mini nucleic acid isolation kit (Qiagen) as per the manufacturer's protocol. Briefly, 400 μ L of serum was used as starting material, and the elution volume was 40 μ L. The concentration of cfDNA was measured using a Multi-ScanGo spectrophotometer (Thermo Fischer), and the cfDNA purity was analysed using the A260/A280 ratio.

2.3 Genomic DNA isolation and PCR amplification of ALU, LINE-1, GAPDH, and β -actin gene small and large fragments

The genomic DNAs from whole blood was isolated using the QIAamp mini genomic DNA isolation kit (Qiagen), and the genomic DNA from the Huh-7 hepatoma cell line was isolated using the Proteinase-K digestion, phenol-chloroform extraction, and ethanol precipitation method. These genomic DNAs were used as a template for amplification of small and large fragments of ALU (115 bp and 247 bp), LINE-1 (97 bp and 266 bp), β -actin (100 bp and 400 bp), and GAPDH (110 bp and 205 bp) genes. The primers for amplification of ALU, LINE-1, β -actin and GAPDH gene small and large fragments were custom designed using NCBI reference sequence, verified with previously published primer sequence (Sobhani et al., 2018) and mentioned in Table 1. The desired fragment size was confirmed by 2% agarose gel electrophoresis (Figure 1).

TABLE 1 Primers for amplification of large and small fragments of ALU, LINE-1, GAPDH and β -actin gene.

| Gene | Primer sequence (5' → 3') | Amplicon size |
|----------------|--|----------------|
| ALU | CCTGAGGTCAGGAGTTCGAG (Forward) | Small (115 bp) |
| | CCCAGTAGCTGGGATTACA (Reverse) | |
| | GTGGCTCACGCCTGTAATC (Forward) | Large (247 bp) |
| | CAGGCTGGAGTGCAGTGG (Reverse) | |
| LINE-1 | TGGCACATATACACCATGGAA (Forward) | Small (97 bp) |
| | TGAGAATGATGGTTTCCAATTTC (Reverse) | |
| | ACTTGGAACCAACCCAAATG (Forward) | Large (266 bp) |
| | CACCACAGTCCCCAGAGTGT (Reverse) | |
| β -Actin | GCACCACACCTTCTACAATGA (Forward) [*] | Small (100 bp) |
| | GTCATCTTCTCGGGTTGGC (Reverse) | |
| | GCACCACACCTTCTACAATGA (Forward) | Large (400 bp) |
| | TGTCACGCACGATTTCCC (Reverse) | |
| GAPDH | TGGCACATATACACCATGGAA (Forward) | Small (110 bp) |
| | TGAGAATGATGGTTTCCAATTTC (Reverse) | |
| | GGATTGGTCGTATTGGG (Forward) | Large (205 bp) |
| | GGAAGATGGTGATGGGATT (Reverse) | |



2.4 Concentration of cfDNA small and large fragments by real-time quantitative PCR of ALU, LINE-1, GAPDH, and β -actin gene

Real-time qPCR for ALU, LINE-1, β -actin, and GAPDH genes using SYBR green chemistry was used to determine the

concentration of large (>205 bp) and small (110 bp) fragments of cfDNA in the sera of healthy, CLD, and HCC patients. RT-qPCR was carried out in a 10 μ L final reaction volume using the Rotor-Gene Q real-time PCR (Qiagen) System. Each reaction mixture contained 2 μ L (~10 ng) of cfDNA template, 0.2 μ L forward and reverse primer (10 μ M), 5 μ L 2X SYBR green (Agilent), and 2.4 μ L nuclease-free water (Qiagen). The reaction condition

was 95°C for 5s (seconds), followed by 40 cycles of 95°C for 15 s, annealing (58°C, 52°C, 55°C, and the 50°C) for 20s, and extension at 72°C for 30s. The Ct value of the RT-qPCR test was used to determine the absolute concentration of fragments by interpolating it from the genomic DNA standard curve of known DNA concentrations ranging from 20 ng to 0.02 pg. The concentration of smaller fragments relative to larger fragments was determined by the comparative Ct ($2^{-\Delta\Delta Ct}$) method. Normalization using the mean Ct value of Huh-7 cell genomic DNA was carried out. Assuming a small fragment as target and a large fragment as a calibrator, normalization was carried out using a reference Ct value of Huh-7 cell genomic DNA or healthy control cfDNA.

2.5 DNA integrity index and total cfDNA concentration

The cfDII is derived from the ratio of large-to-small (L/S) DNA fragments and named as L/S ratio or cfDII-integrity. It was measured for ALU 247/115, LINE 266/97, β -actin 400/100 and GAPDH 205/110 genes in healthy, CLD and HCC patients. The cfDII was derived using the absolute concentration of large to small fragments as described earlier (Umetani et al., 2006b). The absolute concentration of small fragments is indicative of total serum cfDNA concentration. The GAPDH gene small (110 bp) fragment concentration was measured to compare cfDNA concentration in healthy, CLD, and HCC patients. An L/S ratio or cfDII-integrity closer to 1 is indicative of better DNA integrity using the absolute quantitation method, which was determined for all four genes in healthy, CLD, and HCC patients.

The cfDII determined by comparative Ct method (Wang et al., 2003) as per formula $DII = \text{exponential of } (-\Delta\Delta Ct * \ln 2)$ or $e^{-\Delta\Delta Ct * \ln 2}$ (0.693) or $2^{(-\Delta\Delta Ct)}$ is named as cfDII-fragmentation. The cfDII-fragmentation measures the true level of fragmented cfDNA (smaller fragment) by subtracting large fragment from total cfDNA. Normalization using Huh-7 cell genomic DNA mean Ct value and healthy control cfDNA mean Ct value was carried out for comparison of cfDII-fragmentation among healthy, CLD, and HCC patients.

2.6 Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.4.1 and SPSS software. The clinical, demographic profile data of patients expressed as mean \pm standard deviation. All the parametric data among groups were compared using ANOVA and between two groups were compared by *t*-test. The cfDII-fragmentation and cfDII-integrity in different groups were non-parametric and expressed as a median (IQR). These non-parametric data among healthy, CLD and HCC groups were compared using Kruskal–Wallis test followed by Dunns multiple

comparison test for posthoc analysis. The cfDII-fragmentation (normalization with healthy control cfDNA) of CLD and HCC groups as non-parametric data were compared by Mann-Whitney *U* Test. The pool sample size was calculated considering HCC patients as cases and CLD patients as controls using Alu-cfDII as the quantitative variable to determine differences in mean (*d*) and standard deviation (SD). Assuming 80% power (Z_β), 5% level of significance ($Z_{\alpha/2}$), and case: control ratio (*r*) = 1, the required sample size was calculated to be 79 for both case and controls using the formula: $(r+1/r)[SD^2 * (Z_{\alpha/2} + Z_\beta)^2 / d^2]$. To evaluate the diagnostic utility of the cfDNA integrity index (DII), the area under the receiver-operating characteristic (AUROC) curve was plotted. The sensitivity, specificity, and accuracy were calculated accordingly using the cut-off value when the Youden index was maximal. All *p* values were two-sided, and *p* < 0.05 was considered statistically significant.

3 Results

3.1 Demographic, clinical, and biochemical profiles of the study population

HCC patients had a higher mean age (54 ± 13.14 years, *n* = 100) than CLD patients (36.08 ± 14.22 years, *n* = 100), with a significant male predominance in both cases (CLD, 67% and HCC, 84%). The major etiologies for both cases were viral infection (CLD, 87% and HCC, 61%) followed by chronic alcohol consumption (CLD, 6% and HCC, 16%). The percentages of liver cirrhosis in CLD and HCC patients were 32% and 89%, respectively. Other biochemical and clinical parameters were mentioned in Table 2.

3.2 Total cfDNA concentration in healthy, CLD, and HCC patients

Total cfDNA concentration in the serum of healthy controls, CLD, and HCC patients was assessed using qRT-PCR of GAPDH's smaller (110 bp) fragment. The abundance of repetitive elements (ALU and LINE-1) and β -actin gene concentration as compared to GAPDH was determined using the large fragment concentration of genomic DNA. The ratio of β -actin, LINE1 and ALU to GAPDH concentrations was 0.05: 2500: 5833. In the healthy control, the median cfDNA total concentration in the sera was 16.88 ng/ml (IQR: 1.66–62.59 ng/ml). Total cfDNA concentrations in CLD and HCC patients were 33 ng/ml (IQR: 0.21–105 ng/ml) and 244 ng/ml (IQR: 179–287 ng/ml), respectively. The total cfDNA concentration in HCC patients was significantly higher than in healthy and chronic liver disease controls (*p* = 0.0001).

TABLE 2 Demographic, biochemical and clinical profile of study population and comparison between HCC, CLD and Healthy cohorts.

| Sr. No | Variable | Healthy (n = 30) | CLD (n = 100) | HCC (n = 100) | p value |
|--------|----------------------------------|------------------|--------------------|--------------------------|---------------|
| 1 | Age (Year, Mean \pm SD) | 29.62 \pm 7.63 | 36.08 \pm 14.22 | 54 \pm 13.14 | 0.001 |
| 2 | Sex (n, %) | 8 (27%) | 67 (67%) | 84 (84%) | 0.008 |
| | Male | 22 (73%) | 33 (33%) | 16 (16%) | |
| | Female | | | | |
| 3 | Etiologies (n, %) | - | 65 (65%) | 45 (45%) | 0.004 |
| | HBV | - | 22 (22%) | 16 (16%) | 0.28 |
| | HCV | - | 6 (6%) | 8 (8%) | 0.58 |
| | Alcohol | - | - | 6 (6%) | ---- |
| | HBV + Alcohol | - | - | 2 (2%) | ----- |
| | HCV + Alcohol | - | 2 (2%) | 5 (5%) | 0.25 |
| | HVOTO | - | 5 (5%) | 11 (11%) | 0.11 |
| | NASH | - | - | 3 (3%) | ---- |
| | HBV + HCV | - | - | 4 (4%) | ----- |
| | Cryptogenic | | | | |
| 4 | Child's Score | - | - | 88 (88%) | NA |
| | A | - | - | 11 (11%) | |
| | B | - | - | 1 (1%) | |
| | C | | | | |
| 5 | BCLC Stage | - | - | 45 (45%) | NA |
| | A | - | - | 38 (38%) | |
| | B | - | - | 15 (15%) | |
| | C | - | - | 2 (2%) | |
| | D | | | | |
| 6 | PST Score | - | - | 71 (71%) | NA |
| | 0 | - | - | 24 (24%) | |
| | 1 | - | - | 03 (3%) | |
| | 2 | - | - | 02 (2%) | |
| | 3 | | | | |
| 7 | Type II Diabetes Mellitus (n, %) | - | 8 (8%) | 21 (21%) | 0.07 |
| 8 | Cirrhosis | - | 32 (32%) | 89 (89%) | 0.001 |
| 9 | AFP (ng/ml) | - | - | 14,835.2 \pm 113,567.5 | NA |
| | <20 (ng/ml) | - | - | 22 (22%) | |
| | >20 (ng/ml) | | | 78 (78%) | |
| 10 | Serum Albumin (g/dl) | - | 4.45 \pm 0.68 | 4.25 \pm 4.52 | 0.64 |
| 11 | Bilirubin (mg/dl) | - | 0.85 \pm 0.59 | 1.34 \pm 1.49 | 0.0025 |
| 12 | AST (IU/ml) | - | 81.75 \pm 63.78 | 76.46 \pm 83.03 | 0.61 |
| 13 | ALT (IU/ml) | - | 83.70 \pm 104.88 | 60.91 \pm 83.98 | 0.08 |
| 14 | SAP (IU/ml) | - | 234.23 \pm 112 | 316.96 \pm 321.62 | 0.016 |
| 15 | Total Protein (g/dl) | - | 7.5 \pm 0.49 | 7.4 \pm 0.61 | 0.20 |

