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Indian Journal of
Forensic Medicine and Pathology

January - March 2020
Volume 13 Number 1

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Review Article

Case Report

Patterns of Lung Lesions in Autopsy: A Histopathological Study

Anisha TS[1], Shashikala K[2], Ramya T[3], Sharmila PS[4]

Abstract

Introdution: Autopsy is recognized as a necessary part of medicine to establish final diagnosis, and relate the cause of death to the associated pathologies and the interaction between the two.

Aim: To study and highlight the histopathological changes in lungs seen in autopsy cases.

Methods: A cross-sectional study was done in the department of Pathology in a tertiary care hospital in Bangalore. A total of 100 lung specimens received from the department of forensic medicine for clinical autopsy over 3 years (January 2016 to January 2019) were studied. After noting the patient details and autopsy findings, the specimens were examined grossly and based on the gross findings, representative bits were given for histopathological examination. The histopathological findings were noted and results were statistically analyzed.

Results: Amongst the 100 lungs studied, the commonest lesion was Congestion and/ Pulmonary edema (64%), followed by non-tuberculous pneumonia (15%), chronic bronchitis (5%), emphysema (4%), tuberculous pneumonia (4%), autolytic changes (2%), pulmonary embolism (2%), lung abscess (1%) and an immature lung (1%).

Conclusion: This study highlights the various lesions in lungs from patients with history of varied causes of death and the lung lesions contributing directly or indirectly to the cause of death.

Keywords: Autopsy; Lung lesions; Histopathology

Introduction

Autopsy is examination of the body after death in order to determine the cause and manner of death as well as to evaluate any disease or injury that may be present. There are two main types of autopsies: forensic and clinical. The first one is performed in case of suspicious, violent or unknown case of death and the second one is performed by a pathologist in the hospital.[1] Clinical autopsy, loosely termed as pathological autopsy, is carried out to diagnose the disease which has caused the mortality when antemortem efforts have failed. Many a time clinical autopsy is done despite the cause of death having been established antemortem, to study the disease process in situ, thus enriching medical knowledge.[2]

The lungs are affected by various infectious, inflammatory, occupational and neoplastic conditions. Lungs are involved secondarily in almost all the terminal events.[3] Lung disorders have varied and complex presentations. As a result, despite availability of modern advanced diagnostic methods, diagnosis is often challenging task for clinicians.[4]

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Gross pathologic examination of autopsy lungs yields information regarding status of lung-collapsed or hyperinflated, presence of scarring, fibrosis, bullae, consolidation, nodules, infarction, secretions, edema, congestion, granuloma/abscess formation and also provides information regarding status of bronchi and pleura which may provide hint to the diagnosis.[5]

The aim of this study was to emphasize and study the histopathological alteration in lungs in autopsies of patients with varied causes of death.

Materials and Methods

This is a cross-sectional study done in the department of pathology in a tertiary care hospital in Bangalore for a period of 3 years (January 2016 to January 2019). The study was conducted on lung specimens of 100 autopsies where the specimen of lung was sent for pathological examination. Patient information regarding age, sex, brief history of illness, any medical/clinical findings, investigations done, and in situ postmortem findings were obtained from the request form. All specimens were adequately fixed in 10% formalin. Gross examination of lungs included size, weight, color, consistency and presence of any pathological findings were noted and sections from representative areas were taken. After processing and paraffin embedding, sections were cut and stained with Hematoxylin and eosin (H&E) stain according to standard procedure. Special stains were used whenever required. All the histological sections were examined microscopically, and findings were noted. Pathological findings were then correlated with the findings in other organs to know the systemic involvement.

Results

A total of 100 cases of autopsy lung were received from January 2016 to January 2019 and histopathological examination were done in all the cases. The age of the patients ranged from a preterm baby to 65 years, with majority of the cases being in the age group of 30 to 39 yr (26%) and the least were from ages 0 to 9 (3%). Cases with clinical histories of sudden death, suspiciously found dead at home/street, assault, road traffic accident, death due to underlying illness, suicide, electrocution, drowning, snake bite, etc. were sent for clinical autopsy to the department of pathology (Table 1).

Table 1: Ditribution of cases based on the clinical history

History	Percentage (%)
Sudden death	30
Found dead at home/street	27
Due to underlying illness	11
Road traffic accident	10
Assault	09
Suicide	08
Drowning	02
Electrocution	01
Snakebite	01
Stillborn baby	01

A wide spectrum of microscopic findings were seen which included congestion and oedema, non tuberculous pneumonia, tuberculous pneumonia, emphysematous changes, chronic bronchitis, immature lung tissue, lung abscess, autolyzed lung and lungs with no specific pathology. Congestion and edema (Fig. 1) were the maximum with 64 cases (64%) followed by non-tuberculous pneumonia with 15 cases (15%) (Fig. 2), chronic bronchitis 5 cases (5%) (Fig. 3), tuberculous pneumonia 4 cases (4%) (Figs. 4, 5), emphysematous changes in 4 cases (4%) (Fig. 6), lung abscess in one case (1%) and pulmonary embolism Fig. 7, autolytic changes and no specific pathology in two cases each (2%) (Table 2).

Table 2: Distribution of cases based on histopathology diagnosis

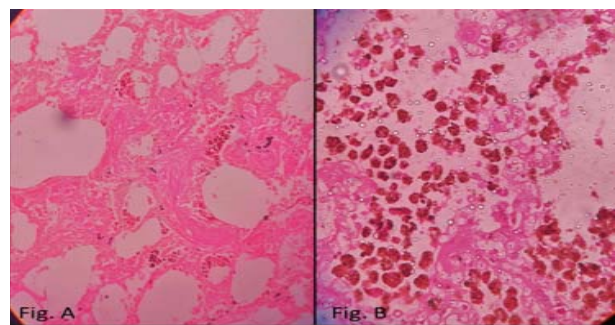| Histopathology Diagnosis	Percentage (%)
Chronic venous congestion/pulmonary edema	64
Non-tuberculous pneumonia	15
Chronic bronchitis	5
Tuberculous pneumonia	4
Emphysema	4
Pulmonary embolism	2
Autolytic changes	2
No pathology/unremarkable	2
Lung abscess	1
Immature lung	1

Fig. 1: Photomicrographs show lung with features of congestion and presence of heart failure cells (hemosiderin laden macrophages) within the alveoli. Fig. A [H&E, 100X], Fig. B [H&E, 400X]
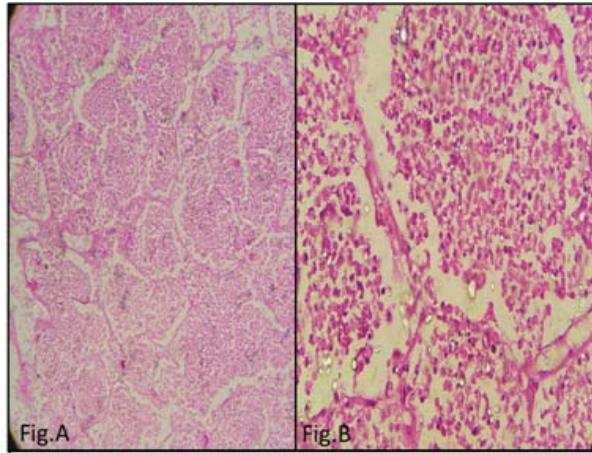
Fig. 2: Photomicrographs show lung with features of lobar pneumonia. Fig. A [H&E, 100X], Fig. B [H&E, 400X]

Fig. 5: Shows gross picture of lung with the presence of multiple grey white nodules in the lower lobe (arrow), from a patient who was a known case of pulmonary tuberculosis
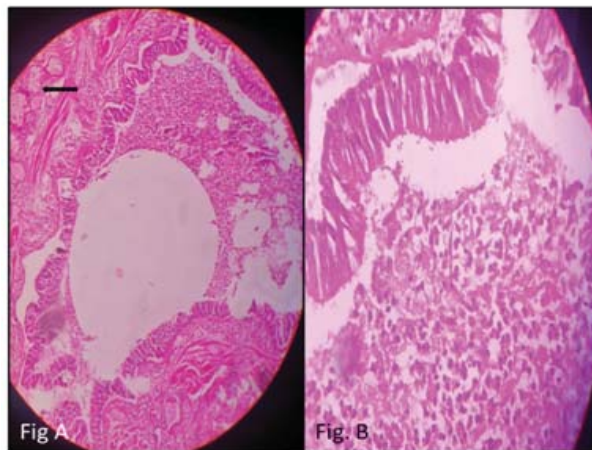
Fig. 3: Photomicrographs show features of chronic bronchitis. (A & B) Bronchi with increased mucous glands (arrow) and presence of chronic inflammatory infiltrate within the bronchi. Fig. A [H&E, 100X], Fig. B [H&E, 400X]
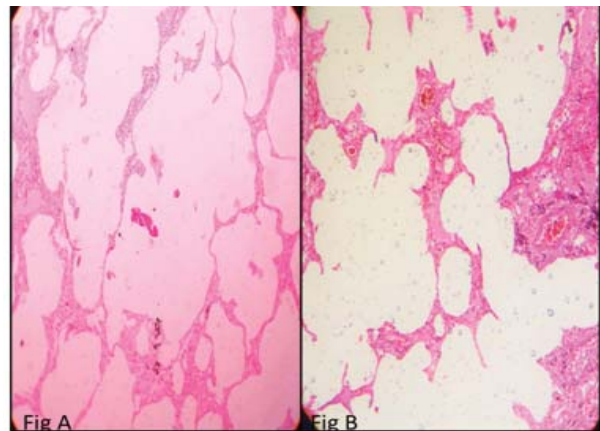
Fig. 6: (A & B) Photomicrographs show lung with emphysematous changes [H&E, 100X]
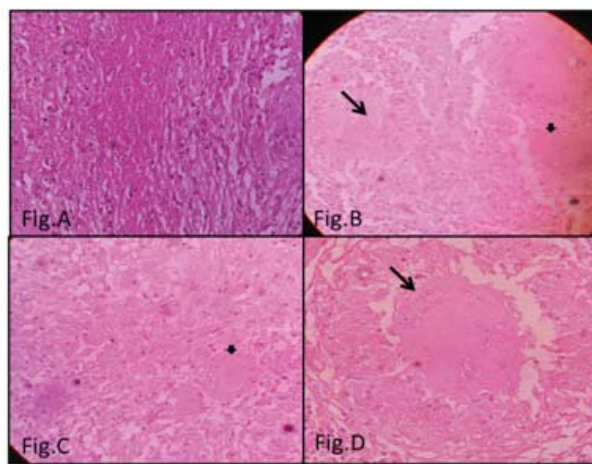
Fig. 4: (A) Photomicrograph show caseous necrosis [H&E, 100X], (B & C) Photomicrographs show presence of granulomas with epithelioid cells (large arrow) and Langhans giant cell (arrow head) [H&E, 100X], (D) Photomicrograph show a well formed granuloma with epithelioid cells and central necrosis (arrow) [H&E, 400X]
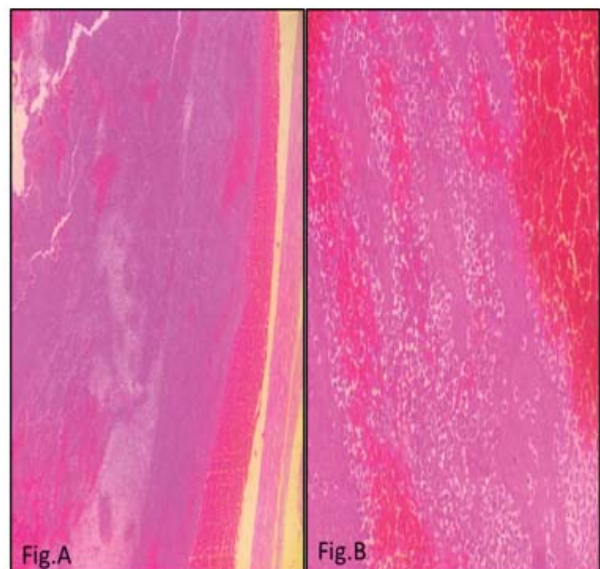
Fig. 7: Photomicrograph of pulmonary emboli. (A) shows a pulmonary vessel with an embolus [H&E, 100X], (B) shows lines of zahn [H&E, 400X].

Table 3: Age wise distribution of cases

Lesions	0–9 yr	10–19 yr	20–29 yr	30–39 yr	40–49 yr	50–59 yr	60–69 yr	>70 yr
CVC	2%	4%	16%	18%	13%	9%	2%	–
Emphysema	–	–	–	1%	1%	1%	1%	–
Non-TB Pneumonia	–	2%	2%	4%	3%	2%	2%	–
TB pneumonia	–	–	–	1%	2%	–	1%	–
Chronic Bronchitis	–	–	3%	–	1%	–	1%	–
Lung Abscess	–	–	–	1%	–	–	–	–
Pulmonary embolism	–	–	–	–	–	–	2%	–
Autolytic change	–	–	–	–	–	2 %	–	–
No specific pathology	–	–	1%	–	–	–	1%	–
Immature lung	1%	–	–	–	–	–	–	–

Majority of the cases of congestion and edema, non tuberculous pneumonia and lung abscess were of the age group 30 to 39 years, emphysematous changes were seen in age groups ranging from 30 to 60 years, tubercular lesions were seen more in the age group of 40 to 49 years while both cases of pulmonary embolism were of the age group of 60 to 69 years. We had one perinatal autopsy with histopathological features suggestive of an immature lung (Table 3).

Amongst these 100 cases, majority were male patients (81%) and female patients were 19% (which included a female preterm perinatal autopsy) (Table 4) and most of the female patients came with the history of sudden death.

Table 4: Sex wise distribution of cases.

Histopathology Diagnosis	Male	Female
Chronic venous congestion/pulmonary edema	56%	09%
Non-tuberculous pneumonia	09%	06%
Tuberculous pneumonia	05%	Nil
Chronic bronchitis	03%	02%
Emphysema	04%	Nil
Autolytic changes	02%	Nil
No pathology/unremarkable	01%	01%
Lung abscess	01%	Nil
Immature lung	Nil	01%

Discussion

In a medicolegal autopsy, the histopathological examination is done to establish the cause of death if any morbid anatomical change in the tissue is observed.

This study was done on the clinical autopsy specimens of lungs received in the department of pathology from the department of forensic medicine after forensic autopsy.

Of the 100 specimens of lungs received over a three-year period (January 2016 to January 2019), majority of the specimens were from the patients with a clinical history of sudden death (28%) and suspicious death, found dead at home/street (25%).

Histopathological examination of the specimens showed that, of the 100 lung specimens, 64% showed features of congestion and/pulmonary edema. Our study was in concordance with the study conducted by Pulak Chakma et al.[6], Hanmante et al.[7], Selvam et al.[8] and Mangal et al.[9] where pulmonary edema and/ congestion were seen in 62.91%, 21.7%, 31.5% and 76.26% respectively. In our study, the incidence of pulmonary edema and congestion were more in cases with histories of sudden death, found dead at home/street, assault and road traffic accident.

In the present study, non-tuberculous pneumonia was the second most common lung lesion seen with 15% of the total number of cases. Our study was comparable to the study done by Chauhan et al.[10], Rupali et al.[11] and Selvam et al.[8] with their total non tuberculous pneumonia cases being 14.62%, 19.16% and 10.2% respectively. In our study males were more commonly affected by pneumonia compared to females, which was similar to the study conducted by Kurawar et al.[12], Chauhan et al.[10] and Bal et al.[13]

Tuberculous pneumonia was seen in 4% of the total number of cases in our study, which was comparable to the study done by Mangal et al.[9] (4.08%), Chauhan et al.[10] (6.26%), Selvam et al.[8] (2.8%) and Kurawar et al.[12] Of the 11 cases which came with a history of underlying illness, four has respiratory symptoms of cough and difficulty in breathing and one was a known case of pulmonary tuberculosis on treatment.

Emphysematous changes were noted in 4% of the total number of cases and chronic bronchitis in 5% of the cases, whereas the incidence of emphysema

was higher in the study conducted by Selvam et al.[8] at 50% and Chauhan et al.[10] at 7.06%.

In our study, autolytic changes were seen in only 2% of the cases, whereas the study conducted by Bal et al.[13] showed a higher incidence of autolytic changes. Our study was comparable to Pratima et al.[14] where they had a lower number of autolytic changes. The two cases with autolytic changes in our study were brought for autopsy days after their death, vowing to the autolytic changes and also the lesser number in our entire study vowing to facility of forensic autopsy being available in our hospital.

We had two cases of pulmonary emboli in our study, and both the cases came with a history of sudden death and belonged to the age group of 60–69 years. One of the case had a triple vessel disease of heart and the other came with a history of road traffic accident.

Most of the cases which came with history of sudden death, suspicious death, assault, road traffic accident, electrocution and drowning showed pulmonary edema and/congestion of lung on histopathology, incidental finding of pneumonia were seen in 9% of the cases which came with a history of sudden death and suspicious death.

Conclusion

Autopsy has remained an important complimentary tool for identifying and understanding respiratory diseases. It also serves as a reassuring and educative tool in identifying and establishing the underlying cause of death. In our study, pulmonary edema and/congestion was the highest incidence of all the lung lesions contributing directly or indirectly to the cause of death.

Source(s) of support: Nil

Presentation at a meeting: Nil

Conflicting Interest: None

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Profile of Cases Brought to the Forensic Medicine Department for Age Estimation Under the POCSO Act, 2012

Ropmay AD[1], Patowary AJ[2], Slong D[3], Bhattacharyya H[4]

Abstract

Background: The Protection of Children from Sexual Offences (POCSO) Act was enacted in 2012 with a view to curbing the menace of sexual assault, sexual harassment and pornography on children. The present study gives a profile of cases registered under the Act and subsequently brought to the Forensic Medicine Department for the purpose of medicolegal age estimation.