All values are expressed as n (%) or (mean \pm SD) unless otherwise specified. *p*-value obtained from statistical analysis shows comparison between HCC, CLD, and Healthy subjects. Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; SAP, Serum alkaline phosphatase; PT, Prothrombin time; Hb, Hemoglobin; TLC, Total leucocyte count; PLT, Platelet count; AFP, Alpha-feto protein; HBV, Hepatitis B virus; HCV, Hepatitis C virus; NASH, Non-alcoholic steatosis Hepatitis; HVOTO, Hepatic venous outflow tract obstruction. Bold values are statistically significant ($p < 0.05$).

3.3 The cfDII-integrity or L/S ratio as liquid biopsy marker to differentiate HCC from healthy and CLD patients

The ratio of large to small DNA fragment concentration (L/S ratio) as a measure of cfDII-integrity was determined by

absolute quantification of RT-qPCR and mentioned in Table 3A for ALU, LINE-1, GAPDH and β -actin. The median value of ALU gene cfDII-integrity was found significant ($p < 0.0001$) in Kruskal–Wallis test among groups: healthy (0.1408), CLD (0.1275) and HCC (0.0145) patients. In post hoc analysis, the cfDII-integrity of HCC vs.

TABLE 3 Comparison of ALU, LINE-1, GAPDH and β -actin gene cfDII-integrity and cfDII-fragmentation among healthy, CLD and HCC patients.

| Gene | Healthy (Llovet et al., 2021) | CLD (Sung et al., 2021) | HCC (Dikshit et al., 2012) | <i>p</i> -value | Posthoc, <i>p</i> value |
|---|-------------------------------|-------------------------|----------------------------|---------------------|--|
| (A) The cfDII-integrity (L/S ratio): Absolute quantification, median (IQR) value | | | | Kruskal-wallis test | Dunns multiple comparison test |
| ALU | 0.141 (0.015–0.501) | 0.127 (0.029–0.256) | 0.014 (0.003–0.163) | <0.0001 | 1 vs. 2, 1.0 1 vs. 3, 0.0051 2 vs. 3, <0.0001 |
| LINE-1 | 0.022 (0.001–0.204) | 0.025 (0.005–0.058) | 0.022 (0.006–0.103) | 0.809 | 1 vs. 2, >0.9999 1 vs. 3, 0.8653 2 vs. 3, >0.9999 |
| GAPDH | 0.078 (0.017–0.429) | 0.058 (0.008–0.523) | 0.001 (0.00007–0.093) | 0.0001 | 1 vs. 2, 1.0 1 vs. 3, <0.0001 2 vs. 3, <0.0001 |
| β -Actin | 0.210 (0.031–0.453) | 0.066 (0.016–0.220) | 0.093 (0.032–1.000) | 0.042 | 1 vs. 2, 0.1774 1 vs. 3, 1.0 2 vs. 3, 0.0822 |
| (B) The cfDII-fragmentation normalization with gDNA: Relative quantification, median (IQR) value | | | | Kruskal-Wallis test | Dunns Multiple comparison Test |
| ALU | 2.334 (0.815–14.446) | 2.522 (1.414–8.430) | 15.189 (2.067–47.752) | <0.0001 | 1 vs. 2, 1.0 1 vs. 3, 0.005 2 vs. 3, <0.0001 |
| LINE-1 | 2.930 (0.310–53.629) | 2.808 (1.208–12.104) | 3.295 (0.686–11.196) | 0.827 | 1 vs. 2, 1.0 1 vs. 3, 1.0 2 vs. 3, 1.0 |
| GAPDH | 7.412 (1.942–24.199) | 6.233 (1.268–27.57) | 64.222 (1.855–831.366) | <0.0001 | 1 vs. 2, 1.0 1 vs. 3, 0.01 2 vs. 3, <0.0001 |
| β -Actin | 1.607 (0.817–8.477) | 4.423 (1.539–14.898) | 3.271 (0.406–8.414) | 0.034 | 1 vs. 2, 0.13 1 vs. 3, 1.0 2 vs. 3, 0.07 |
| (C) The cfDII-fragmentation normalization with healthy control cfDNA: Relative quantification, median (IQR) value | | | | Mann-Whitney U Test | |
| ALU | | 0.729 (0.408–2.436) | 4.389 (0.597–13.799) | <0.0001 | |
| LINE-1 | | 0.566 (0.243–2.441) | 0.664 (0.138–2.257) | 0.531 | |
| GAPDH | | 1.248 (0.228–5.856) | 13.784 (0.398–178.445) | <0.0001 | |
| β -Actin | | 1.586 (0.551–5.341) | 1.173 (0.145–3.016) | 0.086 | |

Statistical significance is the *p* value < 0.05 and significant values are mentioned in bold font.

healthy ($p = 0.0051$), HCC vs. CLD ($p < 0.0001$) was found significant (Figure 2A). The AUROC differentiating healthy and CLD from HCC was found significant. The AUROC for healthy vs. HCC was 0.67, $p = 0.005$, and the cut-off point is 0.05 at 68% sensitivity and 70% specificity (Figure 2B). The AUROC for CLD vs. HCC was 0.67, $p < 0.0001$ (Figure 2C) with the cut off value <0.05 at 68% sensitivity and 67% specificity.

The median value of GAPDH gene cfDII-integrity was found significant ($p = 0.0001$) in Kruskal-Wallis test among groups: healthy (0.078), CLD (0.058) and HCC (0.001) patients. In post hoc analysis, the cfDII-integrity of HCC vs. healthy ($p < 0.0001$), HCC vs. CLD ($p < 0.0001$) was found significant (Figure 2D). The GAPDH gene cfDII-integrity AUROC was 0.7453, $p < 0.000$, which can differentiate healthy vs. HCC and the cut-off point is <0.02 at 67% sensitivity and 73.3% specificity (Figure 2E). The

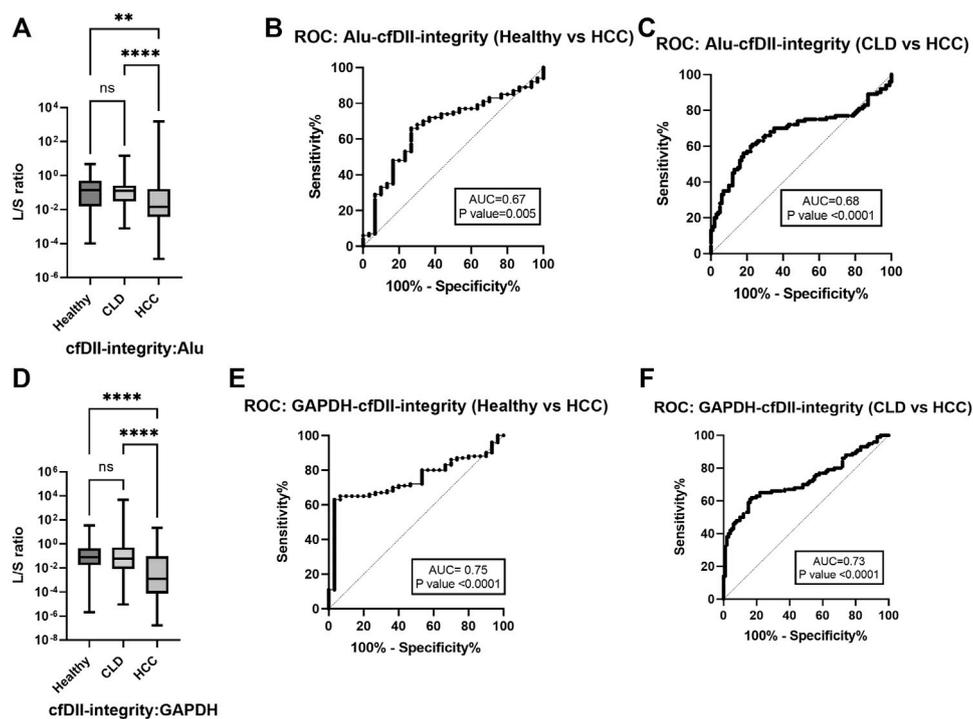


FIGURE 2

The cfDII-integrity (L/S ratio) differentiating HCC from CLD and healthy subjects. (A) ALU gene cfDII-integrity (L/S ratio) among healthy, CLD and HCC patients; (B) The ROC plot of ALU gene cfDII-integrity differentiating HCC from healthy subjects; (C) The ROC plot of ALU gene cfDII-integrity differentiating HCC from CLD patients, (D) GAPDH gene cfDII-integrity (L/S ratio) among healthy, CLD and HCC groups; (E) The ROC plot of GAPDH gene cfDII-integrity differentiating HCC from healthy subjects; (F) The ROC plot of GAPDH gene cfDII-integrity differentiating HCC from CLD patients, ns- Non significant, **- p value = 0.005, ****- p value < 0.0001.