Material and methods: Research was conducted in the Department of Forensic Medicine, NEIGRIHMS, Shillong. Data was extracted from records maintained in the office and analyzed using statistical software SPSS version 11 by descriptive analysis. All cases brought for age estimation under the POCSO Act, 2012 for the period May 2013 to June 2018 were included.

Results: Medical examinations were performed in compliance to police requisitions from four districts of the state of Meghalaya. A total of 26 (twenty-six) individuals were brought for age estimation during the study period. Among those examined, 81% were victims and 19% accused in sexual offences. The majority of victims were female children in the 6–15 age group.

Conclusion: These findings should draw the attention of doctors and healthcare workers to the problem of sexual crime against children and prompt them to work together with law enforcement and social organizations in securing justice for the vulnerable.

Keywords: POCSO Act; Sexual offences; Forensic medicine department; Age estimation

Introduction

Children are the building blocks of the nation and it is our duty as citizens to help them realize their dreams of a bright and hopeful future. A child is legally defined as any person below the age of eighteen. It is disheartening to note that in recent years we have witnessed so much exploitation and abuse of the most vulnerable members of our society. The Government of India, in its commitment to securing the best interests of children in the midst of escalating injustice against them, took the initiative of introducing a law in this regard. Thus, the protection of children from sexual offences (POCSO) Act was passed in Parliament, received the assent of the President on June 19th and was published in the Official Gazette on June 20th 2012.[1] It has since been implemented in letter and spirit in the state of Meghalaya in northeast India.

The current paper presents a profile of cases registered under the Act and subsequently brought to the forensic medicine department of our institution for the purpose of age estimation from May 2013 to June 2018. Our findings gave us an idea about the caseload and magnitude of the

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problem as a whole which would then enable us to improve our services by strengthening liaison with law enforcement and local government in dealing with forensic issues.

Materials and Methods

The present study is a retrospective observational one conducted in the Department of Forensic Medicine, NEIGRIHMS, Shillong. All cases which were brought for age estimation under the POCSO Act, 2012 for the period May 2013 to June 2018 were included in the study. The cases which were registered under sections of the Indian Penal Code (IPC) alone were excluded from the same. The parameters studied were sociodemographic characteristics, and possible correlation between stated age and estimated age of persons examined.

Data analysis

Data was extracted from records maintained in the department, entered in Microsoft Office Excel 2007 sheet and analyzed using statistical software SPSS version 11 by descriptive analysis. Pearson's correlation coefficient test was applied to find out if there is any relationship between the stated age and the estimated age.

Ethical considerations

Relevant data was collected and stored in confidentiality with the principal investigator. Anonymity of cases was strictly maintained during the course of the study. Approval for the project was obtained from the Institutional Ethics Committee (IEC) on 11th June, 2018.

Results

Medical examination for the purpose of age estimation was conducted in compliance to police requisitions from four districts of the state of Meghalaya, i.e. East Khasi Hills, West Khasi Hills, Ri Bhoi and West Jaiñtia Hills. Altogether, 26 individuals were brought to the Forensic Medicine Department under the Protection of Children from Sexual Offences (POCSO) Act 2012 during the study period out of which 21 were victims and 5 accused. All the accused were males (Table 1).

Among the victims, 20 were females and 1 was a male child. The majority (66.6%) of victims examined were in the 6–15 age group (Table 2). We observed that 62% of sexual assaults occurred in rural areas of the state. We also found that 67% of victims hailed from villages in and around Meghalaya. The value of Pearson's correlation coefficient was calculated to determine the correlation between stated age and estimated age ($R^2 = 0.9$). This indicates that there is a strongly positive association between stated age and estimated age in our study (Fig. 1).

The detailed results of the study are demonstrated in Tables 1 and 2 and Figure 1.

Table 1: Characteristics of persons examined

Criminal profile	Number	Percentage (%)
Victim	21	81
Accused	5	19
Total	26	100
Gender profile		
Male	6	23
Female	20	77
Total	26	100
Age profile		
≤ 5 years	1	4
6–8 years	7	27
12–15 years	8	31
16–18 years	10	38
Total	26	100

Table 2: Characteristics of victims examined

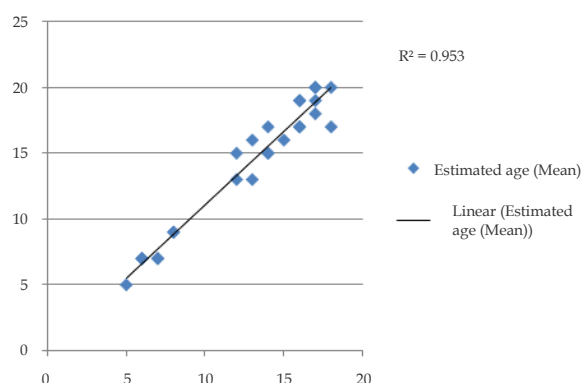| Gender profile	Number	Percentage (%)
Male	1	4.8
Female	20	95.2
Total	21	100
Age profile		
≤ 5 years	1	4.8
6–8 years	7	33.3
12–15 years	7	33.3
16–18 years	6	28.6
Total	21	100
Origin		
Rural	14	67
Urban	7	33
Total	21	100

Fig. 1: Correlation between stated age and estimated age (Mean)

Discussion

The Department of Forensic Medicine at NEIGRIHMS started taking up cases of age estimation from the year 2012 concurrently about the same time the POCSO Act was enacted. Medical age estimation from doctors is necessary in cases where documents or certificates are either missing or found to be fabricated or manipulated.[2] According to Indian law, even consensual sexual intercourse amounts to an offence if the woman in question is less than 18 years of age.[3] Hence, when minor adolescent girls involved in romantic sexual relationships with their boyfriends are brought for medical examination, they tend to claim to be older than they actually are. It was observed that perpetrators were more likely to lower their age in attempts to pass off as juveniles to avoid being tried as adults for the same crime. The occurrence of sexual assaults in rural areas could be due to lack of privacy and long absences of working parents or guardians from home leaving children exposed, unsupervised and vulnerable to unwanted invasion of their personal space. The victims native to rural areas were probably unsure about their age for want of documentation. Again, it is seen in this research that victims hailing from villages are susceptible to exploitation especially when they reside in towns and cities away from their place of origin.

A study on the working of Special Courts under the POCSO Act, 2012 in Assam shows that the majority of victims (40%) were in the age group 12–15 years which is consistent with our findings. In 50.58% of cases, age was determined by way of medical examination that included physical, dental, and secondary sexual characteristics.[4] Similarly, Yadukul S et al. found that the 13–18 age group constituted 91.4% of cases booked under the POCSO Act.[5] Research conducted by Kulkarni KV et al. reveal that the maximum incidence in female victims is 11–14 years with a minimum age of 2 years and maximum age of 17 years.[6] On the other hand, the research findings of a study done in Bangalore are not in agreement with ours in that 68.5% of female victims (n = 35) were in the older age group of 15–20 years.[7] A golden rule to medical professionals working with children is to report all reasonable degree of suspicion in child sexual abuse to the legal authorities.[8] The Government of India has issued specific guidelines for responding to children, both boys and girls, facing sexual abuse as its prevalence in the country is known to be high.[9] According to Dr. Uwom O. Eze of Nigeria, sexual assault is also not discriminatory to sex but studies have shown that the number of female victims is far greater than males.[10] In contrast, the findings of a study by Elgendy IS et al. in Cairo, Egypt demonstrated that most of the victims (71.8%) were males.[11]

In our center, bone age is estimated by referring to Galstaun's chart for Indian subjects.[12] However, it is important to appreciate that skeletal and chronological ages are not the same measurement of time since birth, and depending on the analytical approaches applied, there will be an inherent source of variation between estimated (biological) age and actual (legal) age.[13] Mughal AM et al. observed a strong positive correlation between chronological age and bone age in both the genders.[14] Similarly, a study conducted at Manipal among children aged 9–14 years showed a statistically significant (p < 0.01) correlation between dental age, skeletal age and chronological age.[15] In our research, we made an attempt to compare the stated age with the estimated age using Pearson's correlation coefficient. It is to be noted that the age as stated by the examined individual is not necessarily the actual age and this is especially so in the absence of valid supporting documents. Nevertheless, we did find a positive correlation between the two variables (Fig. 1).

The Protection of Children from Sexual Offences (POCSO) Act, 2012 has provisions to ensure that hospital and courtroom procedures are child-friendly and carried out in a congenial environment in the best interests of survivors. According to Phad LG et al., not only medical examination but also counseling of the survivor by a social worker and psychologist is most important and should be made mandatory in all cases of childhood sexual abuse.[16]

Conclusion

It can be concluded that most child survivors of sexual offences are girls in the age group of 6–15 years while perpetrators tend to be older male children or adults. These crimes occur more in rural areas and in children of rural origin. The Forensic Medicine Department has a role in medical examination and age estimation of both the accused and victims of an offence. As doctors and healthcare workers, we need to be aware of the growing incidence of this problem in the minor population and work closely with law enforcement and social organizations to strengthen our resources and strive towards a common goal of securing health and justice for the vulnerable.

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Histopathological Spectrum of Ovarian Tumors: A 3-Year Retrospective Study in a Tertiary Care Centre in Southern India

Anugnya P Ranjoalkar[1], Swati Sharma[2], Manna Valiathan[3], Kanthilatha Pai[4], Muralidhar Pai[5], Shankar M Bakkannavar[6]

Abstract

Introduction: Ovarian tumors are a group of diverse neoplasms with a varied clinical, morphological and histological feature. The varied anatomy, histogenesis and its peculiar physiology including the cyclical changes from puberty to menopause give rise to number of cell types, each of which may give rise to tumors. *Materials and methods:* A 3-year retrospective study of histologically proven ovarian neoplasms where the tumors were classified according to World Health Organization (WHO) 2014 classification and their clinical and histopathologic parameters were analyzed. *Results:* Of all 138 ovarian tumors studied, 94 (68.12%) were benign, 13 (9.42%) borderline and 31 (22.5%) were malignant in nature. Benign tumors chiefly presented with abdominal pain with median age of 39. Mature cystic teratoma was found to be the most common benign tumor. Borderline tumors presented at a median age of 37. Borderline serous and mucinous tumors (30.76%) were the most common borderline tumors. Malignant tumors presented frequently with abdominal mass and at median age of 48. According to WHO classification of tumors based on cell of origin, surface epithelial tumor were the most common ovarian neoplasms, accounting for 63.04% cases, followed by germ cell tumor (24%) and sex-cord stromal tumors (8.7%). *Conclusion:* Surface epithelial tumors were the most common histopathological subtype of ovarian tumors. Benign and borderline tumors were predominantly found in reproductive age, whereas malignant tumors were seen in perimenopausal and postmenopausal women. Since the prognosis, therapeutic strategies including multidisciplinary approach depend primarily on the histopathologic diagnosis, an accurate pathological evaluation and classification is of prime importance. The multidisciplinary approach employed has its own medico legal implications.

Keywords: Neoplasm; Epithelial Cancer; Ovarian

Introduction

Ovarian cancer is second most common genital tract malignancy accounting for 3% of total cancer in females[1] and 25% of all gynecological malignancies.[2,3] Ovarian tumors often go unnoticed or the patients present with nonspecific symptoms and present at advanced stage[1,4,5,6] with an overall survival rate of 30–40%.[7] Due to absence of early screening modalities, unknown precursor lesions and no specific clinical features, ovarian tumors are often missed.[8–10] These tumors are diverse with low and high grade subtypes and widely divergent clinicopathologic features which develop independently along different molecular pathways.[11] Histogenesis of ovarian tumors includes a wide spectrum of neoplasm depending upon the origin of cell, i.e. tumor arising from epithelium, germ cell, sex cord stromal and connective tissue.[12,13] Decreased risk is associated with increased parity, oral contraceptive pills and history of hysterectomy or tubal ligation.[7] Since ovarian tumors cannot

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be clearly differentiated from one another just on the basis of their clinical, radiological or gross characteristics, there is definitely a requirement to consider and study their histopathological pattern. This may help the clinician to decide appropriate treatment modality. The aims and objectives of this study are to study the prevalence and demographic characteristics of ovarian neoplasms, to classify the ovarian neoplasms according to WHO 2014 classification and to analyze the histomorphological spectrum of benign, borderline and malignant ovarian neoplasms.

Materials and Methods

This is a 3-year retrospective study of histologically proven ovarian neoplasms diagnosed at Department of Pathology, Kasturba Medical College, Manipal, India from January 2015 to December 2017. This study has been approved by Institutional Ethical committee (No: 90-2019) and informed consent has been obtained from the cases pertaining to this study. The tumors were classified according to the WHO classification of Ovarian tumors 2014.[14] The clinical data collected from Medical Records Department consisted of information about age and clinical presentation of the patient. Histopathological analysis including macroscopy and microscopy along with ancillary studies like immunohistochemistry (IHC) for cases wherever available were retrieved from the pathology database. *Inclusion criteria:* Resected specimens of histologically proven benign, borderline, malignant tumors of ovary. *Exclusion criteria:* Trucut/ non-

resected biopsy specimens, non-neoplastic lesions of ovary, cases where clinical details could not be retrieved and cases of which H & E slides/ blocks were not available.

Results

One hundred and thirty-eight cases of ovarian tumors were studied retrospectively from January 2015 to December 2017.

Out of 138 cases, 94 (68.12%) were benign, 13 (9.42%) were borderline, 31 (22.5%) were malignant in nature (Fig. 1).
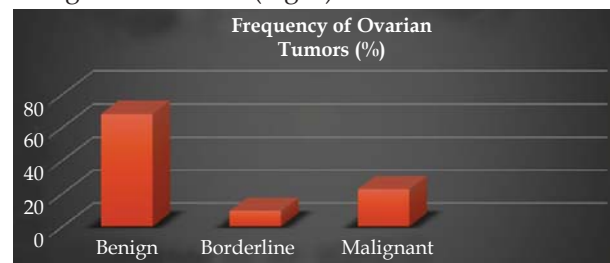
Fig. 1: Frequency of benign, borderline and malignant tumors (%)

The most common clinical presentation in benign tumors was pain in abdomen (58%) followed by other symptoms like urinary urgency and vomiting (18%). Patients with malignant tumors presented with mass per abdomen (40%) as the most common symptom followed by abdominal pain (24%) and others (24%) including menstrual irregularities and increased urinary frequency due to pressure symptoms (Figs. 2 & 3).
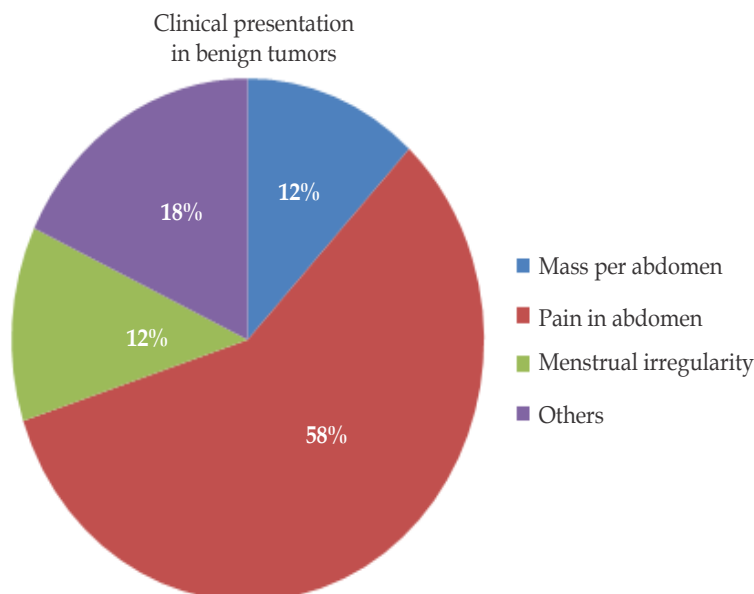
Fig. 2: Clinical presentation in benign cases.

Majority of the tumors were in the age group 31–40 years (26.4%) and 41–50 years (26.08%) (Fig 3). Most of the benign tumors were falling in the age group 31–40 years (27.7%) followed by 41–50 years (23.4%). Borderline tumors mostly occurred in younger age group 21–30 years (38.5%) (Table 1). Majority of the malignant tumors were found to be in perimenopausal and postmenopausal age group i.e 41–50 (32.3%) followed by 51–60 (29%) (Table 1). The youngest case in our study was 1-year-old

child with unilateral mature cystic teratoma and the oldest case was unilateral high grade endometrioid carcinoma seen in 79-year-old female.

In the present study, nearly 87.2% of benign tumors were unilateral, however 12.8% were found to be bilateral. Borderline tumors also had predominantly unilateral presentation as seen in 77% cases whereas malignant tumors presented with nearly equal number of unilateral and bilateral cases (Table 2).