AUROC for CLD vs. HCC was 0.7257, $p < 0.0001$ (Figure 2F), with the cut off value <0.02 at 66% sensitivity and specificity. The cfDII-integrity of LINE-1 and β -actin gene was not found significant (Table 3A).

3.4 The cfDII-fragmentation as liquid biopsy marker to differentiate HCC from healthy and CLD patients following normalization with cellular genomic DNA

The cfDII-fragmentation which determine fragmentation of cfDNA was calculated among healthy, CLD, and HCC patient groups following normalization with Huh-7 cell genomic DNA. The cfDII-fragmentation as a measure of cfDNA fragmentation was determined by relative quantification method using RT-qPCR. The cfDII-fragmentation median and IQR value for ALU, LINE-1, GAPDH and β -actin genes were mentioned in Table 3B. The median value of ALU gene cfDII-fragmentation was found significant ($p < 0.0001$) in Kruskal–Wallis test among groups: healthy (2.334), CLD (2.522) and HCC (15.189) patients. In post

hoc analysis, the cfDII-fragmentation was found significant for HCC vs. healthy ($p = 0.005$), HCC vs. CLD ($p < 0.0001$) (Figure 3A). The AUROC for healthy vs. HCC patients cfDII-fragmentation was 0.67 and found significant $p < 0.0001$ with cut-off value >5.4 at 68% sensitivity and 70% specificity (Figure 3B). The AUROC for CLD vs. HCC patients cfDII-fragmentation was 0.68, $p < 0.0001$ with the cut off value 5.5 at 68% sensitivity and 67% specificity (Figure 3C).

The median value of GAPDH gene cfDII-fragmentation was found significant ($p < 0.0001$) in Kruskal–Wallis test among groups: healthy (7.412), CLD (6.233) and HCC (64.222) patients. In post hoc analysis, the cfDII-fragmentation was found significant for HCC vs. healthy ($p = 0.01$) and HCC vs. CLD ($p < 0.0001$) (Table 3B and Figure 3D). The AUROC of GAPDH gene cfDII-fragmentation was 0.67, $p = 0.004$, which can differentiate healthy vs. HCC with a cut-off value of >10.14 and a sensitivity and specificity of 62% (Figure 3E). The AUROC of GAPDH gene cfDII-fragmentation for CLD vs. HCC was 0.674, $p < 0.0001$ with a cut off value of 11.08 at 62% sensitivity and specificity (Figure 3F). The cfDII-fragmentation of LINE-1 and β -actin gene was not found significant (Table 3B).

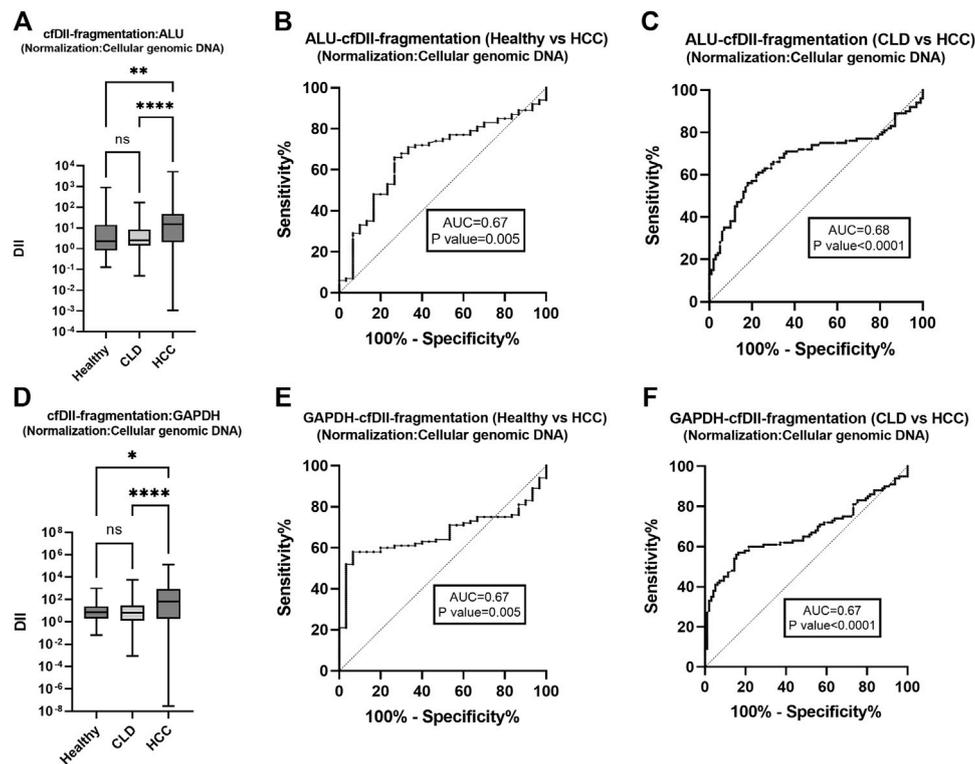


FIGURE 3

The cfDII-fragmentation following normalization with cellular genomic DNA for comparison among healthy, CLD and HCC patients. The ALU gene cfDII-fragmentation among healthy, CLD and HCC patients following normalization with cellular genomic DNA (A). The ROC plot of ALU gene cfDII-fragmentation differentiating HCC from healthy subjects (B), HCC from CLD patients (C). The GAPDH gene cfDII-fragmentation among healthy, CLD and HCC patients following normalization with cellular genomic DNA (D). The ROC plot of ALU gene cfDII-fragmentation differentiating HCC from healthy subjects (E), HCC from CLD patients (F). ns- Non significant, * - p value = 0.01, ** - p value = 0.005, **** - p value < 0.0001.

3.5 The cfDII-fragmentation as liquid biopsy marker to differentiate HCC from CLD patients following normalization with healthy control cfDNA

The cfDII-fragmentation following normalization with healthy control cfDNA was calculated by relative quantification method using RT-qPCR for comparison between two groups (CLD and HCC). The cfDII-fragmentation of ALU, LINE-1, GAPDH and β -actin genes median and IQR value were mentioned in Table 3C. The non-parametric data between two groups were compared using Mann-Whitney test and found significant ($p < 0.0001$) for ALU (Figure 4A) and GAPDH (Figure 4C) genes (Table 3C). The ALU gene cfDII-fragmentation of HCC patients (4.389, IQR-0.60–13.83) was significantly ($p < 0.0001$) higher than that of CLD (0.729, IQR-0.40–2.4) patients (Table 3C; Figure 4A). The AUROC that distinguished HCC from CLD was 0.68 ($p = 0.0001$) and

the cut off value of cfDII-fragmentation > 2.03 with 65.6% sensitivity and 71% specificity (Figure 4B).

The GAPDH gene cfDII-fragmentation following normalization with healthy control cfDNA found significantly ($p < 0.0001$) higher in HCC patients (13.784, IQR-0.398–178.44) than that of CLD (1.248, IQR-0.228–5.856) patients (Table 3C; Figure 4C). The AUROC for CLD vs. HCC was 0.67, $p < 0.0001$ (Figure 4D). The cut-off value for distinguishing CLD from HCC was found to be 2.37, with 62.6% sensitivity and 63.64% specificity. The cfDII-fragmentations of LINE-1 and β -actin genes for CLD and HCC patients were not found significant (Table 3C).

3.6 The cfDII-fragmentation trends in different etiologies of HCC

The cfDII-fragmentations following normalization with healthy control cfDNA were further evaluated in HCC

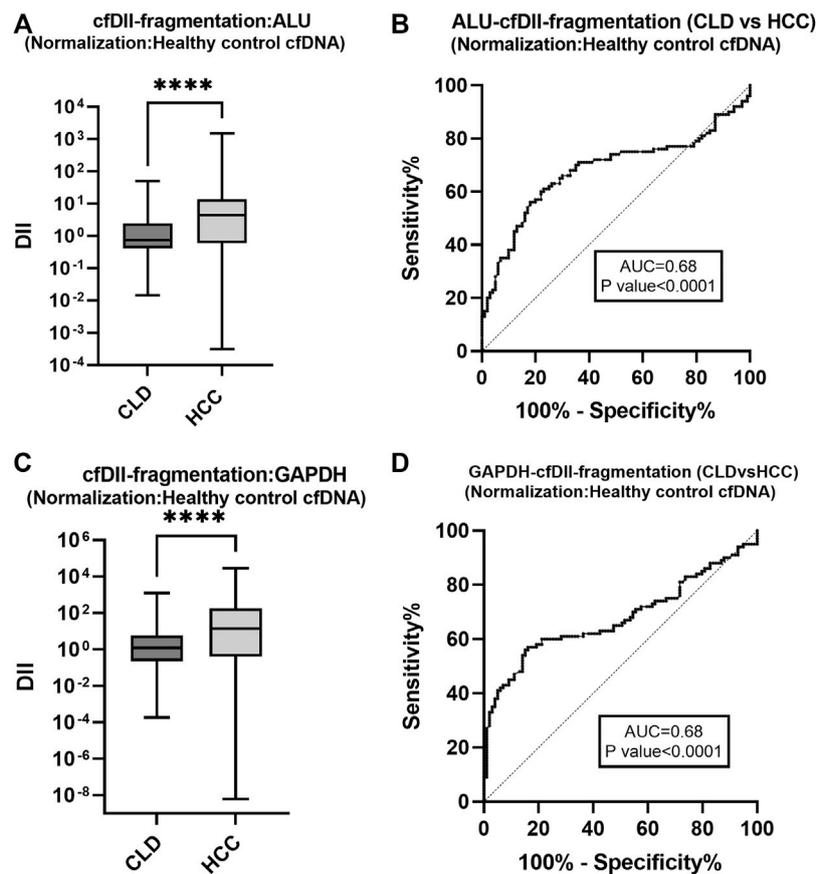


FIGURE 4

The cfDII-fragmentation following normalization with healthy control cfDNA for comparison between CLD and HCC patients. The cfDII-fragmentation median value of CLD and HCC patients following normalization with healthy control cfDNA for ALU element (**A**) and GAPDH gene (**C**). The ROC plot of ALU element cfDII-fragmentation differentiating HCC from CLD patients (**B**). The GAPDH gene cfDII-fragmentation ROC plot differentiating HCC from CLD patients (**D**). There is an obvious similarity observed between **Figure 4B** with **Figure 3C** for ALU element and between **Figure 4D** with **Figure 3F** for GAPDH gene. Both of these genes cfDII-fragmentation differentiate HCC from CLD. The primary data are the same for both genes, only the normalization method is different one is with cellular genomic DNA (**Figures 3C,F**) other is with healthy control cfDNA (**Figures 4B,D**). Therefore, the ROC pattern is similar even though data and cut-off points are different after normalization. ****- p value < 0.001.

patients with various etiologies, including viral and non-viral. In Kruskal–Wallis test, the data were not found significant and only trends were depicted in **Figure 5**. The median cfDII-fragmentation of ALU elements (**Figure 5A**) were HBV, 15.14; HCV, 10.81; HBV + HCV, 21.41; Cryptogenic, 13.19; Alcohol, 13.36; HVOTO, 29.04, NASH, 13.98. The median cfDII of LINE1 elements (**Figure 5B**) were HBV, 2.928; HCV, 1.057; HBV + HCV, 9.448; Cryptogenic, 5.697; Alcohol, 1.05; HVOTO, 6.589, NASH, 8.15. Higher cfDII-fragmentation was observed for ALU than LINE elements for HCC of different etiologies.

The median cfDII-fragmentation of GAPDH gene (**Figure 5C**) in HCC patients of different etiologies were HBV, 117.0; HCV, 61.67; HBV + HCV, 6.916; Cryptogenic, 313.1; Alcohol, 39.12; HVOTO, 11.16, NASH, 611.1. The median cfDII-fragmentation of -actin gene in HCC (**Figure 5D**) patients were

HBV, 3.793; HCV, 0.8594; HBV + HCV, 3.317; Cryptogenic, 4.653; Alcohol, 6.277; HVOTO, 3.182, NASH, 1.974. For all etiologies of HCC, GAPDH had a higher cfDII-fragmentation value than β -actin gene.

4 Discussion

Chronic liver diseases that occur due to HBV and HCV infections, alcohol consumption, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, and hemochromatosis are all strongly associated with cirrhosis, HCC, and an increased risk of mortality. These CLD patients require routine viral load estimation, treatment, and monitoring for HCC. The liquid biopsy markers such as cfDNA or ctDNA can be useful for HCC screening due to the ease of the RT-qPCR

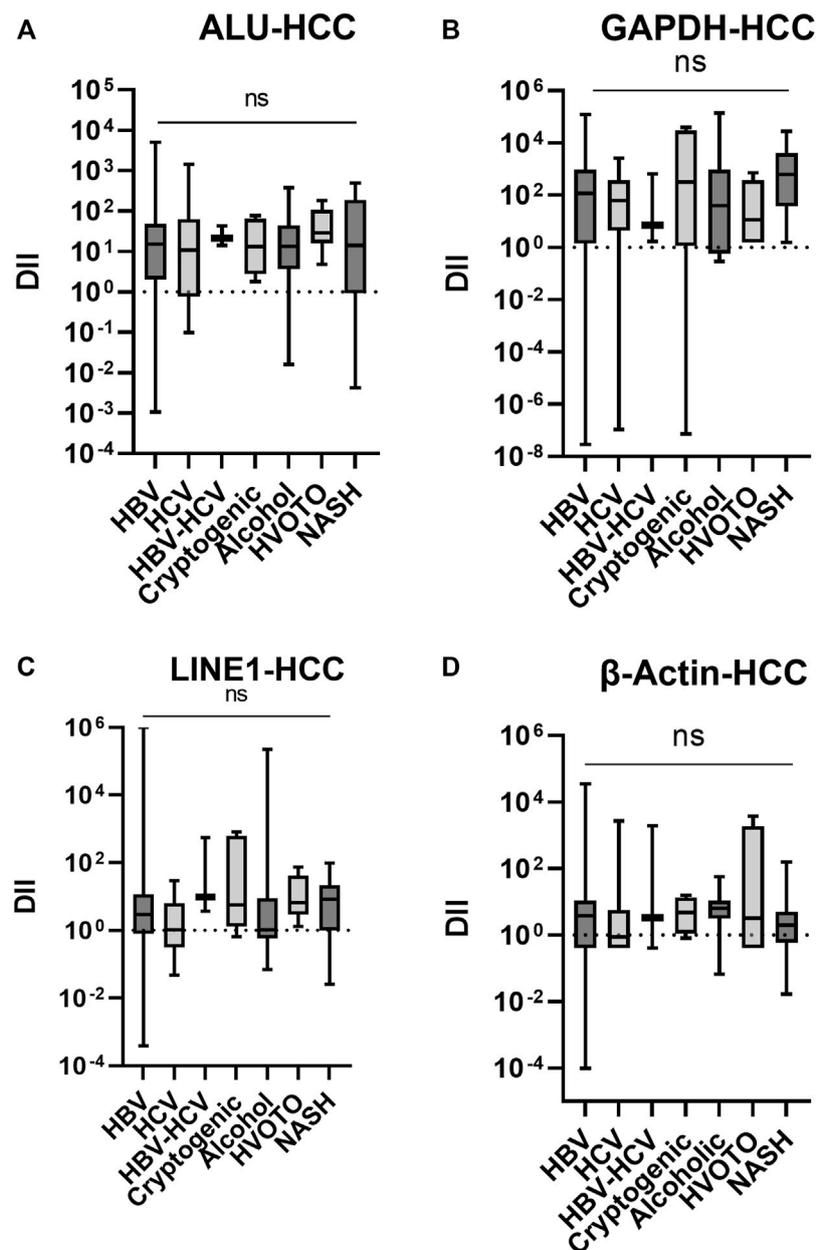


FIGURE 5

The cfDII-fragmentation trends in HCC patients following normalization with healthy control cfDNA for ALU (A), LINE (B), GAPDH (C) and β -actin (D) gene. ns, non significant.

technique, cost-effectiveness, frequent mass sample screening, and early detection of cancer. In this study, we used RT-qPCR to evaluate cfDII for ALU, LINE-1, β -actin, and GAPDH gene in healthy, CLD, and HCC patients as measures of cell-free DNA integrity (cfDII-integrity or L/S ratio) by absolute quantification methods and as measures of cell-free DNA fragmentation (cfDII-fragmentation) by relative quantification methods. We observed low cfDII-integrity (L/S ratio) and high cfDII-

fragmentation in HCC patients as compared to CLD and healthy subjects. Using the ROC curve of both the cfDII-integrity and -fragmentation, we have shown that it can differentiate HCC from healthy individuals and CLD patients. Among the four selected genes, the RT-qPCR assay determining cfDII-integrity and -fragmentation for ALU and GAPDH have shown promising utility as an early biomarker for hepatocarcinogenesis by differentiating HCC from CLD.

Cell-free DNA is important for cancer diagnostics as a major portion of it is originated from the tumor itself resulting quantitative changes in the cfDNA concentration, integrity and fragmentation in the circulation of cancer patients (Schwarzenbach et al., 2011; Diaz and Bardelli, 2014). Total cfDNA concentration can be measured by spectrophotometry but the cfDNA concentration can be easily biased by pre-analytical techniques. Different cfDNA extraction kits/methods have different DNA recovery efficiencies (Page et al., 2013). Cell lysis may lead to increased cfDNA concentration (Page et al., 2006) whereas prolonged storage can decrease cfDNA concentration (Sozzi et al., 2005). The cfDNA concentration in healthy subjects ranges from 0 to 100 ng/ml of blood, with an average of 30 ng/ml whereas, the cfDNA concentration in cancer patients ranges from 0 to 1,000 ng/ml of blood, with an average of 180 ng/ml (Schwarzenbach et al., 2011). Using RT-qPCR of GAPDH gene, we have observed median cfDNA concentration of 16.88 ng/ml (IQR: 1.66–62.59 ng/ml) for healthy control, 33 ng/ml (IQR: 0.21–105 ng/ml) for CLD and 244 ng/ml (IQR: 179–287 ng/ml) for HCC patients. These results are consistent with previous findings of higher cfDNA DNA concentration in HCC patients than in healthy and CLD subjects (Diehl et al., 2005; Schwarzenbach et al., 2011).

The integrity of cfDNA is another parameter that changes in cancer patients. The cfDNA is more fragmented in cancer, with a higher prevalence of tumor-associated mutations in the shorter fragments (~150–180 bp in length) of cfDNA in cancer patients (Diehl et al., 2005; Jiang et al., 2015). Larger fragments derived from apoptosis appear in multiples of ~180-bp DNA fragments and are visible in gel as a DNA ladder, whereas necrotic DNA fragments are more non-specific and appear as a smear. DNA integrity is indicative of more cfDNA larger fragments in circulation due to apoptosis. Integrity of cfDNA expressed as a ratio of large to small fragments or DNA integrity index (DII), which is unaffected by external factors and accurately represents cancer-related DNA fragmentation. However, in cancer, both decreased (Madhavan et al., 2014; Cheng et al., 2017) and increased (Wang et al., 2003; Jiang et al., 2006; Leszinski et al., 2014) cfDII levels have been reported. This is primarily due to different approaches in determining DII. The DII determination was described in two ways: absolute quantification method using standard curve for small and large fragments concentration (L/S) ratio (Umetani et al., 2006b), and the relative quantification or comparative Ct method for large and small fragments (Wang et al., 2003). We have evaluated cfDII in both ways and plotted a ROC curve to differentiate HCC from healthy and CLD patients. The concentration of small fragments using RT-qPCR determines total cfDNA concentration and the L/S ratio closer to 1 indicates better integrity. We have observed cfDII-integrity (L/S ratio) closer to 1 in healthy and CLD subjects than in HCC subjects for ALU (Figure 2A) and GAPDH (Figure 2D), indicating better integrity. The cfDII-integrity was significantly able to differentiate HCC from healthy and CLD with AUROC of

0.67 at a cut-off of 0.05 for ALU elements (Figures 2B,C) and AUROC of 0.74 and 0.72, a cut-off of <0.02 for GAPDH gene (Figures 2E,F).