Table 1: Distribution of tumors in different age group

Age distribution (years)	Benign (%)	Borderline (%)	Malignant (%)
<= 20	5 (5.6)	—	1 (3.2)
21–30	14 (14.9)	5 (38.5)	1 (3.2)
31–40	27 (27.7)	3 (23)	6 (19.4)
41–50	22 (23.4)	3 (23)	10 (32.3)
51–60	14 (14.9)	1 (7.7)	9 (29)
61–70	9 (9.6)	1 (7.7)	3 (9.7)
>= 71	3 (3.2)	—	1 (3.2)

Clinical presentation
in malignant tumors
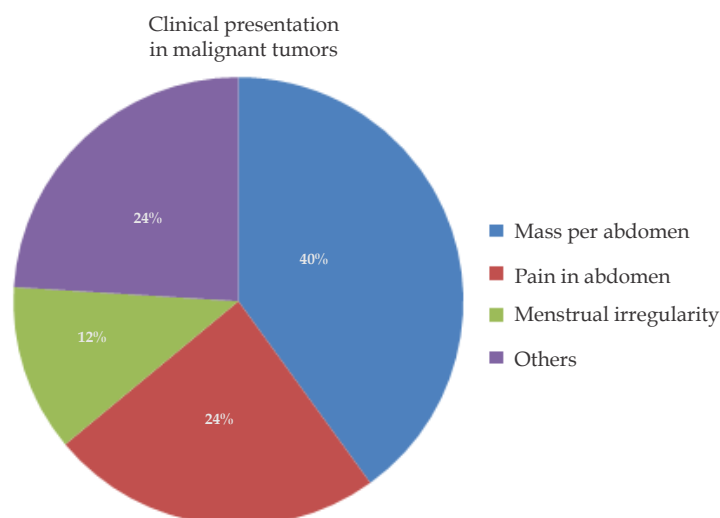
Fig. 3: Clinical presentation in malignant cases.
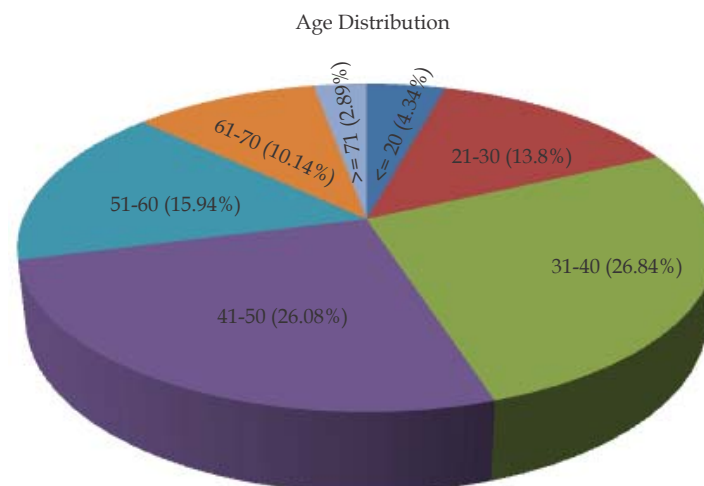
Age Distribution

Fig. 4: Distribution of the tumors in different age groups.

Fig. 5: Gross—Serous cystadenoma
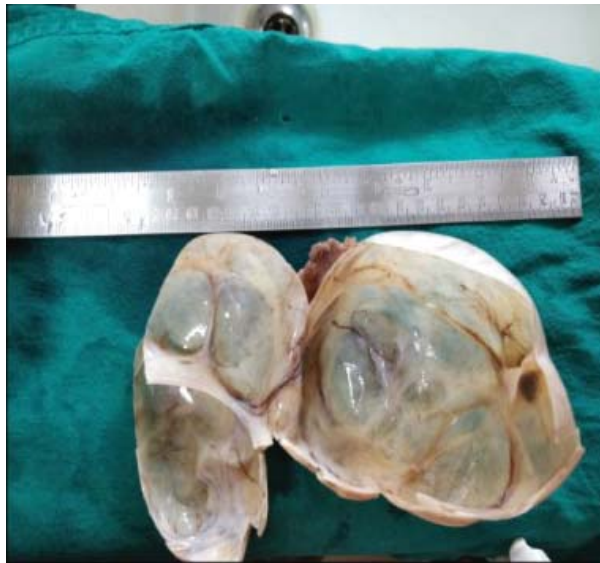
Fig. 6: Gross—Serous cystadenoma, c/s shows uniloculated cyst filled with serous fluid
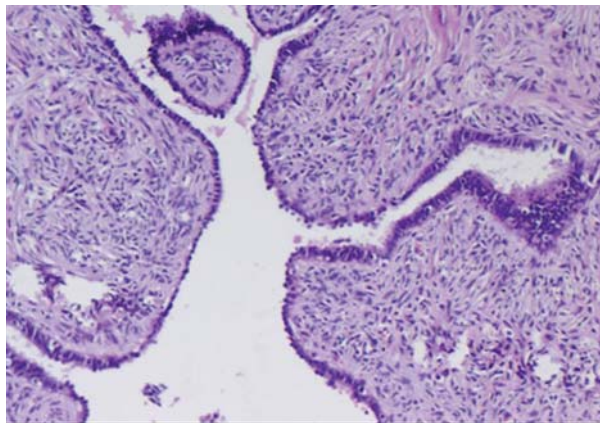
Fig. 7: Microscopy—Serous cystadenoma with cyst lined by benign cuboidal to columnar, focally ciliated epithelium H&E (400X)

Fig. 8: Gross—benign mucinous tumor, multiloculated cysts filled with mucinous material
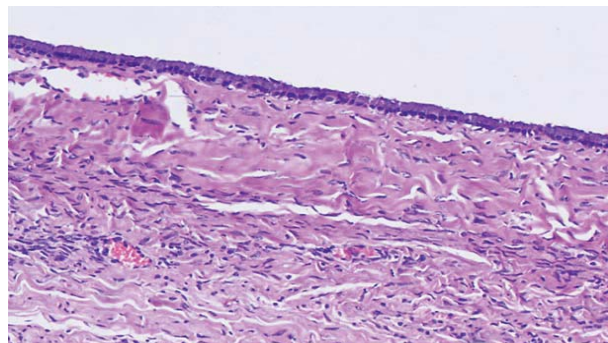
Fig. 9: Microscopy—Mucinous cystadenoma, cyst wall lined by tall columnar mucinous epithelium H&E (400X)
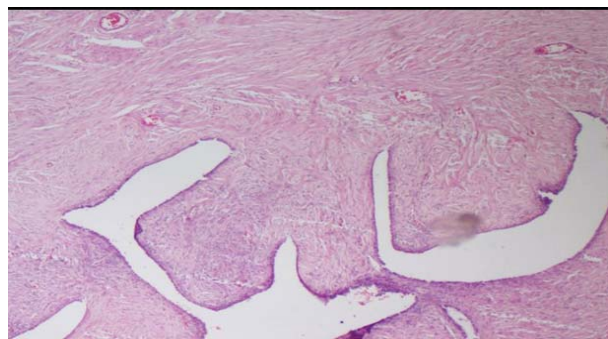
Fig. 10: Microscopy—serous cystadenofibroma, serous epithelium overlying fibrous ovarian stroma H&E (100X)
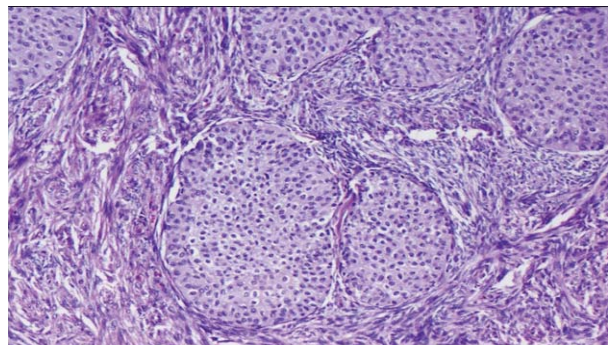
Fig 11: Microscopy—Brenner tumor, Oval to irregular nests of transitional type cells within fibromatous stroma H&E (100X)

Fig. 12: Gross—Borderline mucinous tumor, cystic tumor with cut surface showing multiloculated cyst with mucinous material and focal papillary excrescences
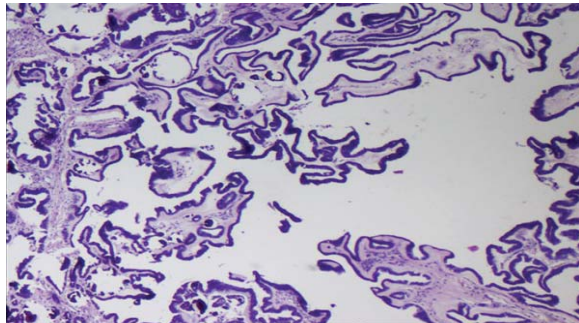
Fig. 13: Microscopy—Serous borderline tumor, branching architecture lined by cuboidal to columnar epithelium with minimal or no atypia H&E (100X)
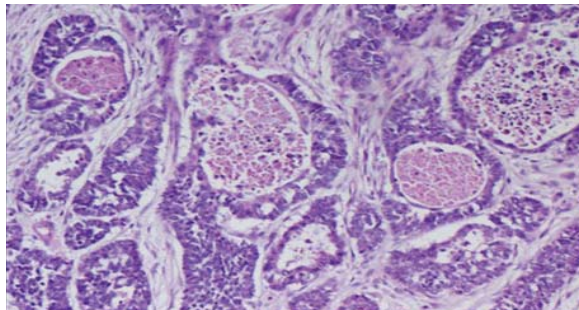
Fig. 14: Microscopy—Borderline endometrioid tumor, crowded glands lined by endometroid epithelium with mild to moderate cytological atypia H&E(400X)

Fig. 15: Gross—Bilateral high-grade serous tumor, hysterectomy with bilateral salpingo-oophorectomy
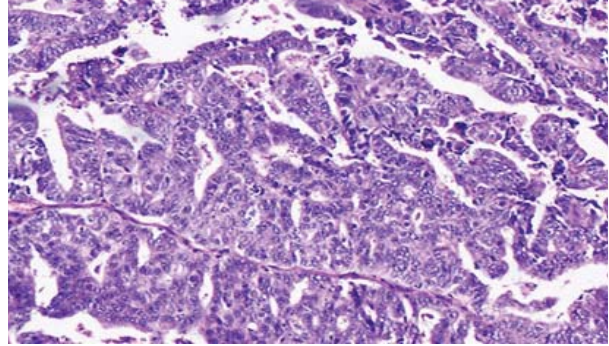
Fig. 16: Microscopy—Serous cystadenocarcinoma, confluent glandular growth with back to back arrangement and loss of intervening stroma H&E (100 X)
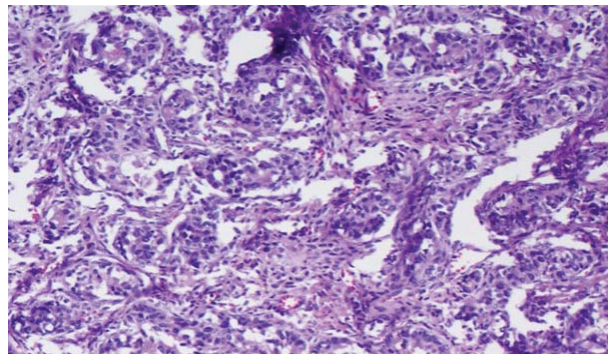
Fig. 17: Microscopy—Mucinous cystadenocarcinoma, infiltrative mucinous tumor invading ovarian stroma in small nests and single cells H&E (100X)
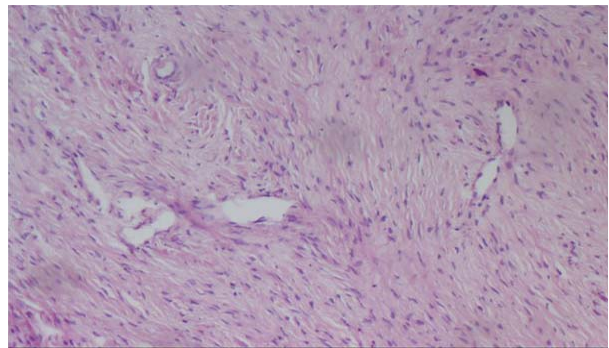
Fig. 18: Microscopy—Fibroma ovary, spindle cells with bland nuclei and scant cytoplasm arranged in intersecting bundles admixed with collagen H&E (100X)
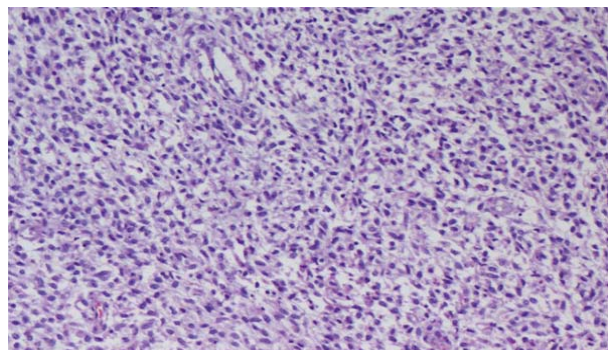
Fig. 19: Microscopy—Luteinized thecoma, spindle cells and weakly luteinized cells H&E (400X)

Fig. 20: Gross—Adult granulosa cell tumor, enlarged ovarian mass with cut section showing haemorrhagic and solid areas

Fig. 24: Microscopy—Immature teratoma, cystic areas and stroma showing immature neural tissue H&E (100X)
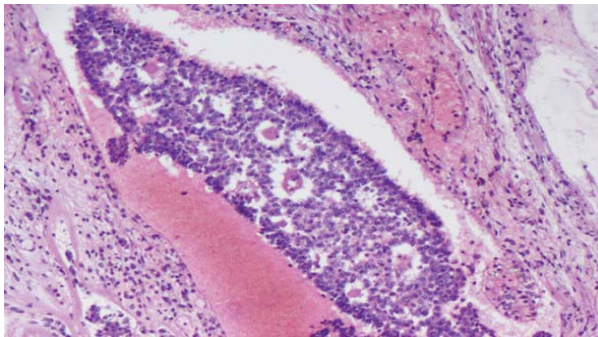
Fig. 21: Microscopy—Adult granulosa cell tumor, Call-Exner bodies- granulosa cells surround small spaces containing eosinophilic secretion H&E (400X)

Fig. 25: Microscopy—Ovarian leiomyoma: well circumscribed tumor showing intersecting fascicles of smooth muscle fibers H&E (100X)
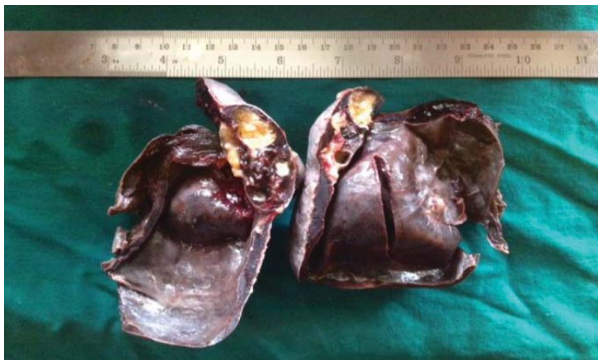
Fig. 22: Gross—Teratoma, Enlarged ovary with multiloculated cysts, c/s shows one cyst with luminal pultaceous and mucoid material

Fig. 26: Gross—Bilateral Krukenberg's tumor, hysterectomy with bilateral salpingo-oophorectomy, C/S of both the ovaries showing solid areas, cystic areas and hemorrhagic areas

Fig. 23: Microscopy—Mature cystic teratoma, cyst wall lined by epithelium overlying epithelial components H&E (100X)
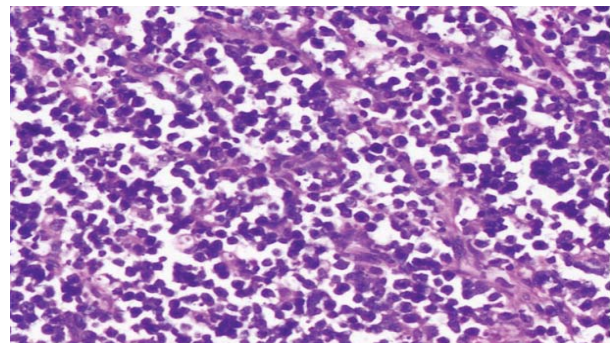
Fig. 27: Microscopy—Primary non- Hodgkin's Lymphoma, diffuse sheets of small to medium sized monomorphic lymphoid population H&E (400 X)

Largest dimension of the tumor was taken in account to categorize and to study the various size distribution of these tumors in benign, borderline and malignant tumors. Majority of the benign tumors were (39.78%) were falling into a size range of 5–9 cm (largest dimension) followed by a size range of 10–19 cm accounting for (26.9%). Borderline tumors had a size range of 5–19 cm, and among malignant tumors, maximum cases had a size rage of 10–19 cm in largest dimension (38.23%) (Table 3).

All the tumors were analyzed macroscopically based on the cystic or solid consistency on cut section with additional features like papillary excrescences if any. Majority of the benign tumors were cystic accounting for 58 cases followed by 29 cases with solid and cystic consistency. 5 cases had additional papillary excrescences. Majority of the borderline tumors had both solid and cystic component. Among the malignant tumors, 3 cases had predominantly solid and cystic morphology each, 18 had both solid and cystic components and 7 cases showed additional papillary excrescences (Table 4),

WHO has reclassified ovarian tumors in the consensus meeting in Lyon, where the borderline tumors were considered as a separate entity. This is because of the variable behavior of these tumors mimicking low grade serous tumor with nodal involvement or may be associated with malignant counterpart.[3] In our study, when classified according to WHO 2014 it was found that, surface epithelial tumor was the commonest tumor, accounting for 87 cases, followed by Germ cell tumor 33 cases and sex-cord stromal tumors being 12 cases. Others 6 cases included ovarian leiomyoma, bilateral Krukenberg tumor, metastatic mucinous adenocarcinoma of colon, neuroendocrine tumor and Non-Hodgkin lymphoma.

Among surface epithelial tumors, most common benign, borderline and malignant tumors were serous cystadenoma, borderline serous tumors and serous cystadenocarcinoma respectively (Table 5). Mature cystic teratoma was overall the most common benign ovarian neoplasm. It was also the most common germ cell tumor. Among secondary tumors, there were 3 cases (2.2%) of bilateral Krukenberg's tumor with 2 having primary from colon and 1 from stomach.

When classified according to WHO (2014), most common among all benign, borderline and malignant epithelial tumors with a largest dimension of ≥ 20 cm were found to be mucinous tumors. Thus, from this it was concluded that mucinous tumors had largest size overall and among surface epithelial tumors irrespective of the nature of the tumor. Mature teratoma which was the commonest germ cell tumor, predominantly had a size range of 5–9 cm (Figs. 5–27).