The other cfDII method proposed by Wang et al. (Wang et al., 2003) is the comparative $\Delta\Delta Ct$ method, which involves normalizing the Ct value of small and large fragments by the healthy control or cultured cell genomic DNA mean Ct value to get ΔCt and then subtracting ΔCt of the large fragment from ΔCt of the small fragment to get $\Delta\Delta Ct$. Finally, DII was calculated to be as $2^{-\Delta\Delta Ct}$. This method yields a higher cfDII (Wang et al., 2003; Madhavan et al., 2014) and measures cfDNA fragmentation. We have observed increased cfDII-fragmentation in HCC as compared to healthy and CLD patients using Alu element (Figures 3A, 4B) and GAPDH (Figures 3D, 4C). We did not observe significant changes in cfDII-fragmentation of LINE1 and β -actin genes among healthy, CLD, and HCC patients (Table 3B). The cfDII-fragmentation of ALU and GAPDH can differentiate HCC from healthy and CLD patients. The AUROC of ALU element in distinguishing HCC from healthy (Figure 3B) and CLD (Figure 3C) is 0.67 and 0.68, with a cut-off value of >5.4 cfDII-fragmentation. Following normalization with healthy control, the AUROC differentiating HCC from CLD was 0.68, with a cut-off value of >2.03 (Figure 4B). Similarly, GAPDH cfDII-fragmentation differentiates HCC from healthy (AUROC, 0.67, Figure 3E) and CLD (AUROC, 0.67, Figure 3F) at cut-offs > 10.14 and >11.08, respectively. Following normalization with healthy control, the AUROC for GAPDH differentiating HCC from CLD was 0.67, with a cut-off value of >2.37 (Figure 4D). Increased levels of cfDNA in the serum or plasma of various HCC patient cohorts have been reported (Iizuka et al., 2006; Huang et al., 2012).

The increased cfDII-fragmentation in HCC is attributed to the cell necrosis within the tumour, resulting in an increased number of highly fragmented (smaller fragment) DNA copies released into circulation (Wang et al., 2003; Andersen et al., 2015). Increased cfDII-fragmentation was also observed in other cancer types, i.e., breast cancer, colorectal cancer, bladder cancer, and pancreatic cancer (Yu et al., 2014). Our results are consistent with previous studies. ALU cfDII-fragmentation has shown differentiating ability in colorectal cancer from healthy (Hao et al., 2014), β -actin cfDII-fragmentation (394/99bp) has shown differentiating ability for breast cancer patients (Salimi and Sedaghati Burkhani, 2019). We did not observe any significant changes in β -actin and LINE1 cfDII-fragmentation (Table 3). This may be attributed to cancer of different organs for β -actin whereas LINE1 mostly relates to the methylation pattern of cfDNA. We have observed a similar pattern of cfDII-fragmentation in HCC patients of different aetiologies, which may indicate similar increased fragmentation of cfDNA in HCC regardless of its aetiology (Huang et al., 2016).

The limitations of our study include clinicopathological correlation of HCC with cfDII; changes in cfDII during hepatocarcinogenesis and disease progression; the effect of therapy on cfDII; and staging of HCC using cfDII. We did

not perform any comparison of RT-qPCR based determination of cfDII with other liquid biopsy-based detections, such as: CTCs, CSCs, microRNAs, and exosomes in cancer. In conclusion, both cfDII-integrity and cfDII-fragmentation determined by RT-qPCR techniques for ALU elements and GAPDH gene can be useful biomarkers to differentiate HCC from CLD and healthy subjects. It has the potential to be an early hepatocarcinogenesis marker. Using cfDII to improve surveillance of CLD patients may help find more people with HCC at a curative stage.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by IEC PG-38/23.01.2019, RT-13/28.02.2019 (All India Institute of Medical Sciences, New Delhi). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SK: Concept, data acquisition, draft writing; NN: data acquisition, draft writing; SP: data acquisition, draft revision; SG: data acquisition; ND: data acquisition, draft revision; PV:

Draft revision, Statistical analysis; AS: Draft writing; S: Patient recruitment, Concept, draft writing; BN: Concept, data acquisition, draft writing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Isolation and identification of metronidazole resistance *Helicobacter pylori* from gastric patients in the southeastern region of India and its advanced antibacterial treatment using biological silver oxide nanoparticles

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ABSTRACT

The present study aimed to identify the drug resistance *Helicobacter pylori* (*H. pylori*), and its advanced treatment using *Digiria muricata* (Dm) leaf mediated silver oxide nanoparticles (Ag₂ONPs). The synthesized Dm-Ag₂ONPs were confirmed by UV-visible spectrometry, X-ray diffraction crystallography (XRD), Fourier transfer infrared spectroscopy (FT-IR), Transmission electron microscopy (TEM), Energy dispersive spectroscopy (EDAX). The fifty gastric biopsy samples were obtained from gastric patients in the Trichy SRM medical College Hospital and Research Centre, Central South-Eastern region of India, Trichy with Gastro endoscopy. *H. pylori* was identified by biopsy crush cytology, histopathology, rapid urease test, and biochemical tests. The resistance pattern was checked by the disk diffusion method. Among 50 biopsy samples, ten patients had positive results for culture, biochemical, and histopathology. The obtained strains showed 10% resistance to the metronidazole and 100% sensitivity toward amoxicillin and clarithromycin. Further, the metronidazole resistance strain was tested against Dm-Ag₂ONPs using a well diffusion assay, which showed (25 µg/mL) 2.4 ± 0.4 and (100 µg/mL) 5.5 ± 0.5 mm antibacterial activity. Overall, the obtained results from the present investigation are promising enough to reveal the effectiveness of Dm-Ag₂ONPs to inhibit the metronidazole resistance *H. pylori*, this may help to develop a novel therapeutic drug against the drug resistance *H. pylori* infections.

1. Introduction

H. pylori is a gram-negative microaerophilic pathogenic bacterium that affects up to half of the global population; compared to the developed countries, the developing countries have more *H. pylori* infection rates. It colonizes the human stomach and is associated with upper gastrointestinal diseases like gastric adenocarcinoma, and peptic ulcers [1,2]. In ambience, the quick emergence to develop alternative drugs against the multi-drug resistance *H. pylori* strains [3]. Shortage of evidence on the studies of sensitivity profile of *H. pylori* linked gastric

infections, which leads to the abandoned management as a result the treatment is ineffective. The study has been reported clarithromycin and metronidazole show more resistance, highly prevalent, and more common [4]. In *H. pylori* diagnosis, stomach biopsy culture is not regularly achieved due to the difficulty in maintenance [5]. The gastric biopsy samples are highly specific for culture, and isolation of *H. pylori* and are also helpful for detecting the susceptibility of antibiotics as well as characterization of isolates [6,7].

Universally antibacterial resistance is a major problem to treat some bacterial infections resulting in weakness and death. The major concern

Abbreviations: Ag₂ONPs, Silver oxide nanoparticles; *H. pylori*, *Helicobacter pylori*; Dm, *Digiria muricata*.

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about *H. pylori* was developed resistance against routinely used drugs such as metronidazole, clarithromycin, and amoxicillin [8,9]. Thus, the researchers concentrate on the discovery of less cost, eco-friendly and new antimicrobial agents. Nanobiotechnology is a growing field of technology for the synthesis of environmentally friendly nanoparticles for antibacterial treatment [10]. The green NPs have developed as an addition to the synthetic methods [11]. Green synthesis of Ag₂ONPs has been extensively used because of their eco-friendly and cost-effective nature than the chemical methods, which contain a broad range of biological applications such as antibacterial and anti-fungal properties [12]. Previously, many studies have reported the synthesis of Ag₂ONPs from different plant extracts such as *Zephyranthes rosea* and *Daphne alpina* [13,14]. In the current study, the silver oxide nanoparticles were synthesized by *Digera muricata* (L) Mart. aqueous leaf extract. This medicinal plant belongs to the *Amaranthaceae* family; originates as a weed all over India. The leaves and shoots are mainly used for medical applications [15].

The bacterial isolates from the human stomach biopsies are the best source for the strain typing method used in different studies. In the south-eastern region of India, Tamil Nadu, there is a lack of reported data regarding *H. pylori* isolation and its treatment using biologically synthesized Ag₂ONPs. Understanding the growing occurrence of antibiotic resistance in different regions and the efficiency of many eradication treatment regimens show a negative impact. Hence, it is necessary to clinical identification and antibiotic susceptibility patterns when selecting exact suppression actions for *H. pylori* infections in the empirical situation. The present study aims to investigate the isolation and identification of *H. pylori* from gastric biopsy using different biochemical and histopathology methods. Further, the resistance strain was checked for advanced Dm-Ag₂ONPs antibacterial activity.

2. Materials and methods

2.1. Patient's data and endoscopic procedure

Fifty biopsy samples with various upper gastric infection symptoms were collected using Endoscopy at the Department of Gastroenterology, Trichy, SRM Medical College Hospital and Research Centre, Trichy, Tamil Nadu, India. The informed written consent was taken in advance from all the patients. The demographic pattern (sex, age) was recorded. Before the endoscopic procedure, patients were recommended to fast for 12 h and did it under local anesthesia. The study was approved by the Institutional Ethical Committee of (CMCH&RC). Ref No: CMCH&RC/IEC-No 33.

2.2. Biopsy sample collection

Four stomach biopsy samples were collected through the endoscopy, in that two bits were fixed in 10% formalin for histopathological studies and the other two bits were put into the sterile container in ice which contain 2 mL Brucella broth with 10% Fetal bovine serum, then immediately within 1 hr we crushed the biopsy samples and processed for microbial culturing.

2.3. Isolation and identification of *H. pylori*

Histopathologic examination was followed by Dixon et al. [16]. Briefly, stomach biopsies were processed for histopathology examination using Giemsa staining. The curved rods were identified on the surface of the liminal surface of epithelial cells. To investigate the microbial culture identification, the biopsies were crushed and inoculated in blood agar plates containing 10% human blood and Skirrow supplement (Skirrow supplement is a selective media for *H. pylori* isolation, which is composed of Trimethoprim, Vancomycin, Polymyxin B sulfate) and incubated at room temperature in the microaerophilic jar (Anaero gas pack HiMedia). Further, rapid urease test, gram staining, urease,

catalase, and oxidase tests were performed.

2.4. Antibiotic susceptibility test

The antibiotics were used in this study amoxicillin (25 mcg), clarithromycin (15 µg⁻¹), metronidazole (5 µg⁻¹), Muller Hinton with 10% FBS, and Skirrow supplement were used for the disk diffusion method [17,18].