Table 2: Laterality of tumors

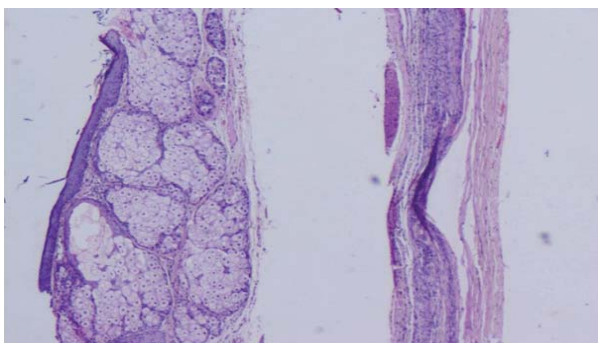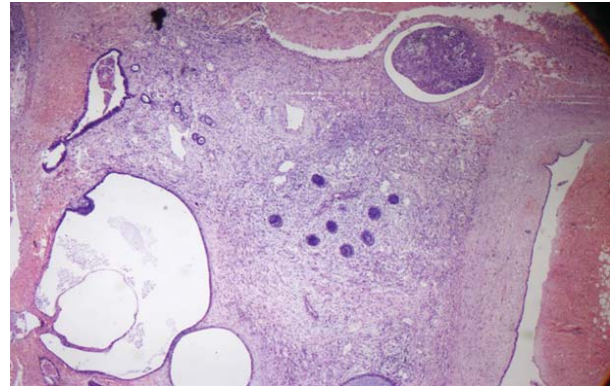| Laterality	Benign (%)	Borderline (%)	Malignant (%)
Unilateral	82 (87.2)	10 (77)	15 (48.4)
Bilateral	12 (12.8)	3 (23)	16 (51.7)
Total	94 (100)	13 (100)	31 (100)

Table 3: Size distribution of ovarian tumors

Size (cm)	≤4	5–9	10–19	≥20
Benign (%)	15.05	39.78	26.89	8.60
Borderline (%)	8.33	50	16.67	8.33
Malignant (%)	17.64	20.68	38.23	8.82

Table 4: Macroscopy of ovarian tumors

Nature of tumor	Solid (%)	Cystic (%)	Solid+cystic (%)	Solid+cystic+papillary excrescences (%)
Benign	2 (2.1)	58 (61.7)	29 (30.9)	5 (5.3)
Borderline	—	1 (7.7)	7 (53.8)	5 (38.5)
Malignant	3 (9.7)	3 (9.7)	18 (58.1)	7 (22.5)

Table 5: Distribution of ovarian tumors according to WHO classification 2014

Histological subtypes	Number of cases	n (%)
Surface epithelial tumors (n = 87)		
Serous cystadenoma	22	15.9
Serous cyst adenofibroma	8	5.8
Serous surface papilloma	1	0.72
Serous borderline tumor	6	4.4
Low-grade serous carcinoma	1	0.72
High-grade serous carcinoma	17	12.32
Mucinous cystadenoma	19	13.8
Mucinous borderline tumor	4	2.9
Mucinous adenocarcinoma	3	2.2
Borderline endometrioid tumor	3	2.2
Endometrioid carcinoma	1	0.72
Brenner tumor	2	1.5
Sex-cord stromal tumors (*n* = 12)		
Fibroma	5	3.62
Thecoma	3	2.2
Luteinized thecoma	1	0.72
Adult granulosa cell tumor	3	2.2
Germ cell tumors (*n* = 33)		
Mature teratoma	31	22.5
Immature teratoma	1	0.72
Mixed germ cell tumor	1	0.72
Soft tissue tumors (*n* = 1)		
Ovarian leiomyoma	1	0.72
Lymphoma (n = 1)		
Non-Hodgkin Lymphoma: Diffuse large B cell lymphoma (DLBCL)	1	0.72
Secondary tumors (*n* = 4)		
Krukenberg-tumor	3	2.2
Neuroendocrine tumor	1	0.72

Table 6: Size distribution of ovarian tumors in various histological subtypes

Histological subtypes	<=4 cm	5–9 cm	10–19 cm	>=20 cm
Surface epithelial tumors				
Serous cystadenoma	6	10	6	—
Serous cyst adenofibroma	2	4	2	—
Serous surface papilloma	1	—	—	—
Serous borderline tumor	2	4	1	—
Low-grade serous carcinoma	—	1	—	—
High-grade serous carcinoma	5	5	4	—
Mucinous cystadenoma	1	7	9	5
Mucinous borderline tumor	—	3	1	1
Mucinous adenocarcinoma	—	1	2	2
Borderline endometrioid tumor	—	—	3	—
Endometrioid carcinoma	1	—	—	—
Brenner's tumor	—	—	1	1
Sex-cord stromal tumor				
Fibroma	1	1	3	2
Thecoma	—	1	—	—
Luteinized thecoma	—	1	—	—
Adult granulosa cell tumor	—	—	3	—
Germ cell tumor				
Mature teratoma	4	18	5	—
Immature teratoma	—	—	1	—
Mixed germ cell tumor	—	—	1	—
Soft tissue tumor				
Ovarian leiomyoma	1	—	—	—
Lymphomas				
DLBCL				
Secondary tumors				
Krukenberg's tumor	—	—	2	1
Neuroendocrine tumor	—	—	1	—

Discussion

The ovary has a compound embryological and histological structure, shows steroidogenesis, with its high potential for malignancy, with different components like epithelial tissue, germ cells, follicular cells and mesenchymal tissue each having different capability to form various tumors.[11,13,16–18] Its history has been known scientifically for over 150 years, not much change has been seen since then in its mortality rate and increased incidence especially in developing countries.[13] Most of the benign tumors are detected as an incidental finding and are more common in reproductive age group.[16] Risk factors for ovarian malignancy can be non-hereditary and hereditary. Among non-hereditary risk factors, strong association is seen with increased age with a peak in fifth decade, low or nulliparity, early menarche and late menopause.[16] Other non-hereditary risk factors include Ashkenazi Jewish population (16–60%), dietary factors like high-fat diet, obesity and use of ovarian-stimulating drugs.[13,20] The hereditary risk factors include BRCA1 and BRCA2 mutations (27–44%), familial syndromes like Li–Fraumeni syndrome and Lynch syndrome (9–12%).[20,21] Factors known to have a protective role against ovarian malignancies are the use of oral contraceptive pills (OCPs) and multiparity.[22]

The clinical features of ovarian tumors are very imprecise and non-specific which include abdominal distention, loss of appetite, abdominal pain.[18] And hence often go overlooked and diagnosed at a very later stage. The laterality of the tumor may also provide a clue to their nature, for example, tumors in the sex cord stromal

category are almost always unilateral while most of the metastatic tumors are bilateral.[22] Biochemical markers and radiological assistance may help in early diagnosis.[23]

Grossly, these tumors vary from being solid or cystic in consistency (with serous/mucinous or serosanguineous fluid) or may have additional features like papillary excrescences or any calcified areas. Benign tumors usually have a smooth contour externally and are primarily cystic in nature.[24] Serous cystadenomas/adenofibromas are commonly uniloculated and filled with clear serous fluid. However, large size multiloculation of the cyst (on cut section) and mucinous material are peculiar features pertaining to mucinous tumors. Mature cystic teratoma may have solid areas suggestive of calcification or bony areas (Rokitansky's-protuberans), cystic areas, may contain hairs and pultaceous material. Borderline and malignant tumors tend to have an irregular contour and are usually solid in consistency. An exception to this is, Krukenberg's tumor having bilateral involvement with smooth external contour.

Histologically, benign tumors are lined by serous (with papillary arrangement), mucinous or may be lined by epidermis as seen in mature cystic teratomas. Mucinous cystadenomas are more commonly associated with Brenner's tumor which shows nests of cells resembling transitional epithelium. The term 'borderline tumor' was introduced by FIGO and approved by WHO in 1973, implicating that these tumors have morphological and clinical behavior intermediate between benign cystadenoma and carcinoma.[3] This was further substantiated in 2014 at Lyon. Along with these clinical and histomorphological factors, features like serum biomarkers and immunohistochemistry (IHC) aids in diagnosis.

In our study, out of 138 cases studied, the clinical and histopathological findings were analyzed in detail and co-related with different studies available in the literature. These tumors were classified according to WHO 2014 classification of ovarian tumors. In concordance with the literature, the frequency of benign ovarian tumors was more than the malignant tumors in present study.

In the present study, 94 (68.12%) cases were benign, 13 (8.7%) were borderline, 31 (22.5%) were malignant in nature which was similar to the studies Garg et al.[1], Sarangan et al.[2] Manoja et al.[5], Singh et al.[25] and Bindal et al.[26] and Phukan et al.[31] However, these studies have reported a higher incidence of benign cases compared to the present study. Our study reports a greater number of borderline and malignant cases (Table 7), this can be probably due to many oncology cases are referred to our center.

Comparing the age distribution, majority of ovarian tumors were found in reproductive age

Table 7: Comparison of frequency of benign, borderline and malignant tumors

Study (n)	Benign (%)	Borderline (%)	Malignant (%)
Manoja et al. (n = 120)[5]	90	–	10
Sarangan et al. (n = 135)[2]	89	4	7
Singh et al. (n = 120)[25]	80.83	1.67	17.5
Garg et al. (n = 85)[1]	81.2	1.2	17.6
Bindal et al. (n = 130)[26]	79.23	1.53	19.23
Hota et al. (n = 230)[19]	83.4	2.6	14
Jha and Karkhi et al. (n = 135)[27]	83.9	–	16.1
Phukan et al. (n = 84)[31]	75	3.6	21.4
Present study (n = 138)	68.12	8.7	22.5

Table 8: Comparison of Age distribution with various other studies

Age in years	Priya et al. (%)[23] n = 77	Garg et al. (%)[1] n = 85	Sarangan et al. (%)[2] n = 135	Manoja et al. (%)[5] n = 120	Hota et al.(%) [19] n = 230	Present study (%) n = 138
Upto 20	3.9	7.1	2	11.7	8.69	4.34
21–30	20.8	17.6	24	25	34.34	13.8
31–40	22.1	41.2	29	29.2	17	26.84
41–50	27.2	22.3	27	18.3	19.5	26.08
51–60	16.9	10.6	13	9.2	4	15.94
61–70	7.8	1.2	3	5.8	5.2	10.14
>70	1.3	–	2	0.8	1.7	2.89

group as also noted in other studies. The highest number of cases were seen between 30–40 years in present study (Table 8).

Overall, the clinical features were divided into 4 main categories–Abdominal mass, pain in abdomen, menstrual irregularities and others including non-specific. It was seen that in our study predominantly patients presented with dull and vague abdominal pain, this was also documented by Lina Baru et al.[28] however they reported higher percentage (Table 9). On further categorization as benign and malignant tumors and comparing with various studies (Table 10 and 11), our study had pain abdomen as the most common presentation in benign tumors whereas other studies showed abdominal mass as the principle presentation. Similarly, for malignant ovarian tumors also our data was different from other researches done. These differences can be explained by the fact that ovarian neoplasms have a wide range of overlapping clinical presentations and may be non-specific.

In our study 87.2% of benign and 48.4% of malignant ovarian tumors were unilateral. In other studies also, benign tumors were found to be predominantly unilateral. However, we had more of bilateral presentation for malignant ovarian tumors which is not concordant with the other studies (Table 12 and 13).

In this study, surface epithelial tumors (63.04%) constitute the most prominent type of ovarian tumors followed by germ cell tumors (24%) and then by, sex-cord stromal tumors (8.7%), this is in concordance with majority of the studies analyzed (Table 14). Overall, in our study we found mature cystic teratoma was the most common benign tumor and germ cell tumor comprising of 31 cases. This feature was comparable to study by Okugawa et al.[15] and Shiekh et al.[35], however most of the other studies by Rajavigneshwari et al.[29] and others have found serous cystadenoma to be the most common benign tumor. Serous cystadenocarcinoma out numbered other malignant tumors (58.06%) which was similar to findings of the studies by Jain et al.[30] and Atanda et al.[32]

According to our study, benign ovarian tumors were more common than malignant tumors.

Surface epithelial tumors are the most common variant based on cell of origin. And Krukenberg's tumor was the most common secondary tumor of ovary. In such cases, clinical data and co-relation with radiological findings aid in precise diagnosis. The ovarian tumors manifest a wide and varied range of clinical and morphological features. Histopathological study along with ancillary techniques like IHC, molecular studies along with radiological corelation, serum tumor biomarkers, together aids in appropriate diagnosis, proper classification and management of ovarian neoplasms. Since the overall mortality of malignant ovarian tumors is high there is a need for screening test to detect ovarian cancer at an early stage.

Table 9: Comparison of clinical presentation of all ovarian neoplasms

Symptoms	Lina baru et al. (%) [28] n = 108	Hota et al. (%)[19] n = 230	Present study (%) n = 138
Abdominal mass	31.17	44	18.84
Pain in abdomen	79.55	31	43.4
Menstrual irregularities	9.1	17.4	12.3
Others	0	0	0

Table 10: Comparison of clinical presentation of benign ovarian neoplasms

Symptoms	Manoja et al.[5] (%) n = 108	Mohapatro et al.[36] (%) n = 59	Jain et al.[30] (%) n = 162	Present study (%) n = 94
Abdominal mass	42.6	55.93%	28.04	12
Pain in abdomen	38.9	23.7	23.07	58
Menstrual irregularities	9.3	18.6	2.95	18
Others	9.2	9.6	34.91	12

Table 11: Comparison of clinical presentation of malignant ovarian neoplasms

Symptoms	Manoja et al. (%)[5] n = 12	Mohapatro et al. (%)[36] n = 32	Jain et al. (%)[30] n = 80	Present study (%) n = 31
Abdominal mass	25	73.68	17.5	40
Pain in abdomen	25	50	40	24
Menstrual abnormalities	8.3	12.5	—	12
Others	25	—	22.5	24

Table 12: Comparison of laterality of benign tumors

Laterality	Jha and Kharkhi et al.[27] (%) n = 135	Pilli et al.[34] (%) n = 212	Present study (%) n = 94
Unilateral	93.3	92.2	87.2
Bilateral	6.67	7.8	12.8

Table 13: Comparison of laterality of malignant tumors

Laterality	Jha and Kharkhi et al.[27] (%) n = 135	Rajgopal et al.[16] (%) n = 200	Present study (%) n = 31
Unilateral	57.69	64.6	48.4
Bilateral	42.3	35.4	51.7

Table 14: Comparative analysis of frequency of ovarian neoplasms based in cell of origin

Tumor type	Sarangan et al. (%)[2] n = 135	Garg et al. (%)[1] n = 85	Parmar et al (%)[3] n=	Ahmed et al. (%)[33] n = 186	Manoja et al. (%)[5] n = 120	Agrawal et al. (%)[22] n = 226	Akakpo et al. (%)[37] n = 706	Hota et al. (%)[19] n = 230	Present study (%) n = 138
Surface epithelial tumor	81	70.6	62	61.83	84.2	72.1	40.07	64.5	63.04
Sex cord stromal tumors	4	8.2	9.33	6.45	4.2	7.1	15.2	27	8.7
Germ cell tumors	15	18.8	24.67	30.64	10	19.2	41.9	5.2	24
Secondary tumors	0	0.83	4	1.08	0.8	0.9	1.1	2.6	2.9
Miscellaneous	0	0	0	0	0.88	0	1.1	0.8	1.44

Conclusion

This is an institution based the study and with a small sample size, the demographic data as well as histological distribution of the tumors may differ from other areas. Hence, further studies in different regions are required to analyze and compare the prevalence, demography in different populations and regions. The multifaceted nature of these ovarian neoplasms require multidisciplinary approach which many institutes are adopting in their management, poses some medicolegal concerns.[38–40] These concerns like consent from the patient for such approach, fixing liability for the proposed management, responsibility of documenting the facts with consensus are the challenges to the physician involved in ovarian cancer care. The complex nature, unpredictable behaviour and prognosis, debated management make ovarian neoplasms a difficult problem for clinicians. The histogenesis of many tumors is interrelated and precise histopathological diagnosis is of utmost importance for effective treatment.

Prior publication: Nil

Conflicts of interest: Nil

Source(s) of support: Nil

Presentation at a meeting: Nil

Conflicts of interest: None to declare

Permissions: Institutional Ethics Committee permission taken

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Cyto-Histologic Correlation in Hashimoto's/ Lymphocytic Thyroiditis With Emphasis on Genetics of Autoimmune Thyroiditis

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Abstract

Introduction: Hashimoto's/Lymphocytic thyroiditis is a common autoimmune disorder which has female preponderance. Autoimmune thyroiditis is considered as a complex interaction and interplay between various genetic and non-genetic factors. Molecular basis of Hashimoto's thyroiditis is not known. Fine needle aspiration cytology being first line of investigation for thyroid lesions is helpful in diagnosing these lesions as well. However, few potential pitfalls in cytology may lead to cytohistologic discordance. *Materials and methods:* This is a retrospective study of one year where all histologic proven cases of Hashimoto's/ Lymphocytic thyroiditis with their corresponding fine needle aspiration cytology slides from pathology database were studied. All FNACs were analyzed in depth and reviewed for cytohistologic correlation. The reasons of cytohistologic discrepancies and discordant cases were analyzed. The genetics of autoimmune thyroiditis was also reviewed from literature. *Results:* Out of total 38 cases of HT/LT analyzed in this study, 89% were females and 11% were males. Mean and median age was found to be 44.4 years and 44 years respectively. Correlation of cytology and histology showed that 50% FNACs' correlated with their respective histologic diagnosis. Major causes of the discrepancies and discordance are reporting on suboptimal smears, cystic fluid samples, and giving over emphasis on a single cytologic feature in rare cell clusters. *Conclusion:* Autoimmune thyroiditis is frequently encountered lesion. Fine needle aspiration cytology is useful in deciding the management of thyroid lesions. In order to restrict the discrepancies and cytodiagnostic errors, one must adhere to the adequacy criterion along with primary fixation, quality of the smear and cellularity. Cytopathologists should be aware of the possible pitfalls and differentiating clues when overlapping features between different lesions are encountered. Also an integrated multidisciplinary approach can be used to minimize potential pitfalls. Many a time the condition can be diagnosed after death as seen in case of few sudden deaths. The unexpectedly brought in thyroid dysfunction is expected in such cases. So the forensic pathologist must keep this entity in mind while dealing with cases of sudden death.

Keywords: Autoimmune; Cyto-histologic discordance; Fine needle aspiration cytology; Genetics; Hashimoto's thyroiditis

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Introduction

Hashimoto's/Lymphocytic thyroiditis (HT/LT) is one of the most common autoimmune disorders. Hashimoto's thyroiditis (HT) has a prevalence rate of 1–4% and reported incidence of 30–60/100000 population per year.[1] This disease is more common in females. Its occurrence may be stimulated in genetically susceptible individuals by a number of factors including female gender, immunological changes occurring in postpartum period, fetal microchimerism, amount of iodine intake by

the individual and other environmental agents. At genetic level, few susceptibility genes have been identified probable causing development and progression of this disease process. Some of these genes are specific for this autoimmune process in thyroid gland while few others are found to be common for various other autoimmune disorders.[2]

Fine needle aspiration cytology (FNAC) is an easy, cost-effective and useful investigation for thyroid lesions. It has become a standard first line of investigation for evaluation of thyroid nodules. Since FNAC gives initial results about the nature of the lesion, it helps in determining if an invasive procedure is required for the patient. Thus, helping to avoid unnecessary surgeries for benign diseases. Although FNAC of thyroid nodules has shown high sensitivity and specificity for detecting neoplasms, still many diagnostic difficulties and limitations exist leading to false-positive and false-negative results. HT/LT is considered as one particular cause of both false-positive and false-negative results[3-5], however, in few studies, FNAC sensitivity for this lesion has been reported up to 92%.[6] Thyroiditis is usually missed or misinterpreted in cytological smears showing cytological evidence of hyperplasia as in Graves' disease or abundant colloid. Also follicular cells that exhibit some features of papillary carcinoma and a minimum lymphoid population in the background can cause a diagnostic pitfall. Marked hürthle cell change with sparse inflammatory cells mimicking Hürthle cell neoplasm can also lead to misinterpretation.[6,7]

Aims and Objectives

The aims and objectives of this one-year retrospective study were.

1. To analyze and correlate the cytological findings with respective histological features in HT/LT cases identified for this study.

2. To study the discordant cases in detail and to evaluate the possible causes of these discrepancies in cytology and histopathology.

3. To study the clinical parameters and review the genetic aspect of autoimmune thyroiditis.

4. To review Medicolegal implications of autoimmune thyroiditis.

Materials and Methods

This is one year retrospective study. As a protocol, permission from institutional ethics committee was taken prior to the commencement of this study (IEC-14.391). A total of 76 cases of HT/LT in one year were retrieved from histopathology database. Corresponding FNACs were available for 38 cases. Inclusion criteria was all histopathological proven cases of HT/LT where prior FNAC has been performed and cytological diagnosis was made. Exclusion criteria included the HT/LT cases where prior cytology has not been done, cytology slides (PAP/MGG stained) and/or paraffin embedded slides/blocks for histopathology studies were not available. Cases with other associated lesions (benign/malignant) were also excluded. The only clinical parameters considered were patient's age and gender which was available from the pathology requisition forms and reports. All the cytology slides, histology slides and blocks and clinical details were studied. All FNACs were analyzed in depth and reviewed for cytohistologic correlation. The reasons of cytohistologic discrepancies and discordant cases were discussed.

Results

Out of total 38 cases of HT/LT analyzed in this study, 89% were females and 11% were males. Mean and median age was found to be 44.4 years and 44 years respectively. All the cases were reviewed for histopathology findings and diagnosis of HT/ LT was confirmed. None of the cases had any coexisting or associated benign or malignant lesions. The results of cytological findings were analyzed and given in the Table 1.

Table 1: Cytology reports of the cases

Cytology report	No of cases (n)
Hashimoto's/Lymphocytic thyroiditis	19
Papillary carcinoma thyroid	2
Follicular neoplasm	3
Colloid nodule with cystic change	10
Inconclusive/Inadequate	4

On reviewing of cytology slides it was found, that out of 10 cases of colloid nodule with cystic changes, 5 were suboptimal smears.Correlation of cytology and histology showed that 19(50%) FNACs' correlated with their respective histologic diagnosis. The discrepancies and discordance occurring, was found to be due to following causes as mentioned in Table 2.

Table 2: Causes of Cytohistologic discrepancies

Discrepancy	No of cases	Percentage
FNA sampling error (Inadequate)	4/38	10.5
Suboptimal smears	5/38	13.2
Cyto-diagnostic error	10/38	26.3
Papillary carcinoma	2	
Follicular neoplasm	3	
Colloid goiter with cystic change	5	

Among the cytodiagnostic errors, major discordance causing significant effect on patient care was seen in 5/38 (13.2%) cases, where 2 and 3 cases were given malignant diagnosis of papillary carcinoma and follicular neoplasm of thyroid respectively. Rest 5 cases were given benign cytologic diagnosis, which did not correlate with histology subsequently. We have not calculated the false positive and false negative cases in our study as we have not included the cases with cytologic diagnosis of HT/LT with a subsequent different histologic diagnosis.

Discussion

HT was originally described by Hakaru Hashimoto in the year 1912.[8] This is the most prevalent autoimmune thyroid disorder. Patients usually present with a diffuse or less frequently nodular non-tender enlargement of the thyroid gland. Biochemistry reveals hypothyroidism, and the presence of thyroglobulin and peroxidase antibodies in the serum of the individual.[9] At times, patients may not develop thyroid swelling but possess characteristic autoantibodies in the serum.[2]

The etio-pathogenesis for autoimmune thyroiditis is yet to be established completely. Few factors known to play a significant role in its development include genetic susceptibility for the disease. The mechanisms underlying the genetic predisposition are unknown. This has been confirmed predominantly by familial and twin studies. Although a hereditary component in the pathogenesis has been recognized, the inheritance process is complex, involving multiple genes with variable penetrances. Moreover, many loci have been identified, the candidate genes had still not been found. Single-nucleotide polymorphism (SNP) of the thyroglobulin gene (TG; OMIM*188450) and Zinc finger gene in autoimmune thyroid disease 1 (ZFAT1; OMIM*610931) has been found to be associated with susceptibility to autoimmune thyroid disease (OMIM #608175) showing linkage to 8q24 region. Several studies have shown the genes which are associated with the occurrence, progression and severity of autoimmune thyroiditis. The implicated genes include genes for human leukocyte antigen (HLA), cytotoxic T lymphocyte antigen-4, protein tyrosine phosphatase nonreceptor-type 22, thyroglobulin, vitamin D receptor, cytokines and many more. Other endogenous factors for the development of autoimmunity are female gender, pregnancy and postpartum period and fetal microchimerism. Exogenous environmental factors which influence HT development include amount of iodine intake, ingestion of certain drugs, associated infections and exposure to different chemicals. It is believed that disturbed self-tolerance accompanied by the increased antigen presentation is a prerequisite whereas interaction of thyroid cells, antigen presenting cells, and T cells are required for the development of thyroid autoimmunity. The cytokines secreted in this process lead to predominantly T-helper type 1 (Th1) as well as Th 17 response. At the end, thyroid destruction occurs due to the apoptotic processes and T-cell mediated cytotoxicity.[2,10] Molecular basis of HT is not known yet.

The overall incidence of HT is known to be increasing in the recent times and has become nearly 10 times more common in this century when compared to early 1990s. This increase is probably due to excess iodine intake, particularly in the coastal areas.[11,12] The abundance of female cases in our series (89%) was consistent with other studies.[13,14]

A diagnosis of HT/LT is often clinical based on presence of serum auto antibodies.[15] Still, ultrasound neck is done to look for the presence of dominant nodules. FNAC is performed if there is a dominant noduleor when there is a recent increase in the size of the swelling.[16] Cytologic features for HT/LT are oxyphilic (Hürthle) cells, infiltration of follicles by lymphoid cells, plasma cells and the presence of moderate amount of colloid in the background. The histologic findings include a diffuse lymphoid infiltration in the thyroid parenchyma, scattered plasma cells or histiocytes, atrophy of follicular cells and oncocytic changes of follicular cells called as Hürthle or Askanazy cells. Eventually, as the disease progresses, destruction of thyroid parenchyma with fibrous replacement occurs.[8,9,17] While giving a diagnosis of HT/LT, a dilemma and difficulty may result from coexistence of a benign or a malignant tumor or changes that occur in epithelial cell morphology in HT/LT mimicking thyroid neoplasms.[15,16,18] The precise and early diagnosis of HT/LT is of paramount importance as patients subsequently develop hypothyroidism and require lifelong supplementation of thyroxin. These

cases also harbor an increased risk of development of extra-nodal marginal B zone lymphoma. Since the frequency of development of malignancy varies between 0.5–23.5%, long term follow up is recommended.[7] One should not over-diagnose this entity as neoplasms or underdiagnose it as some benign lesion as the management would be varied in different lesions.

FNAC is a primary diagnostic tool for evaluation of thyroid nodules and a precis ecytologic diagnosis obviates unrequired surgeries. The important steps to be followed for any FNAC include careful sample procurement, appropriate sample preparation and accurate interpretation by cytopathologists. This will lead to precise cytologic diagnosis and reduce discrepancies.[19] FNAC is cost-effective first line of investigation in diagnosing HT/LT. However, it has got some pitfalls causing diagnostic dilemma. More importantly, there is an overlap in the morphological features of HT on cytological preparations with other thyroid lesions like multinodular goiter with degenerative changes, follicular neoplasm, hürthle cell neoplasm, papillary carcinoma, reactive lymphnode and lymphoma.[20]

Nearly 23.7% of the cases in the present study were either inadequate or suboptimal for opinion on cytology, hence could not be reported. Our result is little higher than others as reported in literature. Previous studies have shown around 10–20% of aspirates as unsatisfactory.[21,22] These include inadequate number of thyroid follicular cells, cystic fluid, bloody smears, poor technique in obtaining the sample or impropercytologic preparation. The Papanicolaou Society of Cytopathology task force on Standards of Practice recommends that "aspirators who persistently produce a high rate of unsatisfactory aspirates (>15%) should be identified and given remedial training".[23] However, in our study, FNAC samples were taken by different operators with varying skill levels and experience. Also poor cellularity of the aspirated samples in cystic lesions and suboptimal preparations can often be misinterpreted as benign lesions, which has also been observed in 5 cases in our study. In this regard, one should remember that cystic change in thyroid lesions is a common diagnostic pitfall and precise diagnosis cannot be offered if sample is taken from cystic areas. Aspiration from multiple sites and from solid areas is useful in preventing sampling error. Ultrasound-guided FNAC of cystic thyroid nodule is recommended for better yield of cells. Finally, strict criteria for specimen adequacy must be followed to reduce the erroneous diagnosis and improve the overall accuracy.[19]

The false negative rate (FNR) is defined as the percentage of patients given benign diagnosis on cytology, where malignancy was later confirmed on histopathology. Literature reports FNR ranging from 1.5–11.5%.[24-26] It is seen that FNR is higher if cases with negative cytological diagnosis were followed up for months or years.[7,24] In our study this parameter was not calculated as we have selected a cohort of cases with known histologic diagnosis of HT/LT where prior FNAC was done.

The false positive rate (FPR) is defined as the percentage of patients with malignant FNAC result but found to have benign lesion on histology. Various authors have reported FPR ranging from 0 to 8% in their studies.[24,27] In one study, two cases were reported as malignant but later on diagnosed to be Hashimoto's thyroiditis and nodular colloid goiter with focal areas of adenomatous hyperplasia.[24] In our study, 5 cases were diagnosed as malignant on cytology but on histologic examination subsequently, were HT/LT. The cytologic challenge in identifying a thyroid neoplasm associated with HT/LT is well established. Many studies done in the past have pointed out the importance of identifying thyroid neoplasm that may be disguised by a background of lymphoid cells of HT/LT on FNAC preparations. Many specific cytologic criteria have also been suggested to differentiate between thyroid neoplasm and changes occurring due to HT/LT.[18,28,29] Potential pitfalls causing false positivity for malignancy in HT/LT cases are cytologic atypia occurring in autoimmune thyroiditis, amount of background inflammation, sparse cell yield, coexisting thyrotoxicity and neoplasms. Features suggesting a possibility of malignancy include dyscohesive cell clusters, epithelial preponderance over inflammation, nuclear crowding and atypia.[20] Cytologic features which may lead to overdiagnosis of papillary carcinoma of thyroid are powdery nuclear chromatin, presence of nuclear grooves or inclusions and paucity of background lymphocytes. One very important clue which may be helpful in differentiating HT/LT from thyroid neoplasms is lymphocytes infiltrating follicular groups. In this regard, papillary carcinomas display characteristic malignant features in multiple cell clusters and these clusters do not possess infiltrating lymphocytes or may rarely have lymphocytes only at their periphery. In our study, the two cases which were false positive for papillary carcinoma thyroid, on review had focal suspicious nuclear features and scant colloid. True lymphocytic infiltration of the follicular cell clusters were present. However, the frozen section for both cases

did not reveal malignancy and hence unnecessary surgeries could be prevented. We found 3 cases of false positive follicular neoplasm in this study. A microfollicular pattern with paucity of background lymphocytes has been considered as the major pitfall in overdiagnosing follicular neoplasm.[3] Again, in this regard, the presence of lymphocytes closely infiltrating follicular groups serve as an important diagnostic clue.It is important to remember that the number of lymphocytes in FNA alone is not a feature that can distinguish HT/LT from a thyroid neoplasm.[18,30] In our study, presence of sparse lymphoid cells along with microfollicular pattern was the predominant reason for this overdiagnosis. It is suggested that even with predominant microfollicular pattern, it is preferable to render a diagnosis of suspicious for follicular neoplasm. And in presence of scattered lymphocytes, even if sparse, search for other features of HT/LT is recommended. The degree of nuclear pleomorphism of the follicular cells is not considered to be an useful feature to differentiate between the two lesions.[3]

Literature review documents many studies stating difficulty in differentiating between HT/LT from Hürthle cell neoplasm on cytology, leading to cytohistologic discordance.[4] The reason for this dilemma is the proportion of Hürthle cells and lymphoid cells. Hürthle cell metaplasia with nodule formation is a known phenomenon and can be seen as a histologic feature in HT/LT. This must not be overlooked while reporting Hürthle cell rich cytological smears. Cytological features which favor thyroiditis over neoplasm in a smear rich with Hürthle cells are absence of poorly organized cell clusters having nuclear pleomorphism, particularly anisonucleosis of Hürthle cells.[4,13,15,19] Another area of potential pitfall is lymphoid cell rich smears, where cytologic examination reveals dense population of lymphoid cells with occasional epithelial cells. Cytologic diagnosis of HT/LT and lymphoma is difficult and a diagnostic challenge because of the presence of heterogenous population of lymphoid cells in both. The differences in these lesions are very subtle, however, presence of polymorphous population of lymphoid cells, predominantly small mature lymphocytes admixed with plasma cells and presence of germinal center cells favor HT/LT.[19,31,32]

In this study, it is noted that most errors occur when too much emphasis is given on a single cytologic feature. We must not overemphasize classic neoplastic features on rare cell clusters. This may cause overdiagnosis and finally result in cytohistologic discrepancy. Multiple aspirations from different parts of the lesion are required to give a clear picture of cytologic features, reduce over interpretation and help in rendering precise diagnosis.[19,30] One should be careful while giving a positive diagnosis on cytology smears with only a few cell clusters of suspicion. It is recommended to inform the clinician about the limitations of FNAC in these diagnostically challenging cases.[3,19]

If such cases are missed during the lifetime or inaccurately diagnosed because of limitations of FNAC and the clinician overlooks into them, the condition could be a reason of fatality in future. There are cases reported in literature regarding sudden unexpected deaths due to Hashimoto's thyroiditis.[33] Such cases are diagnosed only after the postmortem examination. We should keep in mind the various causes of death associate with Hashimoto's thyroiditis like autoimmune myocarditis,[34] undiagnosed spontaneous intracranial hypotension,[35] etc.

Conclusion

HT/LT is commonly encountered thyroid lesion in day-to-day practice. Presently, autoimmune thyroiditis is considered as a complex interaction and interplay between various genetic and non-genetic factors leading to enhancement in antigen presentation and changes in the immune tolerance of the individual, thus developing autoimmunity. This mechanism is the cause for the development of various clinical features which finally lead to the destruction of the thyroid gland. Molecular basis of HT is not known.

FNAC is an easy, economic, safe, sensitive and specific procedure for the initial evaluation of thyroid nodules. In majority of the cases, correct cytologic diagnosis can be rendered, hence, useful in deciding the further management of the patient. In order to restrict the discrepancies and cyto-diagnostic errors, it is recommended to strictly adhere to the FNAC adequacy criterion. Adequacy should take into account components like primary fixation, quality of the smear and cellularity. Offering a definite diagnosis on suboptimal FNA samples is a very significant and avoidable source of cytohistologic discordance. Since cystic change in the thyroid lesions is a common cause of diagnostic pitfall, aspirations from multiple sites, preferably from the solid areas are recommended. Also sincere and meticulous examination by cyto-pathologist aids in reducing the number of discrepant cases and erroneous diagnosis. Pathologists should be aware of these possible pitfalls and differentiating clues when

overlapping features are seen in cytology. This will help in reducing the number of cyto-histologic discordance rates. However, in certain difficult situations an integrated multidisciplinary approach may minimize potential pitfalls.