2.5. Biosynthesis of Ag₂O nanoparticles

2.5.1. Preparation of *Digera muricata* leaf extract and Ag₂O synthesis

Briefly, the fresh leaves of *Digera muricata* were collected around Salem, Tamil Nadu, India. After, the leaves were thoroughly washed with tap and distilled water. Further, leaves were shaded dry for 7 days and the dried leaves were made as a fine powder. 10 g of powder dissolved in 100 mL of water and boiled at 40 °C for 30 min. After allowed to cool at room temperature and filtered using Whatman No.1 paper.

The synthesis of Ag₂ONPs was followed by Sharma and Srivastava [19]. Briefly, with small modifications, *Digera muricata* aqueous leaf extract was used to make Ag₂ONPs in less time, and eco-friendly nature. The 10 mL of filtered leaf extract and (2 mM AgNO₃) 90 mL were mixed for the reaction and heated and stirred at 60–80 °C for 60 min. The early observation of NPs synthesis was confirmed by witnessing the color-changing reaction medium from golden yellow to dark brown. The supernatant was substituted by adding D.D H₂O every time, the suspension was dried and stored as a powder for further analysis.

2.5.2. Characterization of *Digera muricata* synthesized Ag₂O nanoparticles

Ultra-violet visible spectroscopy was used to investigate the surface plasmon resonance of Ag₂ONPs from 200 to 800 nm in range. The functional group and chemical composition of synthesized Ag₂ONPs were studied using FT-IR spectroscopic analysis in the 7800–350 cm⁻¹ range by potassium bromide pellet. The crystal structure of Ag₂ONPs was determined by XRD analysis. The lyophilized Ag₂ONPs were coated on the XRD grid and the corresponding spectrum was recorded. The values were determined by comparing JCPDS files. The particle size measured by below mentioned Scherrer's equation.

$$D = \frac{0.94\lambda}{\beta \cos\theta}$$

Where D= crystal size, λ = X-ray wavelength, θ = Bragg's angle, and β = FWHM of the peak in radians.

In EDAX analysis, the synthesized particles were coated onto the C film (Hitachi S-3400N) (JEOL, JEM 2100 HR with EELS), grids were prepared by placing Ag₂ONPs (5 µl) C-coated Cu grids and drying under the lamp, TEM pattern was investigated to identify the size of NPs.

2.6. Anti-*H. pylori* activity by well diffusion assay

The anti-*H. pylori* activity of Dm-Ag₂ONPs was tested using a well diffusion assay, followed by Sampath et al. [20]. Briefly, with small modifications, the cultured *H. pylori* cells were placed on a Muller-Hinton agar plate containing fetal bovine serum (FBS) and 1% isovitalax in various directions by aseptic swabs. The 7 mm wells were created using stainless borer, after the different concentrations such as 25, 50, 75, and 100 µg/mL of synthesized Ag₂ONPs were added into individual wells. The plates were incubated for 72 h in microaerophilic conditions at room temperature; the inhibitory zone was measured in millimeters (mm).

2.7. Statistical analysis

The statistical analyses were performed in GraphPad Prism 5. All the values are represented as mean, ± standard deviation (SD) values.

Table 1
Demographic pattern and history of *H. pylori* positive patients.

| Patient number | Age | Sex | Tobacco | Smoking | Alcohol | Endoscopy |
|----------------|-----|-----|---------|---------|---------|-----------|
| 03 | 50 | M | Yes | Yes | Yes | GC |
| 07 | 75 | M | No | No | Yes | GC |
| 12 | 43 | M | No | Yes | Yes | DU |
| 14 | 65 | F | No | No | No | DU |
| 16 | 66 | M | No | Yes | Yes | GC |
| 21 | 67 | M | No | No | No | DU |
| 23 | 70 | F | No | No | No | GC |
| 38 | 45 | F | No | No | No | GC |
| 39 | 64 | M | Yes | No | No | GC |
| 45 | 40 | F | No | No | No | DU |

GC- Gastric cancer, DU- Duodenal ulcer.

3. Results

3.1. Data collection analysis

Fifty stomach biopsy samples were collected from gastric infection patients and before investigation, the study was explained to patients and a written consent form was taken. The demographic pattern such as age, sex, and intake of tobacco, and alcohol were noted and endoscopic symptoms details were recorded (Table 1). The results indicated a total of 70% (35 out of 50) patients are males and 30% (15 out of 50) patients are females are involved with 21–82 years in the range. The high prevalence rate of *H. pylori* positive rate was observed in gastric carcinoma patients.

3.2. Identification of *H. pylori* by microbial, biochemical, and histopathology analysis

Among all the 50 biopsies, 10 samples showed positive results for histopathology and microbial culture test. The endoscopic images were

shown in Fig. 1. Further, these 10 biopsy samples were studied for biochemical studies such as catalase, urease, oxidase, and gram staining (Fig. 2), all the 10 samples showed positive results (Table 2). The microbial culture clearly showed the grown colonies are smooth and translucent and the gram staining reveals all the 10 strains are gram negative in nature. The histopathology confirms the presence of rod-shaped bacterium on the luminal surface of the epithelium (Fig. 3), by all these tests we confirmed the *H. pylori* strains and used them for further analysis.

3.3. Anti *H. pylori* activity by disk diffusion method

Ten positive strains were used for antibacterial activity against three routinely used anti-*H. pylori* antibiotics such as clarithromycin, amoxicillin, and metronidazole (Fig. 4A). The results showed 100% sensitivity toward clarithromycin, amoxicillin, and 10% resistance to the metronidazole (Fig. 4B). Further, the metronidazole resistance strains were used for further investigation.

3.4. Biosynthesis of Ag₂O nanoparticles

The *Digera muricata* leaf extract was used to synthesize the Ag₂ONPs. The biosynthesis was confirmed by the changing color from (golden yellowish-dark brown). The silver ions (Ag⁺) are converted into Ag₂ONPs by the addition of plant extract, here the leaf extract act as a capping agent. The formation of Ag₂ONPs was investigated by observing the UV- absorption spectra after 48 h. The results showed that the absorption peaks in the (438 nm) range of synthesized Ag₂ONPs (Fig. 5A) because of the excitation surface plasmon vibration particles.

3.5. Characterization of *Digera muricata* synthesized Ag₂O nanoparticles

The FT-IR spectrum of synthesized Ag₂ONPs by *Digera muricata* leaf extract was performed (Fig. 5B). The spectrum showed characteristic

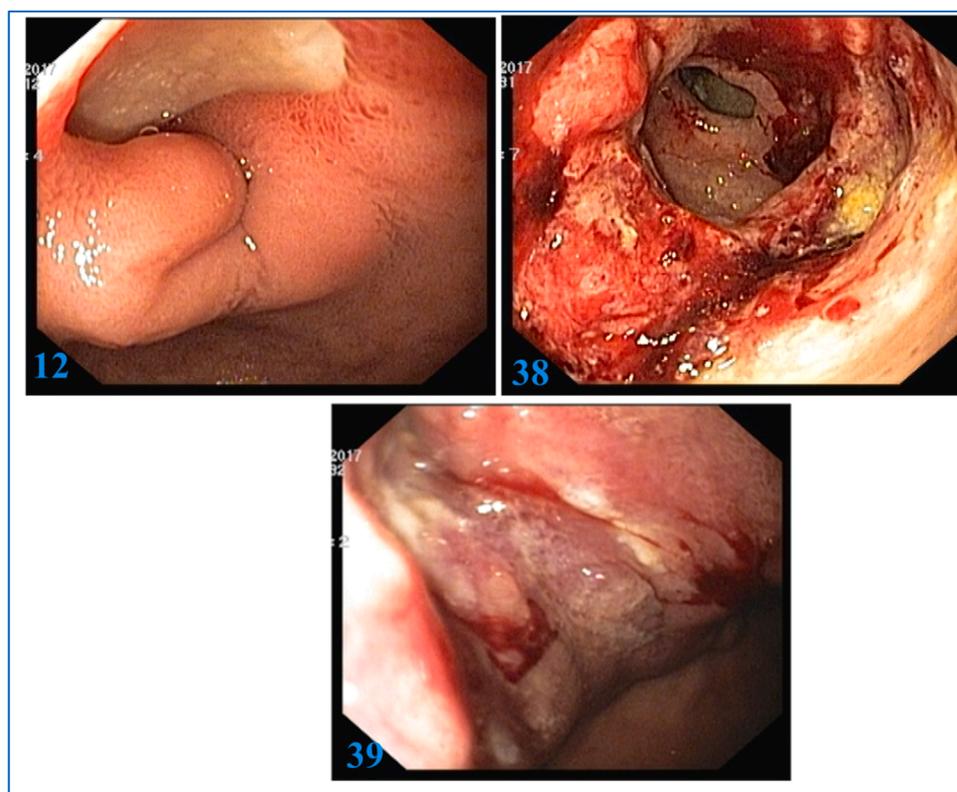


Fig. 1. *H. pylori* positive endoscopic images of patients. Endoscopic findings patient numbers 12, 39 duodenal ulcer and 38 gastric cancer samples.