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Assessment of Dermatoglyphic Pattern in Relation with Blood Group: A Cross-Sectional Study

Ashish Tyagi[1], Hitesh Chawla[2]

Abstract

Objectives: The study was contemplated to ascertain the trends of dermatoglyphic pattern in individuals with different ABO and Rh blood groups and to evaluate the relationship between the pattern of fingerprints and blood groups. *Methodology:* The study was carried out in the Department of Forensic Medicine at Government Medical College of southern Haryana. Medical students of the age group 18–24 years knowing their blood group and considering their accessibility to the department of Forensic Medicine were randomly selected for the study. Plain and rolled fingerprints for all digits of both hands were taken with ink pad on non-glazed paper. *Results:* The study revealed that loop was most frequently seen fingerprint followed by whorl, arch and composite. B positive is the most common blood group, and loops pattern of the fingerprint is predominant in all blood groups and Rh-positive and negative subjects, followed by whorls. *Conclusion:* Each fingerprint is unique; hence, it can be effectively used for corroborative identification of an individual in mass disasters as well as in other forensic applications. More studies with larger sample size should be conducted to enhance the reliability of correlation of dactylographic pattern with sex and blood group.

Keywords: Fingerprint; Dactylography; Dermatoglyphics; ABO blood group; Identification

Introduction

Dermatoglyphics (fingerprint/dactylography) is defined as the scientific study of natural occurring epidermal ridges and their configuration on the volar region of digits, palms, and soles apart from flexion crease and secondary folds. It is derived from the Greek word "Derma meaning Skin, Glyphe meaning Carve." The Anatomist Harold Cummins in 1926 observed that the sole and foot have some ridge designs which are determined by heredity and accidental or environmental influence during intrauterine life and then term dermatoglyphics was first established.[1] The development of fingerprint start developing from 12th to 16th week of intrauterine life and accomplished by 20th week of intrauterine life.[2] Fingerprint pattern is persistent and distinctive even in monozygotic twins from birth till death. Therefore, it is one of the valuable and inimitable personal identification tools of a human being.[3,4] They are supportive in medicolegal cases for recognition of suspect or victims in solving criminal cases. Fingerprint scans also used in the digital mission of India, biometric, validate electronic registration, cashless, library access, and forensic purpose.[5]

Sir Francis Galton was the first to publish a book called Fingerprint in 1892 and categorise dermatoglyphic primary pattern as loop (60–65%), whorl (30–35%), and arches (5%).[6] Loop is of two types: ulnar or radial. It is such a prototype in

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which one or more ridges enter from either-side, re-curve, touch or pass an imaginary line between delta and core, and pass out upon the same side as the ridges entered. A typical concentric design characterises the whorl. The majority of ridges incline to make a consummate circuit around the core, a pivotal feature in the interior of the pattern. The arches are the simplest of all. They are described as patterns in which ridges enter from one side, elevate or curve at the centre and flow out from the opposite side.[7]

Karl Landsteiner, an Austrian physician, revealed Blood group system in 1901.[8] Amongst varied races of human, several blood group systems are recognized till date. Clinically, only "ABO" and "Rhesus" groups are of key significance. "ABO" system is further classified as "A", "B", "AB" and "O" blood groups according to the presence of the corresponding antigen in plasma. "Rhesus" system is categorized into "Rh +ve" and "Rh−ve" accord to the presence or absence of "D" antigen.[9] The inheritance of dermatoglyphic patterns and ABO blood group is polygenic.[10] Bernstein revealed the exact manner of inheritance of the ABO blood group.[11] The study was contemplated with an objective to ascertain the trends of dermatoglyphic pattern in individuals with different ABO and Rh blood groups and to evaluate the relationship between the pattern of fingerprints and blood groups.

Materials and Methods

The study was carried out in the Department of Forensic Medicine at Government Medical College of southern Haryana. Medical students of the age group 18–24 years knowing their blood group and considering their accessibility to the department of Forensic Medicine were randomly selected for the study. Their fingerprints were obtained and studied after taking their informed oral consent.

Inclusion criteria

Participants with known blood group.

Exclusion criteria

Participants with permanent scars, lesion, cuts, bandaged fingers.

Subjects with hand deformity due to injury, congenital defect or disease.

Collection of the fingerprints

Before taking fingerprints, the hands were subjected to thorough washing with soap water and allowed complete drying. The subjects were then asked to press their fingertips on the camel ink pad and then to the plain non-glazed paper to transfer the fingerprint impression. Both rolled, and plane prints of each finger of right and left hand were taken. The same method was followed for all the participants. The necessary details, such as name, age and sex along with blood group, were also noted down.

Assessment of the fingerprints

The pattern of fingerprints was analyzed by using a powerful magnifying hand lens. The fingerprint patterns were identified as loop, whorls, arches and composite based on the appearance of the ridgelines with the help of a magnifying lens. In order to classify the finger-prints, the classification scheme proposed by Galton was used depending upon their primary pattern. The pattern of fingerprints was assessed by two observers separately to remove any observer bias.

The data obtained were evaluated and incorporated on Microsoft Excel sheet, and descriptive analysis in terms of percentage was carried out.

Results

Among 164 students who took part in the study, 133 (81.1%) were male, and 31 (18.9%) were female.

Table 1 shows the distribution of blood groups according to gender. Majority of subjects belonged to blood groups B (36%) followed by A, AB and O. Blood group B was predominantly found in males

Table 1: Distribution of sex and blood groups

Blood group	Sex		Total
	Male	Female	
A	34 (82.9%)	7 (17.1%)	41 (25%)
B	46 (77.9%)	13 (22.1%)	59 (36%)
O	29 (93.5%)	2 (6.5%)	31 (19%)
AB	24 (72.7%)	9 (27.3%)	33 (20%)
Total	133 (81.1%)	31 (18.9%)	164

and females, but in males, B>A>O>AB and females B>AB>A>O was the order of frequency.

Table 2 shows the distribution of subjects according to Rh factors. Among 164 subjects, 155 (94.5%) belong to Rh positive whereas 9 (5.5%) were Rh negative. Out of 155 Rh positive subjects, majority belonged to blood group B followed by A, AB and blood group O.

Table 3 shows the distribution of fingerprint patterns among both the genders. The total number of loops found in all the digits was 920 (57%). Similarly, whorls in all the digits of both the hands were 430 (26.7%), and the number of arches was 256 (15.9%). Frequency of loops, whorls and composite were found to be higher in males.

Table 4 shows the distribution of fingerprint patterns of all the fingers digits in both the hands. Loops were of high frequency on the little finger (71%), whorls on the ring finger (45.4%) and arches were of high frequency on the thumb (24.7%).

Table 5 shows the distribution of fingerprint patterns among Rh factor in all the fingers. Loops were seen in higher frequency in both Rh positive and negative blood group.

Table 6 shows the distribution of fingerprint patterns among ABO blood groups in the entire fingers. Blood group B+ve showed more loops, whorls, arches and composite.

Table 2: Distribution of Rh factor

Rh factor	Blood group				Total
	A	B	O	AB	
Rh positive	38 (24.5%)	58 (37.4%)	28 (18.1%)	31 (20%)	155 (94.5%)
Rh negative	3 (33.3%)	1 (11.1%)	3 (33.3%)	2 (22.3%)	9 (5.5%)

Table 3: Fingerprint pattern sexwise

Fingerprint pattern	Male	Female	Total
Loop	762 (58.2%)	158 (52.1%)	920 (57%)
Whorl	356 (27.2%)	74 (24.4%)	430 (26.7%)
Arches	185 (14.1%)	71 (23.4%)	256 (15.9%)
Composite	7 (0.5%)	0	7 (0.4%)

Table 4: Fingerprint pattern viz-a-viz digits

Finger print pattern	Thumb	Index finger	Middle finger	Ring finger	Little finger
Loop	168 (51.2%)	165 (50.3%)	206 (62.8%)	148 (45.1%)	233 (71%)
Whorl	79 (24.1%)	90 (27.4%)	63 (19.2%)	149 (45.4%)	49 (14.9%)
Arches	81 (24.7%)	70 (21.3%)	59 (18%)	30 (9.1%)	43 (13.1%)
Composite	0	3 (0.9%)	0	1 (0.4%)	3 (0.9%)

Table 5: Fingerprint pattern in Rh factor

	Loop	Whorl	Arches	Composite
Rh positive	859 (55.7%)	409 (26.5%)	275 (17.8%)	6 (0.4%)
Rh negative	61 (62.3%)	21 (21.6%)	12 (12.4%)	3 (3.7%)

Table 6: Fingerprint pattern in different blood groups

Blood group	Loop	Whorl	Arches	Composite
A+ve	206 (22.4%)	94 (21.4%)	78 (27.6%)	2 (28.6%)
A-ve	23 (2.5%)	3 (0.7%)	4 (1.4%)	0
B+ve	310 (33.7%)	138 (31.4%)	128 (45.2%)	3 (42.8%)
B-ve	0	10 (2.3%)	0	0
AB+ve	177 (19.2%)	99 (22.5%)	34 (12%)	0
AB-ve	13 (1.4%)	5 (1.1%)	2 (0.7%)	0
O+ve	166 (18%)	88 (20%)	35 (12.4%)	2 (28.6%)
O-ve	25 (2.7%)	3 (0.7%)	2 (0.7%)	0

Table 7: Summary table of comparative studies of dermatoglyphic in relation to ABO Blood Group

Studied by	Year	Loops		Whorls		Arches	
		Highest	Lowest	Highest	Lowest	Highest	Lowest
Kshirsagar et al.[18]	2003	B	O	O	AB	AB	B
Bhardwaja et al.[13]	2004	A	O	AB	A	B	AB
Rastogi & Pillai[14]	2010	A	−	O	−	O	−
Mehta & Mehta[19]	2011	O	AB	B	O	AB	B
Deopa et al.[12]	2014	O	A	AB	B	A	AB
Singh et al.[15]	2016	B	AB	O	AB	AB	O
Hamid S et al.[17]	2016	B	AB	B	AB	B	AB
Shivhare et al.[16]	2017	B	AB	A	B	AB	B
Current study	2019	B	AB	B	O	B	AB

Discussion

The present study concluded that the majority of subjects belonged to blood groups B (36%) followed by A, AB and O. Blood group B was predominantly found in males and females but in males B>A>O>AB and in females B>AB>A>O was the order of frequency. Similar observations were made by Bhavna et al.[2] and Deopa et al.[12], who concluded in their study that the majority of the subjects belonged to the blood group B in both males and females. In contrast, Bhardwaja et al.[13] and Rastogi and Pillai[14] in their study observed majority of cases belonged to blood group O; followed by blood group B, A and AB.

The present study revealed that among 164 subjects, 155 (94.5%) belong to Rh positive, whereas 9 (5.5%) were Rh negative. Out of 155 Rh-positive subjects, the majority belonged to blood group B followed by A, AB and blood group O. Bhavna et al. in their study observed that out of 200 subjects, the majority (190) belonged to Rh +. Out of 190 Rh+ subjects majority of the subjects, 35% belonged to blood group B, 34% belonged to O, 19.5% belonged to A and only 6.5% belonged to blood group AB.[2] Bhardwaja et al. concluded 95.67% cases were Rh-positive in their study, of which 36.0% each belonged to blood group B and O, and 15.67% cases had A blood group.[13] Rastogi and Pillai also concluded that maximum (96%) subjects in the study were Rh-positive, of which 34.5% belonged to blood group O, 30.5% belonged to blood group B, 26.5% subjects had blood group A while only 4.5% had blood group AB.[14] Deopa et al. concluded that out of maximum number of Rh-positive cases, 39.06% belonged to blood group B, 28.13% belonged to blood group O, 18.75% subjects had blood group A while only 14.06% had blood group AB.[12]

The present study revealed that the number of loops found in all the digits was 920 (57%). Similarly, whorls in all the digits of both the hands were 430 (26.7%), and the number of arches were 256 (15.9%) which is consistent with study results of Bhavna et al.[2] who observed loops 58.9%, whorls 29.6% and arches 11.5% in all the digits. Kanchan and Chattopadhyay[4], Deopa et al.[12], Bhardwaj et al.[13], Rastogi and Pillai[14], Singh et al.[15] and Shivhare et al.[16] in their respective studies also observed that loops were the most common pattern followed by whorls and arches in both hands among males and females. It signifies that loops govern the chart followed by whorls and arches.

The current study indicates that loops were of high frequency on the little finger (71%), whorls on the ring finger (45.4%) and arches were of high frequency on the thumb (24.7%). Our study results were somewhat consistent with the study conducted by Kanchan and Chattopadhyay[4] who concluded that loop pattern was most often found in the little finger (77.7%) followed by middle finger (73.7%) and index finger (49.1 %). Frequency of whorls was maximum on the ring finger (55%) followed by thumb (53.6%) and index finger (38.2%). Contrary to this, Hamid et al. observed that loops were of high frequency on the middle finger, whorls on the thumb, index finger and ring finger, arches on the middle finger, thumb and index finger.[17]

In the current study, the distribution of fingerprint patterns among ABO blood groups in all the fingers depicts that B+ve blood group showed more loops, whorls, arches and composite.However, the results are comparable with the studies conducted by various authors, while few studies differ in this. Table 7 depicts the various comparative studies of dermatoglyphic in relation to ABO Blood Group.

This study revealed that loops are the most commonly occurring fingerprint pattern followed by whorls and arches while composite is the least common fingerprint pattern. Blood group B is the most common blood group in both males and females. Loops are predominant in blood group B

+ve followed by A+ve. Whorls and arches are also most commonly found in B+ve individuals. Thus the prediction of blood group of a person is possible based on his fingerprint pattern.

Limitation of the Study

Small sample size and unequal sex distribution were the main limitations of this study. Similar studies should be conducted on a larger sample to predict the more accurate correlation of dermatoglyphic pattern with sex and ABO blood group.

Conclusion

Each fingerprint is unique; hence, it can be effectively used for corroborative identification of an individual in mass disasters as well as in other forensic applications. Also, retrieving the fingerprints and examination of dactylography pattern is economical, less time consuming and non-invasive, more studies with large sample size should be conducted to enhance the reliability of correlation of dactylographic pattern with sex and blood group.

Key message: Each fingerprint is unique; hence, it can be effectively used for corroborative identification of an individual in mass disasters as well as in other forensic applications.

Prior publication: None

Financial support: None

Conflicts of interest: None

Source(s) of support: None

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Mesiodistal Width of Permanent Anterior Teeth:
A Tool for Sex Determination

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Abstract

Background: Metric and non-metric analysis of the human dentition have played an important role in human biological research and have formed a central focus in the field of dental anthropology for over a century. This study intends assess the degree of sexual dimorphism in permanent anterior teeth in south Indian origin student population. *Aim:* The purpose of this study is to analyze the mesiodistal crown width of permanent anterior teeth and assess the degree of sexual dimorphism in permanent anterior teeth in south Indian origin student population. *Materials and methods:* Materials for this cross-sectional study consisted of 210 students belonging to various parts of South India (Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, and; union territories of Lakshadweep and Pondicherry) comprising of 100 males and 110 females in the age group of 18–25 years studying at A. J. Institute of Medical Sciences and Research Centre, Mangalore, Karnataka who are willing to participate in the study. Methods for the study consisted of measuring the maxillary and mandibular incisor and canine widths of these students using a digital caliper with a resolution of 0.01mm with the provision to fix it in position to the desired position so as to avoid any errors in recording the exact measurements of canines. *Results:* 100 males and 110 female students in the age group of 18–25 years, from various parts of South India were examined to predict the sex from the mesiodistal widths of permanent anterior teeth for both the arches. The mean mesiodistal width of mandibular central incisor was greater in males (Right: 5.55 ± 0.39 mm; Left: 5.59 ± 0.38 mm) than females (Right: 5.43 ± 0.34 mm; Left: 5.44 ± 0.34 mm). The mean Mesiodistal width of maxillary central incisor was greater in males (Right: 8.60 ± 0.52 mm; Left: 8.60 ± 0.58 mm) than females (Right: 8.29 ± 0.52 mm; Left: 8.24 ± 0.67 mm). The mean Mesiodistal width of mandibular lateral incisor was greater in males (Right: 6.07 ± 0.45 mm; Left: 6.10 ± 0.38 mm) than females (Right: 5.83 ± 0.41 mm; Left: 5.86 ± 0.37 mm). The mean Mesiodistal width of maxillary lateral incisor was greater in males (Right: 6.90 ± 0.62 mm; Left: 6.86 ± 0.63 mm) than females (Right: 6.58 ± 0.57 mm; Left: 6.59 ± 0.56 mm). The mean Mesiodistal width of mandibular canine was greater in males (Right: 6.62 ± 0.54 mm; Left: 6.78 ± 0.51 mm) than females (Right: 6.21 ± 0.54 mm; Left: 6.35 ± 0.47 mm). The mean Mesiodistal width of maxillary canine was greater in males (Right: 7.52 ± 0.58 mm; Left: 7.60 ± 0.54 mm) than females (Right: 7.33 ± 0.45 mm; Left: 7.28 ± 0.49 mm). *Conclusion:* This study shows that the Mesiodistal crown width of permanent anterior teeth can be used as tool for determining the sex.

Keywords: Dentition; Mesiodistal crown width; Metric and non-metric analysis; Permanent anterior teeth; Sexual dimorphism.