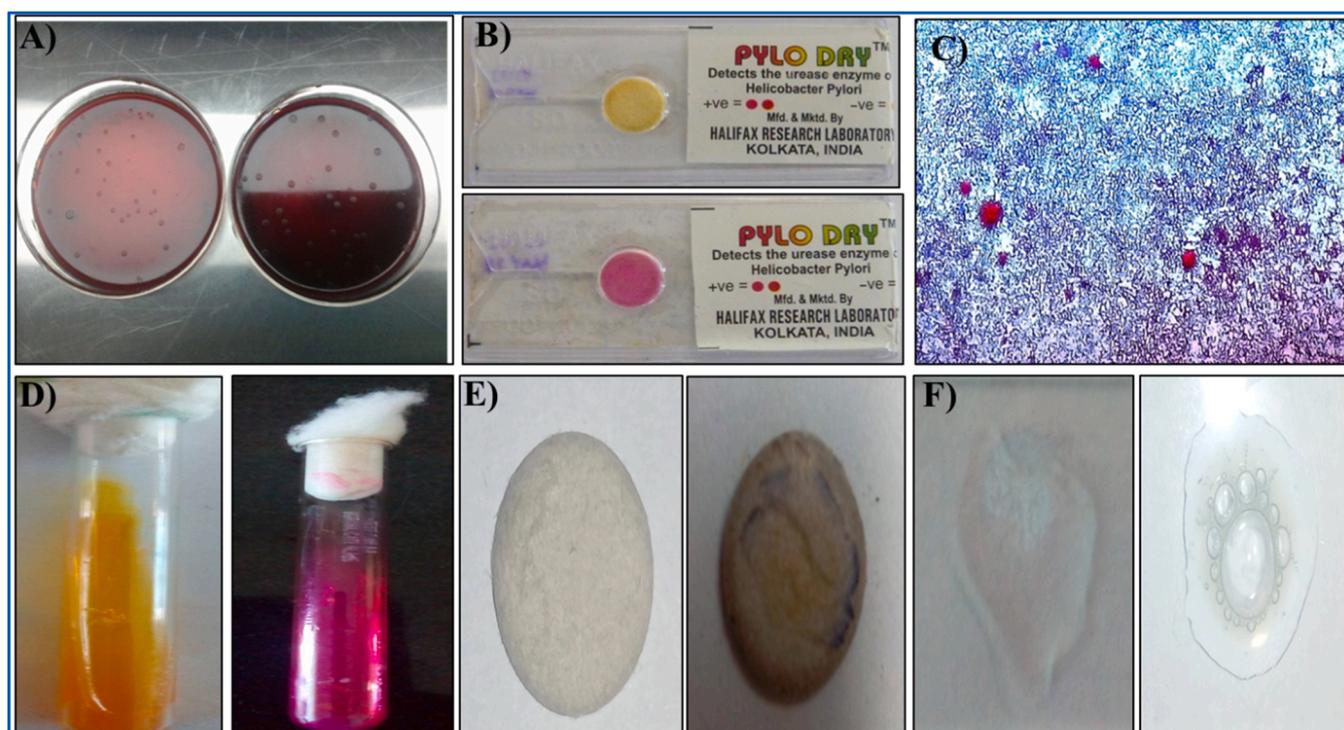


Fig. 2. A) Translucent round shaped colony morphology of *H. pylori* B) RUT test by CLO kit C) Gram staining indicating the presence of spiral shaped bacteria D) Indicating the urease, E) catalase F) oxidase positive tests.

Table 2

Biochemical, histopathological and endoscopic result of *H. pylori* positive patients (GC- Gastric cancer, Du- Duodenal ulcer).

| Patient number | Biochemical test | | | | | | Histopathology |
|----------------|------------------|---------|---------|----------|--------|---------------|----------------|
| | RUT | Culture | Oxidase | Catalase | Urease | Gram staining | |
| 03 | + | + | + | + | + | - | + |
| 07 | + | + | + | + | + | - | + |
| 12 | + | + | + | + | + | - | + |
| 14 | + | + | + | + | + | - | + |
| 16 | + | + | + | + | + | - | + |
| 21 | + | + | + | + | + | - | + |
| 23 | + | + | + | + | + | - | + |
| 38 | + | + | + | + | + | - | + |
| 39 | + | + | + | + | + | - | + |
| 45 | + | + | + | + | + | - | + |

features of Ag₂ONPs. The major peaks at (3300.40 cm⁻¹, 2920.98 cm⁻¹, 2353.99 cm⁻¹, 1638.24 cm⁻¹, 1034.97 cm⁻¹, 675.59 cm⁻¹) were observed. The spectrum shows the band at 3300.40 cm⁻¹ conforming to the OH stretching vibration [21], peak at 2920.98 cm⁻¹ due to C-N and C-H stretching [22]. Other bands at 2353.99 cm⁻¹ due to the flavonoids and carboxylic acid [23] and 1638.24 cm⁻¹ correspond to the C=C stretching. The band observed at 1034.97 cm⁻¹ represent the CH₃ rocking vibrations. The band at 675.59 cm⁻¹ corresponds to the c-Br (Alkyl) group. The FT-IR results of synthesized particles confirmed the capping of *Digera muricata* leaf extract.

XRD analysis of Dm-Ag₂ONPs showed a few intensive peaks of 2θ values ranging from 20 to 80. Fig. 5C showed major distinct diffraction peaks corresponding to the (110), (111), (200), (211), and (220) planes which could be indexed to the Ag₂ONPs based on the comparison with the near-standard given by JCPDS (file no.65–6811). The obtained results indicate that the obtained Ag₂ONPs are crystalline in nature with 32.38 nm in size.

The morphological features were characterized by TEM analysis. Fig. 5D showed Ag₂ONPs with an average particle size of 11–35.6 nm in the range of TEM, which also reveals that NPs are fixed in a dense

matrix, confirming the capping and stabilizing components of the *Digera muricata* leaf extract.

The EDAX result reveals the formation of strong signals for Ag atoms in the range of 2.7–3.9 keV. The elemental analysis of Dm-Ag₂ONPs revealed the highest proportion of silver followed by N and O atoms (Fig. 5E).

3.6. Anti *H. pylori* activity by well diffusion assay

In the current study, Dm-Ag₂ONPs showed good anti-*H. pylori* activity against metronidazole resistance strain with increasing (25–100 µg/mL) concentration. The zone of inhibition was calculated in millimeters, the higher zone was observed at 5 ± 0.5 mm in 100 µg/mL, and 50, 25 µg/mL showed 4.1 ± 0.8, 2.4 ± 0.4 respectively. The D.D H₂O was used as a negative control, which does not show any antibacterial activity (Fig. 6).

4. Discussion

The multidrug resistance of *H. pylori* is the major factor and reason

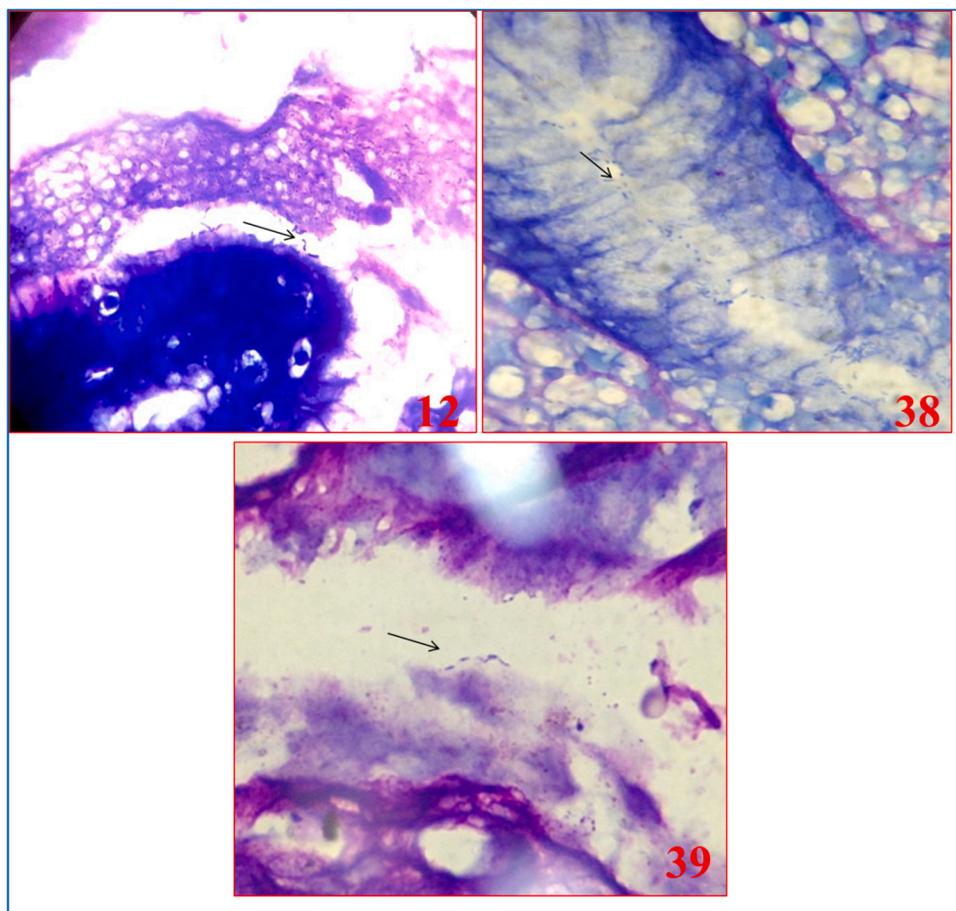


Fig. 3. Histopathology of *H. pylori* positive patients Giemsa stained biopsy samples. Arrow marks indicating the presence of *H. pylori*.

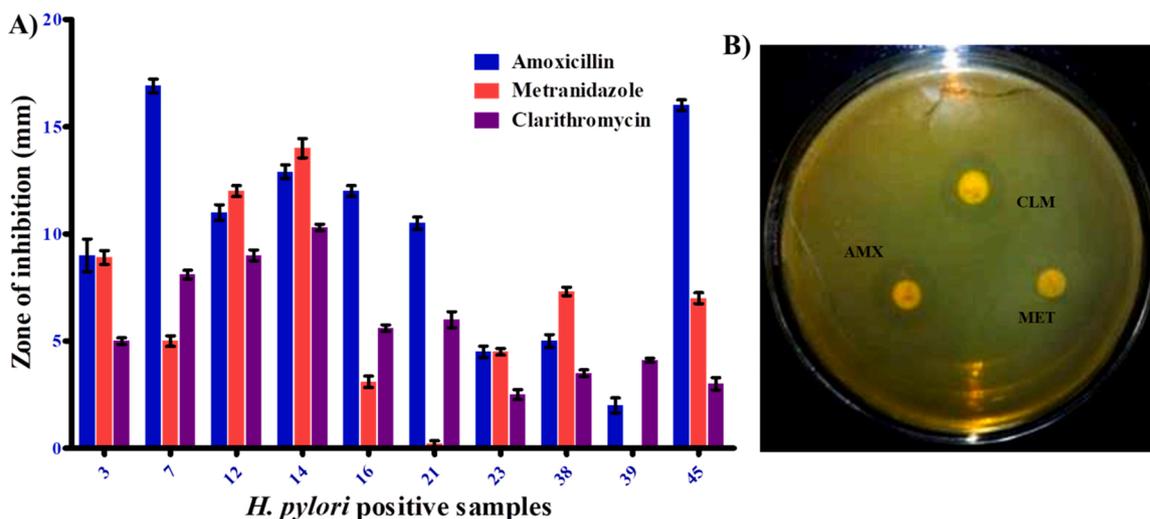


Fig. 4. A) Antibacterial activity of *H. pylori* by disk diffusion assay. (Mean values designated with different superscripts indicate that differences between treatments are significant according to the Tukey's honestly significantly different (HSD) multiple comparison test ($P < 0.05$). B) Strain number 39 showed resistance to the metronidazole (AMX-Amoxicillin, MET-Metronidazole, CLM-Clarithromycin).

for the failure of eradication treatment. The study of the drug resistance profile on *H. pylori* is compulsory to track and acquaint the antibiotic grouping with local resistance designs. The current issue is to identify the interlink between *H. pylori* and the development of different upper gastrointestinal diseases such as gastric ulcers, gastritis, and gastric cancer [24]. To this point, the present study showed a significant

association between the incidences of *H. pylori* with higher risks of gastric infections. The results are in good agreement with the previously published report [25].