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Received on 05.10.2019, **Accepted on** 13.11.2019

Introduction

During legal investigations, especially in crimes resulting in fatalities or when unknown human remains are recovered by investigating agencies, the forensic pathologist is often required to give an opinion regarding personal identification of the deceased. Sex determination is considered as one of the parameters for personal identification and one of the 'big fours' of forensic anthropology. It is an important step in reconstructing the biological profile of unknown individuals from the forensic context. Assessment of sex differences from human remains will be of immense help to the investigating officer as it would narrow down his field of search to 50%.[1] The most commonly used techniques for sex determination are based on the assessment of the morphological characteristics of the pelvis and skull.[2] However, it is not uncommon to recover the pelvis and the skull in a fragmentary state in forensic settings. In this case, teeth can be used as an additional tool for sex determination. Their durability in the face of fire, trauma and bacterial decomposition makes them invaluable for identification.[3]

Metric and non-metric analysis of the human dentition have played an important role in human biological research and have formed a central focus in the field of dental anthropology for over a century.[4] Identification of humans using the unique features of teeth and jaws has been used since Roman times, because humans show dimorphism in jaw and teeth dimensions so also the dietary habits.[5,6] Whenever the jaws with the teeth, fragmented jaws with teeth or teeth alone are available at the crime scene or accident, then sex determination can be made using teeth alone. This identification of gender using odontometric techniques is of real interest in case of major catastrophes when bodies are often damaged beyond recognition.[7] As sexual dimorphism varies between different populations the collection of population specific data is of major importance. The purpose of this study is to analyze the mesiodistal crown width of permanent anterior teeth and assess the degree of sexual dimorphism in permanent anterior teeth in south Indian origin student population.

Materials and Methods

Materials for this cross-sectional study consisted of 210 students belonging to various parts of South India (Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, and; union territories of Lakshadweep and Pondicherry) comprising of 100 males and 110 females in the age group of 18–25 years studying at A.J. Institute of Medical Sciences and Research Centre, Mangalore, Karnataka who are willing to participate in the study. The research conducted between September 2011 and August 2013. Due permission was taken from Institutional Ethics Committee of A.J. institute of Medical Sciences and Research Centre, Mangalore for the conduct of the study. This age group was selected as all the canines would have erupted by this age and attrition is expected to be minimal.[7] Methods for the study consisted of measuring the maxillary and mandibular incisor and canine widths of these students.

Written informed consent was taken prior to the recording of dental measurements, after detailed information given to the participants regarding the study. The measurements of anterior permanent teeth (incisors and canine) were taken intra orally on either side of the jaw using digital calipers with a resolution of 0.01 millimeter with the provision to fix it in position to the desired position so as to avoid any errors in recording the exact measurements. The maxillary and mandibular permanent anterior teeth measurement so collected in millimeter is recorded on a pre-structured proforma.

Mesiodistal crown width of mandibular and maxillary permanent anterior teeth, i.e., the greatest mesiodistal width of the crown between the contact points of the teeth on either side of the jaw was measured three times and the average of the three values were noted.[8]

Subject having fragmentary teeth, abnormal teeth alignment, missing anterior teeth, crowded or excessive spacing in the anterior teeth, abnormal overjet and overbite, caries teeth, subjects with bad/poor oral hygiene, anterior teeth with high degree of attrition, subjects with orthodontic treatment and any trauma to anterior teeth were excluded from the study.

Statistical analysis was carried out using IBM SPSS Statistics (IBM Inc., version 17 for Windows) software package.

In this study the percentage of sexual dimorphism was used as an indicator to describe the differences between males and females. This index is calculated using the formula of Garn et al.[19]

$$\frac{\text{Male mean} - \text{Female mean}}{\text{Female mean}} \times 100$$

Results

This study comprises a total of 210 subjects, comprising of 100 males and 110 female students in the age group of 18–25 years. The agewise distribution of subjects is depicted in Table 1.

Table 1: Age wise distribution of subjects

Age group	Subjects	
	Males	**Females**
18–21 years	77 (36.67%)	103 (49.05%)
22–25 years	23 (10.95%)	7 (3.33%)
Total (n = 210)	100	110

Total 2520 anterior teeth of which 1200 teeth were that of males and 1320 teeth were that of females: 840 central incisors of which 400 were that of males and 440 were that of females, 840 lateral incisors of which 400 were that of males and 440 were that of females and 840 canines of which 400 were that of males and 440 were that of females. The measurements included Mesiodistal crown width of central incisors, lateral incisors and canines in the respective arches.

It was observed that the mean value of the mesiodistal crown width of right mandibular central incisor was 5.55 ± 0.39 mm in males and 5.43 ± 0.34 mm in females, while the mean value of the mesiodistal crown width of left mandibular central incisor was 5.59 ± 0.38 mm in males and 5.44 ± 0.34 mm in females as depicted in Table 2. This value was statistically highly significant (p < 0.01).

Table 2: Mesiodistal crown width of mandibular central incisor

Mesiodistal width (mm)	Sex	Mean	± S.D.
Right central incisor	Male (n = 100)	5.55	0.39
	Female (n = 110)	5.43	0.34
Left central incisor	Male (n = 100)	5.59	0.38
	Female (n = 110)	5.44	0.34

The mean value of mesiodistal crown width of right maxillary central incisor was 8.60 ± 0.52 mm in males and 8.29 ± 0.52 mm in females and the mean value of mesiodistal crown width of left maxillary central incisor was 8.60 ± 0.58 mm in males and was 8.24 ± 0.67 mm in females as depicted in Table 3. This value was statistically very highly significant (p <0.001).

Table 3: Mesiodistal crown width of maxillary central incisor

Mesiodistal width (mm)	Sex	Mean	± S.D.
Right central incisor	Male (n = 100)	8.60	0.52
	Female (n = 110)	8.29	0.52
Left central incisor	Male (n = 100)	8.60	0.58
	Female (n = 110)	8.24	0.67

It was observed that the mean value of the Mesiodistal crown width of right mandibular lateral incisor was 6.07 ± 0.45 mm in males and 5.83 ± 0.41 mm in females, while the mean value of the Mesiodistal crown width of left mandibular lateral incisor was 6.10 ± 0.38 mm in males and 5.86 ± 0.37 mm in females as depicted in Table 4. This value was statistically very highly significant (p <0.001).

Table 4: Mesiodistal crown width of mandibular lateral incisor

Mesiodistal width (mm)	Sex	Mean	± S.D.
Right lateral incisor	Male (n = 100)	6.07	0.45
	Female (n = 110)	5.83	0.41
Left lateral incisor	Male (n = 100)	6.10	0.38
	Female (n = 110)	5.86	0.37

The mean value of Mesiodistal crown width of right maxillary lateral incisor was 6.90 ± 0.62 mm in males and 6.58 ± 0.57 mm in females and the mean value of Mesiodistal crown width of left maxillary lateral incisor was 6.86 ± 0.63 mm in males and was 6.59 ± 0.56 mm in females as depicted in Table 5. This value was statistically highly significant (p <0.01).

Table 5: Mesiodistal crown width of maxillary lateral incisor

Mesiodistal width (mm)	Sex	Mean	± S.D.
Right lateral incisor	Male (n = 100)	6.90	0.62
	Female (n = 110)	6.58	0.57
Left lateral incisor	Male (n = 100)	6.86	0.63
	Female (n = 110)	6.59	0.56

It was observed that the mean value of the Mesiodistal crown width of right mandibular canine was 6.62 ± 0.54 mm in males and 6.21 ± 0.54 mm in females, while the mean value of the Mesiodistal crown width of left mandibular canine was 6.78 ± 0.51 mm in males and 6.35 ± 0.47 mm in females as depicted in Table 6. This value was statistically very highly significant (p < 0.001).

Table 6: Mesiodistal crown width of mandibular canines

Mesiodistal width (mm)	Sex	Mean	± S.D.
Right canine	Male (n = 100)	6.62	0.54
	Female (n = 110)	6.21	0.54
Left canine	Male (n = 100)	6.78	0.51
	Female (n = 110)	6.35	0.47

The mean value of Mesiodistal crown width of right maxillary canine was 7.52 ± 0.58 mm in males and 7.33 ± 0.45 mm in females and the mean value of Mesiodistal crown width of left maxillary canine was 7.60 ± 0.54 mm in males and was 7.28 ± 0.49 mm in females as depicted in Table 7. This value was statistically highly significant (p < 0.01).

Table 7: Mesiodistal crown width of maxillary canines

Mesiodistal width (mm)	Sex	Mean	± S.D.
Right canine	Male (*n* = 100)	7.52	0.58
	Female (*n* = 110)	7.33	0.45
Left canine	Male (*n* = 100)	7.60	0.54
	Female (*n* = 110)	7.28	0.49

The sexual dimorphism, from Mesiodistal crown width of canine tooth was calculated by the formula Xm / Xf -1 X100; Xm is the mean Mesiodistal width of canines in males and Xf is the mean Mesiodistal width of canines in females. The sexual dimorphism was 2.27% for right mandibular central incisor and 2.79% for left mandibular central incisor as shown in Table 8.

Table 8: Sexual dimorphism — Mandibular central incisor

Mandibular tooth	Sexual dimorphism
Right central incisor	2.27%
Left central incisor	2.79%

The sexual dimorphism of right maxillary central incisor was 3.81% and that of left maxillary central incisor was 4.28% as shown in Table 9.

Table 9: Sexual dimorphism — Maxillary central incisor

Maxillary tooth	Sexual dimorphism
Right central incisor	3.81%
Left central incisor	4.28%

The sexual dimorphism was 3.99% for right mandibular lateral incisor and 4.06% for left mandibular lateral incisor as shown in Table 10.

Table 10: Sexual dimorphism — Mandibular lateral incisor

Mandibular tooth	Sexual dimorphism
Right lateral incisor	3.99%
Left lateral incisor	4.06%

The sexual dimorphism of right maxillary lateral incisor was 4.86% and that of left maxillary central incisor was 4.18% as shown in Table 11.

Table 11: Sexual dimorphism — Maxillary lateral incisor

Maxillary tooth	Sexual dimorphism
Right lateral incisor	4.86%
Left lateral incisor	4.18%

The sexual dimorphism was 6.53% for right mandibular canine and 6.78% for left mandibular canine as shown in Table 12.

Table 12: Sexual dimorphism — Mandibular canine

Mandibular tooth	Sexual dimorphism
Right canine	6.53%
Left canine	6.78%

The sexual dimorphism of right maxillary canine was 2.58% and that of left maxillary canine was 4.29% as shown in Table 13.

Table 13: Sexual dimorphism — Maxillary canine

Maxillary tooth	Sexual dimorphism
Right canine	2.58%
Left canine	4.29%

Discussion

Dental identification is the most common and reliable method of human identification especially for identifying burnt, decomposed, skeletonized and fragmented remains. Since teeth survive prolonged immersion, decomposition, desiccation, extensive trauma and direct heat in excess of 1000°F.[10]

Teeth can help us to determine age, ancestry, gender, and habits, past and present systemic disease, occupation, country or area of origin or residence and socio economic status. This study makes an attempt to establish gender of an individual by using Mesiodistal crown width of permanent anterior teeth and to assess the degree of sexual dimorphism in south Indian student population.

In our study, there were no significant differences between the Mesiodistal crown width of right and left, mandibular and maxillary central incisors, lateral incisors and canines among males. Similar observations were made amongst the female counterparts. The difference between the mean Mesiodistal dimension of any individual tooth on the right and left hand side were very small and ranged from 0.01 mm to 0.16 mm. These findings were in agreement with the studies conducted in the different parts of the world.[7,11-16] Lundstrom found a definite significant difference between left and right tooth measurements.[17] These findings indicate that right or left side measurements, for both sexes, could be taken to represent Mesiodistal crown dimension in this population. Harper provides evidence that the right-left differences between homologous teeth are smaller than the differences between the teeth of monozygotic twins, suggesting that the side differences can be attributed to environmental influences.[18] According to Garn, intra-individual variations in crown size and similarities between isomers and antimeres might be derived from specific intrauterine events during odontogenesis and less from genetic effects.[19]

The mean Mesiodistal crown dimensions of the anterior permanent teeth of males were larger than that of females in the maxillary and mandibular arches. The mean Mesiodistal crown dimensions of the anterior permanent teeth of maxillary arch were larger than that of mandibular arches in both the genders. These findings were in agreement with the similar studies conducted on South Indian population, North Indian population and Nepalee population.[7,20,21] But in contrast with the study on Bangladeshi population where there was no significant difference between males and females.[16] The larger dimensions of Mesiodistal width of tooth in males can be attributed to 'Y chromosome' which controls the thickness of dentine, which in turn determines the width of a tooth. Whereas the X chromosome, which was considered to be the chromosome responsible, is only concerned with the thickness of enamel.[2,12,22,,23]

In our study, among anterior permanent teeth, the greatest percentage of sexual dimorphism is demonstrated by left mandibular canine (6.78%) and lowest percentage of sexual dimorphism is by right mandibular central incisor (2.27%). The mandibular canines showed greatest percentage of sexual dimorphism amongst all teeth in their Mesiodistal width in our study in concurrence with the similar studies conducted on Tristanite, Ohio Caucasians, Australian aborigines, Pima Indians, South Indian and North Indian population.[7,12,24] The greatest percentage of sexual dimorphism demonstrated by mandibular teeth amongst all teeth in their Mesiodistal width is in agreement with the studies conducted globally.[7,12,25]

Conclusion

100 males and 110 female students in the age group of 18–25 years, from various parts of South India (Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, and; union territories of Lakshadweep and Pondicherry) were examined to predict the sex from the Mesiodistal widths of permanent anterior teeth for both the arches. It is evident from our study that the Mesiodistal crown width of permanent anterior teeth can be used as tool for determining the sex.

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Granulomatous Mastitis — Clinicopathological Review of 38 Cases: A 3 Year Study with a Brief Review of Literature

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Abstract

Introduction: Granulomatous mastitis (GM) is a rare, chronic inflammatory condition of obscure etiology, varied clinical and pathological features. *Methods:* This was a restrospective, three-year analysis, of the clinical and histological parameters of diagnosed cases of GM. *Results:* An analysis of age at presentation revealed the mean age as 35.3 yr. Clinical data was available for 26 patients and the mean lesional size was 5.3 x 4.3 cm. The most common presenting complaint was as a lump, with diffuse swelling and nipple discharge next in frequency. Clinical diagnosis varied widely from benign to malignant. Treatment administered included incision and curettage, lumpectomy and simple mastectomy. Thirty-eight cases reviewed showed granulomas in all the cases. Caseous necrosis was absent. Special stains for tubercular bacilli and fungi had been done in 26 cases and were negative. Follow-up ranged from 3–5.5 years. Recurrence was documented in 57.6% of patients. *Conclusion:* GM, generally, is a disease of young women that is of particular significance since it can easily be mistaken for malignancy. Histopathological diagnosis is confirmatory.

Keywords: Corticosteroids; Granuloma; Lobulocentric; Mastitis; Necrosis.

Introduction

Granulomatous mastitis (GM) is a rare, chronic inflammatory disease of the breast, of obscure etiology and varied clinicopathological features. Initially described by Kessler and Wooloch[1] in 1972, and further elaborated by Cohen[2] in 1977, GM characteristically affects women in the reproductive age group, and is also associated with use of oral contraceptives. An immune basis for the disease is also postulated. The histopathological picture is characterised by lobulocentric non-necrotizing granulomatous inflammation. The clinical and radiologic findings of GM can be mistaken for breast cancer, leading to misdiagnosis and erroneous treatment. Thirty-eight cases of granulomatous mastitis were reviewed. Clinical and pathologic features of GM are discussed along with a brief review of literature.

Materials and Methods

This study was approved by the Manipal Institutional Ethical committee (IEC no 482-2019). A retrospective review of records from our institute for a period of three years yielded 38 cases of GM between the ages of 23 and 66. Clinical details were available for 26 patients. The archived histopathological (H & E) slides for all 38 patients were analyzed. Special stains for tubercular bacilli and fungi were also accessed.

Results

GM constituted 38 cases (2.37%) of total breast specimens received during the three-year-period.

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Mean patient age was 35.3 years (range 23–66 yr). Mean lesional size was 5.3 x 4.3 cm. Presentation with breast lump, fever and pain were noted in 20, 10 and 17 cases respectively. Symptomatology ranged from a week to 6 months. Associated diabetes and hypothyroidism in was recorded in 2 cases respectively. No history of specific infection or oral contraceptive use was obtained. Diffuse swelling was noted in 6 and nipple discharge in 5 cases. Erythema ($n = 8$), retraction of nipple ($n = 4$), sinus formation ($n = 2$) and peau d' orange appearance ($n = 2$) were recorded. Axillary lymphadenopathy was present in 4 cases. Clinical suspicions varied widely abscess ($n = 13$), tubercular mastitis ($n = 1$), galactocele ($n = 1$), fibroadenoma ($n = 1$) and carcinoma ($n = 5$). Ultrasonography and mammography were done in 10 and 3 cases respectively with suspicion ranging from benign disease to carcinoma. Fine needle aspiration cytology was done in 8 cases with 2/8 showing GM, benign cystic disease ($n = 1$), epitheliosis with atypia ($n = 1$), abscess ($n = 3$) and 1 case was suspicious of malignancy. Incision and curettage, lumpectomy and simple mastectomy were done in 12, 13 and 1 cases respectively.Histologically, 38 cases reviewed showed a lymphocyte predominant infiltrate in 26 cases, with neutrophils, plasma cells and histiocytes predominating in 10, 1 and 1 cases respectively Granulomas were universal. Multinucleated giant cells were present in 37 cases, abscess in 30, fibrosis in 24 and dystrophic calcification in 1 case. Caseous necrosis was conspicuously absent. Special stains for tubercular bacilli and fungi available in 26 cases were negative. Surgical treatment formed the mainstay and was the sole mode of treatment in 17 cases, with added ATT and steroids in 6 and 3 cases respectively. Follow up ranged from 3–5.5 years. 57.6% of patients developed recurrence.

Discussion

GM is a rare inflammatory disease of the breast. As it is often unreported, the exact incidence is unknown.[3] In our series, GM constituted 2.37% of total breast specimens. According to Tuli et al.[4] most reports of GM have come from outside the United States and the reason for this is lower prevalence or under diagnosis in developed countries or increased index of suspicion in developing countries, or a combination of both.