The prevalence rate of *H. pylori* is varied in different countries and the higher rate of infection is associated with low socioeconomic status [26]. The variations in susceptibility and resistance profile may be due

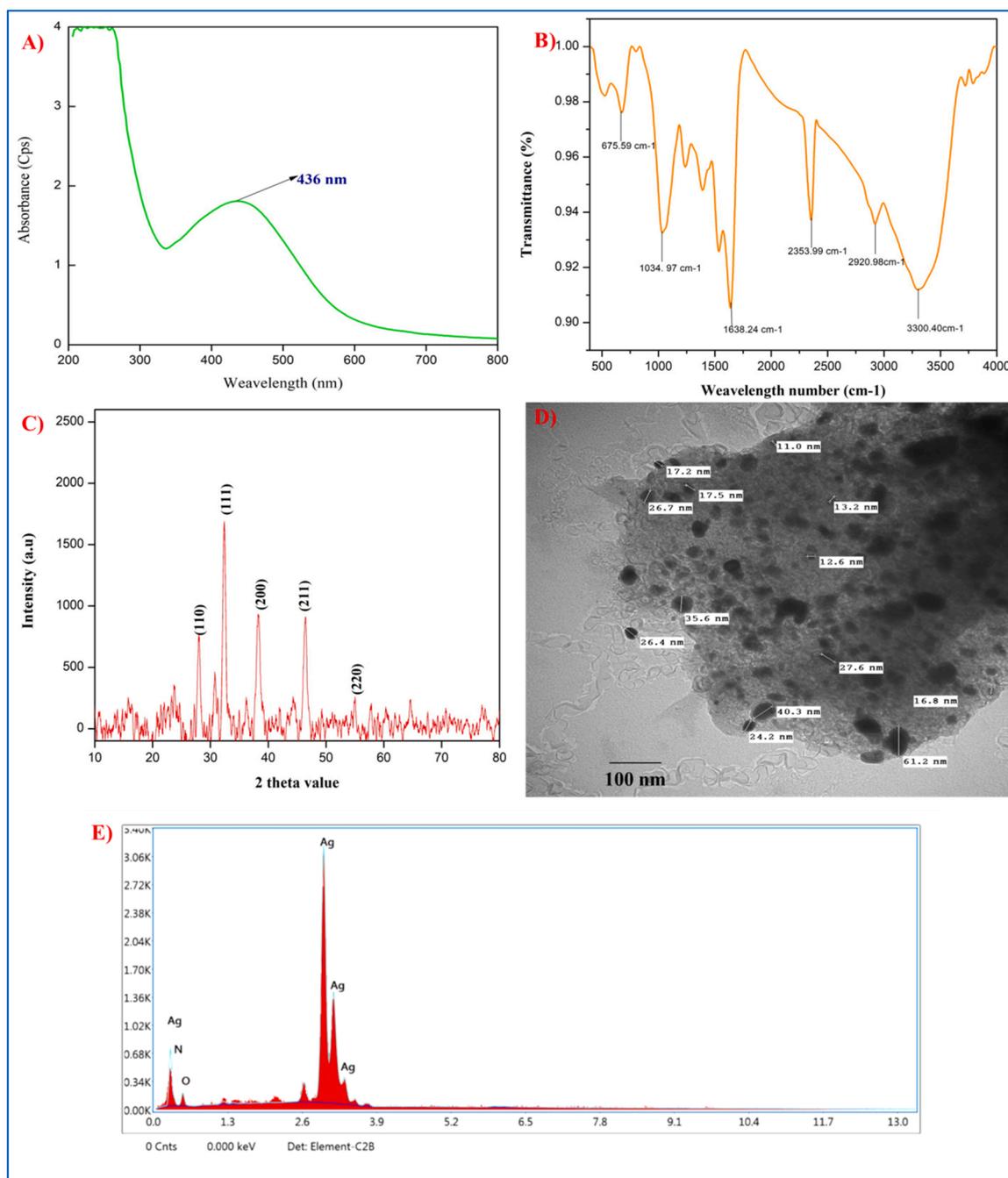


Fig. 5. Characterization of Ag₂O nanoparticles using *Digeria muricata* leaf extract A) UV-visible spectrophotometry B) FT-IR spectrometry C) XRD D) TEM E) EDAX analysis.

to the differences in local antibiotics used. The current investigation has provided evidence that was before inaccessible concerning the occurrence of *H. pylori* infection in central south-eastern region patients in India. It would be of attention to carry on the designated study on a larger group of patients and to assess in additional studies the molecular epidemiology of *H. pylori* infection in the general population. The intended work takes the first step in that way by if data on the molecular characteristics of strains that can assist physicians in taking the correct steps for patient care and treatment.

According to the World Health Organization, gastric cancer is one of the most common types of cancer, it is estimated at 76,9000 deaths in 2020 (W.H.O 2021) [27]. Current treatment options for *H. pylori* infection and gastric cancer such as different chemotherapeutic drugs. However, in recent days *H. pylori* infections resulted, in a major clinical

challenge and substantial encumbrance to individuals due to the resistance developed by bacteria against routinely used antibiotics and the scarcity of diagnosis and treatment options [28]. Therefore, in search of alternative medicines with superior effects, and low cost, no side effect treatment will need. Medicinal plants contain phytochemical compounds, it was helpful to fight against different diseases with minimum side effects (Bhatia et al.) [29]. Tripathi et al. explained the various therapeutic activities of *D. muricata* plant extract [30].

Many reported studies explained the nanoparticle synthesized from biological methods showed significant antimicrobial and anticancer properties [31–34]. Previously Pradeesh et al. reported the synthesis of Ag₂ONPs from *Cyathea nilgiriensis* Holttum plantextract [35]. In the present study, we synthesize the Ag₂ONPs from *Digeria muricata* aqueous leaf extract. Initially, the synthesis of Ag₂ONPs was confirmed by the

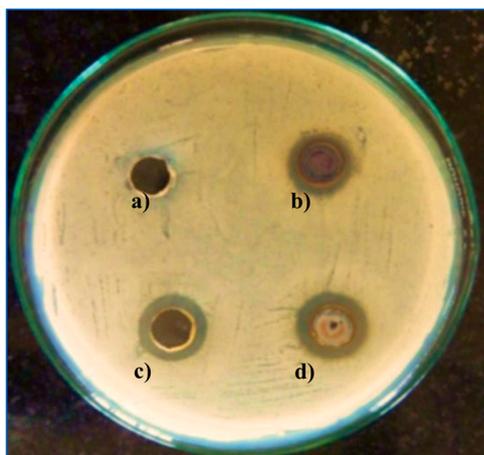


Fig. 6. Antimicrobial activity of synthesized nanoparticle against metronidazole resistance *H. pylori*, a) negative control b) 25 µg/mL c) 50 µg/mL d) 100 µg/mL.

color changing of the solution due to the surface plasmon excitation and surface plasmon resonance (SPR) [36,37]. The silver ions (Ag^+) are converted into Ag_2ONPs by the addition of plant extract, here the leaf extract act as a capping agent. The formation of Ag_2O was investigated by observing the UV-absorption spectra at 48 h however, after 24 h no responsive peak was observed. The results showed that the absorption peaks in the (438 nm) range of synthesized Ag_2ONPs (Fig. 5A) because of the excitation surface plasmon vibration particles, an increased absorbance was identified by increasing incubation time duration explains the enhancement of Ag_2ONPs formation. The XRD results indicated that the obtained Ag_2ONPs are crystalline with 32.28 nm in size these results were in good agreement with previous reports of *Rhamnus virgate* aqueous leaf extract 2θ values [38]. Fig. 5B FT-IR results of synthesized particles through *D. muricata* confirmed the capping of *D. muricata* extract which are like previously reported studies, 3300.40 cm^{-1} conforming to the OH, peak at 2920.98 cm^{-1} due to C-N and C-H stretching and 2353.99 cm^{-1} due to the flavonoids and carboxylic acid [21–23], which confirmed the flavonoids and carboxylic acids are the major responsible for Dm- Ag_2ONPs synthesis. Previously, Sarvanakumar et al. (2019) studied the synthesis of AgNPs using *Toxicodendron vernicifuum* plant bark extract and its anti-*H. pylori* activity [39].

5. Conclusion

In conclusion, this investigation reports on the first successful isolation of metronidazole resistance *H. pylori* from a southeastern region of India gastric infection patient. Further, the synthesis of Dm- Ag_2ONPs was confirmed by forming UV-spectrum at 428 nm, XRD reveals the Dm- Ag_2ONPs are crystalline in nature with 32.38 nm in size. It also describes the fact that *D. muricata* aqueous leaf extract mediated Ag_2ONPs showed antibacterial activity against metronidazole resistance clinical isolate of *H. pylori*. These findings suggest that Dm- Ag_2ONPs act as a good bactericidal agent against *H. pylori* and are presumed to be a promising therapeutic agent in gastric diseases.

Ethics approval and consent to participate

Applicable.

CRediT authorship contribution statement

Gattu Sampath Conceptualization, Methodology, Data curation, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, **Muthusamy Govarthanam**:

Methodology, Data curation, Validation, Formal analysis, Visualization, Writing – review & editing, **Sridharan K**: Methodology, Data curation, Validation, Formal analysis, Investigation, Visualization, Writing – review & editing, **Prabhusaran N**: Methodology, Data curation, Validation, Formal analysis, Investigation, Visualization, Writing – review & editing, **Neelamegam Rameshkumar**: Methodology, Data curation, Validation, Visualization, Writing – review & editing, **Muthukalingan Krishnan**: Methodology, Data curation, Validation, Visualization, Writing – review & editing, **Kayalvizhi Nagarajan**: Methodology, Data curation, Validation, Conceptualization, Investigation, Writing – original draft, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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