GM usually afflicts women in the reproductive age group.[3,5] The mean age reported in literature is variable, but the average age of presentation is in the third decade of life with a wide range of 11 to 83 years. Symptoms are often recorded a few years subsequent to pregnancy.[4–7] In our study the mean age of presentation was 35.3.

The etiology of GM is obscure. An autoimmune reaction, triggered by proteinaceous duct secretions has been suggested and the response to steroid therapy supports this hypothesis. Associations with the use of oral contraceptives, pregnancy, hyperprolactinemia and alpha-1-antitrypsin deficiency have been postulated. The documented percentages of patients of GM using oral contraception ranges from 0% to 33%.[4–6] however none of our cases were on oral contraceptives.

GM usually presents as a painful breast mass. Chronicity may lead to development of abscesses, sinus, inversion of the nipple, skin inflammation, thickening and ulceration with axillary adenopathy.[5] Lai et al.[8] concluded that all women with a histopathological diagnosis of GM presented with palpable breast masses and 56% were had a clinical suspicion of malignancy. This parallels most of the other studies.[5,6] In our study 20 cases presented with breast lump while 6 had diffuse breast swelling. Malignancy was suspected in 5 cases (13.2%).

GM may mimic carcinoma in mammography, ultrasound and even in fine needle aspiration cytology leading to unnecessary mastectomies.[4,5] This attributes a level of importance to the initial correct diagnosis. In our study FNA was diagnostic in 25% cases. Other studies have documented diagnostic FNA in 21%[5] of cases studied. In a study by Kocaoglu et al.[9] the possible utility of dynamic contrast enhanced MRI in diagnosing GM was suggested, along with limitations in diagnostic utility, observed by other authors.[6]

GM is characterized by lobulocentric non-necrotizing granulomas (Figs. 1,2,3) along with a chronic inflammatory infiltrate composed of lymphocytes, plasma cells, epithelioid histiocytes, multinucleated giant cells and neutrophils (Fig. 4). Granulomas may be confluent, obliterating lobulocentricity. Microabscess formation involving the entire lobule, intense fibroblastic activity and metaplastic squamous change of lobular and ductal epithelium may also occur.[4–6] In 1 case calcification was noted (Fig. 5). The diagnosis of GM is one of exclusion, and the differential diagnosis includes infectious etiology like bacteria, mycobacteria or fungi which can be confirmed by culture and special stains. Non infectious conditions include sarcoidosis which has characteristic naked granulomas, traumatic fat necrosis which has

foamy macrophages and non-lobular pattern of involvement, ruptured cyst which has non-lobular pattern, duct ectasia with characteristic periductal fibrosis, plasma cell mastitis, polyangiitis with granulomatosis which is usually associated with vasculitis, and most importantly carcinoma which has characteristic histology.[5]

Treatment options remain a subject of controversy. Histopathological confirmation of GM is of paramount in the prevention of inappropriate and unnecessary treatment. Currently, treatment includes the options of surgical management, systemic steroids, or methotrexate. More research remains to be done to determine the best treatment option associated with the lowest recurrence rates Nearly 50% of cases undergo spontaneous resolution.[4,10–13]

When medicolegal aspect of mastitis is considered, whether granulomatous or other verity, the prompt, early and accurate diagnosis is important. The delay or missed diagnosis itself is the reason for potential litigation. Granulomatous mastitis usually presenting as a breast mass greatly misdiagnosed as breast cancer and core biopsy and histology are the only definitive diagnostic techniques left in absence of specific radiologic features.[14,15] Hence the role of a pathologist is important in managing these cases.

Fig. 1: Lobulocentric involvement of the lesion H&E, X40
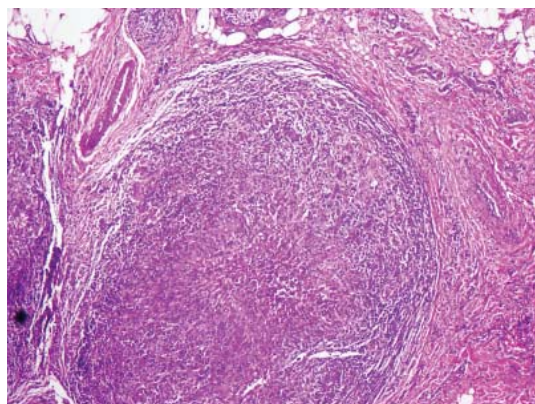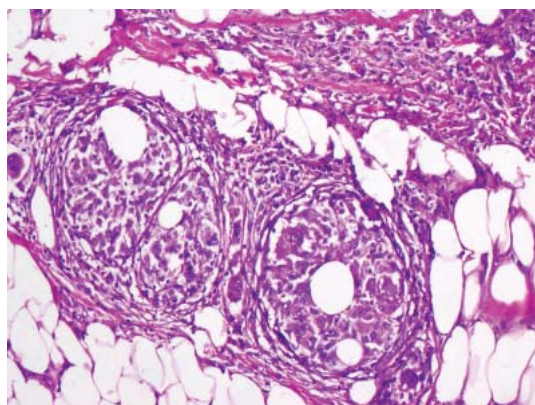

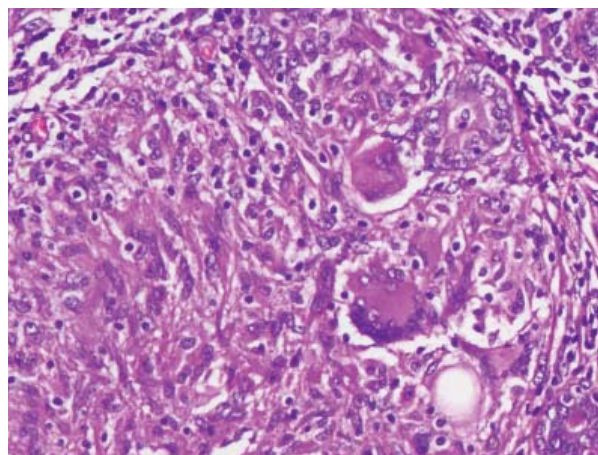**Fig. 2:** Non-caseating granulomas H&E, X100

Fig. 3: Non-caseating granuloma composed of epithelioid cells, giant cells and lymphocytes H&E, X200
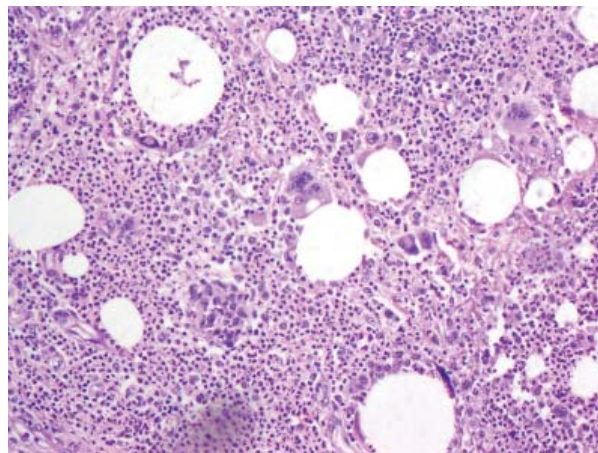

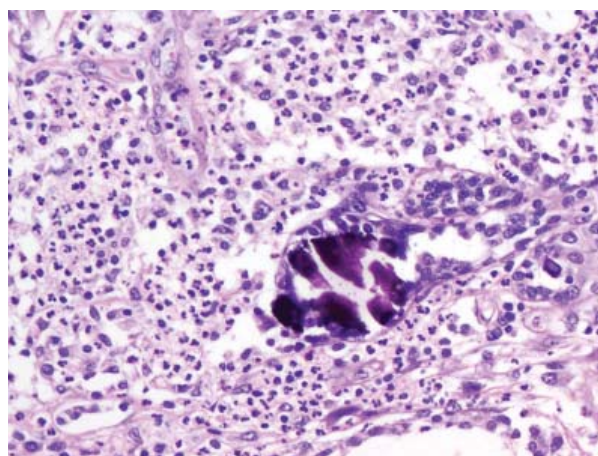**Fig. 4:** Mixed chronic inflammatory infiltrate along with giant cells H&E, X100

Fig. 5: Calcific deposit along with inflammatory cells H&E, X200

Conclusion

Granulomatous mastitis is a rare, chronic

inflammatory process with diverse modes of presentation. It generally affects young women and is of great significance in that it can mimic malignancy clinically and radiologically. The Gold standard for diagnosis is histopathology. Infectious etiology must be excluded before making a diagnosis of GM. A high index of suspicion is required to prevent misdiagnosis and unnecessary radical surgery. The exact etiology and treatment modalities are yet to be defined.

Prior publication: Nil

Conflicts of interest: Nil

Source(s) of support: Nil

Presentation at a meeting: Nil

Conflicts of interest: None to declare

Permissions: Institutional Ethics Committee permission taken

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Therapeutic Perception of Access to Medicines and Health Care in Government Hospital of Union Territory of Jammu and Kashmir

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Abstract

The therapeutic perception of access to medicines and health care in government hospital of Union Territory of Jammu and Kashmir (UToJ&K) is an empirical study of Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar in right based approach underpinned in *Patents (Amendment) Act*, 1970, *Patents (Amendment) Act*, 1999, *Patents (Amendment) Act*, 2002 and *Patents (Amendment) Act*, 2005, *Essential Commodities Act*, 1955 and *Drug Price Control Order*, 1995. On the other hand consumer law perspective are rooted in *Consumer Protection Act*, 1986, J&K *Consumer Protection Act*, 1987 and *Consumer Protection Act*, 2019. The broad parameters are access to medicines, health care system, health delivery services, patient satisfaction and utility and doctor – patient relationship. It is estimated that substantial section of population in Union Territories of Jammu and Kashmir is health deficient and medicine starved due to unavoidable contingency, spiraling cost shifting and inordinate health care infrastructures. The therapeutic perception needs a closer analysis of attendance and care of patients, diagonostic methods of treatment and access to medicine in the context of medico-legal profiling of SKIMS, Srinagar.

Keywords: Therapeutic perception; Access to medicines; Health care; Diagnostic methods of treatment; Doctor–Patient relationship.

Introduction

The access to medicines refers to the ability of all persons to receive the medicines necessary for the treatment of any condition afflicting them. It includes that these medicines are available, accessible, acceptable under physical, informational and economic access to vulnerable and marginalized sections of the population.[1] The access to medicine has been a key ingredient of desirable health policies however in India it is below 35% due to several barriers and aggravating circumstances.[2] Globally the subject is attended by *Universal Declaration on Human Rights,* 1948, *International Covenant on Economic, Social and Cultural Rights*, 1966. *The TRIPS Agreement*, 1995 flexibilities under the *Doha Declaration on Public Health,* 2001 and *United Nations Sustainable Development Goals* 2015–2030[3] and *United Nations Secretary-General's* High-Level Panel On Access *To Medicines: Promoting Innovation And Access To Heath Technologies Which Review And Assess The Situations of Health Technologies Report*, 2016.[4] The legal and intellectual property dimension of health and access to medicines in India is governed by the *TRIPS Agreement*, 1995 and the *Patents (Amendment) Act*, 1970, *Patents (Amendment) Act*, 1999, *Patents (Amendment) Act*, 2002 and *Patents (Amendment) Act*, 2005, *Essential Commodities Act*, 1955 and *Drug Price Control Order*, 1995.[5] The health care delivery system is guided by *Consumer Protection Act*, 1986, *Jammu and Kashmir Consumer Protection Act*, 1987 and *Consumer Protection Act*, 2019 in terms of consumer right awareness.[6] Such access is deemed to be part of the right to health and supplemented by *National IPR Policy*, 2016 and *National Health Policy*, 2017 in balancing the public interest in health

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care system.[7] On the ground of reality, a silent crisis is confronted by patients seeking treatment of acute and chronic diseases in India. Firstly, 40% of Indians live on less than US$1 per day and most of them pay out of pocket for using healthcare. Secondly out-of-pocket spending in India is over four times higher than public spending on healthcare. Thirdly the direct out-of pocket payments could push 2.2% of all healthcare users and one-fourth of all hospitalized patients, into poverty in a year.[8] A study has shown that patients belonging to the low income group in urban India were spending 27% of their annual income and those in rural India 34% of their annual income on diabetes care and purchase of medicines.[9] A recent study calculated the expenditure incurred on outpatient treatment of community-acquired pneumonia as a proportion of the mean per capita expenditure on food.[10] On the other hand the urban patients spent 17.6% of their mean per capita expenditure on food (rural patients spent 23.4%) on the medicines prescribed for community-acquired pneumonia.[11] The lack of access to essential medicines 348 drugs are listed in the national list of essential medicines of India give rise to unexpected illness having a catastrophic effect on the family of the ill person.[12]

Materials and Methods

The materials and methods applied for the study include analytical method of legal research by undertaking the legislative survey and scrutiny of health care laws at international, national, regional and state levels. The comparative law study of international health and consumer laws is based on established canons of statutory interpretation.[13] These laws are studied under Brint and Williams' pragmatism in law and society of Union Territories of Jammu and Kashmir.[14] While undertaking this study the behavioral approach is focused on changing risk factors and lifestyle behaviours along with the determinants approach which situates health and social problems in the broader social, structural and cultural conditions of our society and informs public health and health promotion approaches. Thus penultimately health promotion approach is the process of enabling people to increase control over, and to improve their health. The material and method reveals that health promotion work is strongly influenced by the knowledge derived from the determinants of health approach and consumer right awareness. The study partake the empirical framework of SKIMS, Srinagar a premier medical institution in the UToJ&K in terms of access to medicines, health care system, health delivery services, patient satisfaction and utility, doctor–patient relationship.

Results

The health care services in the UToJ&K are important not only for human resource development, but also for restoring the faith of the people in the institutions of governance. At present there are 5,534 health institutions (4,433 governments and 1,101 private) functional in the UToJ&K. Among these there are two notable medical colleges, namely Government Medical College, Bakshinagar, SKIMS whereas four new medical colleges have been set up in Anantnag, Baramulla, Kathua and Rajouri districts of the state.[15] (Fig. 1).
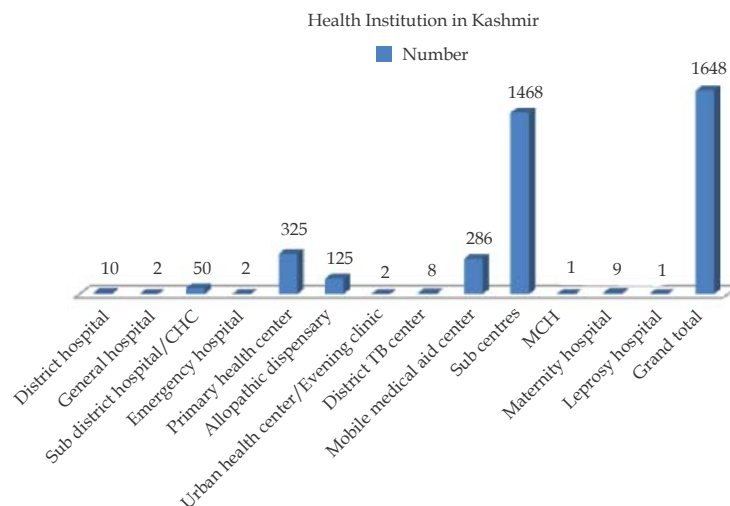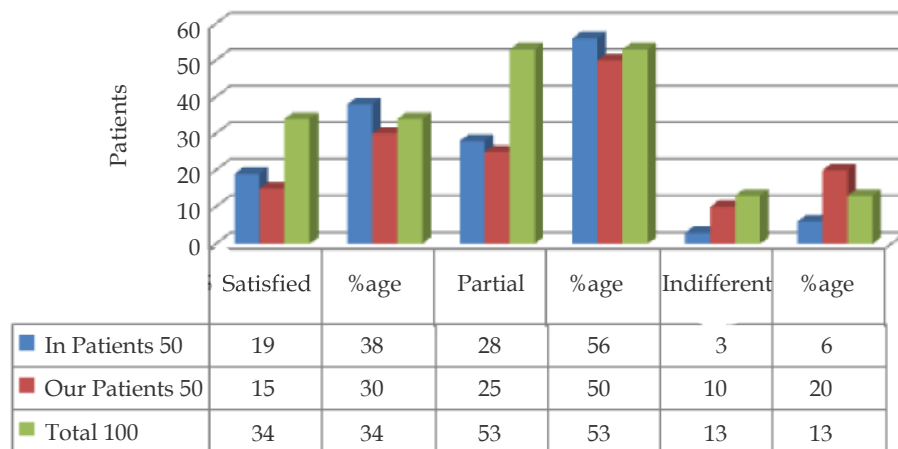

Fig. 1: Health Institution in Kashmir
Source: http://jkhealth.org/new2017/

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Satisfaction towards health care delivery: To assess the health care services and patient satisfaction a survey of 100 patients admitted to SKIMS was conducted regarding by applying randomized sampling method. The following Table 1 and Chart 1 shows the responses of patients having varying degres of satisfaction towards medical care in SKIMS.

In health care services the patient satisfaction is an important and commonly used indicator for measuring the quality in health governance. The above table clearly shows that 34% respondents were quite satisfied and 53% respondents said that they are partially satisfied. When we asked patients about the medical care they received 13% respondents is oblivious of any opinion on the subject.

Table 1 & Chart 1: Satisfaction towards health care delivery

	Satisfied	%age	Partial	%age	Indifferent	%age
■ In Patients 50	19	38	28	56	3	6
■ Our Patients 50	15	30	25	50	10	20
■ Total 100	34	34	53	53	13	13

Source: *Field work*

Patient–Doctor relationship and health care: The attendance and attention constitutes an important segment of health care system under patient–doctor relationship.[16] The adequacy of time given to patients presumably considered indices for better for health care and staisfaction to the patients.[17] A simple question put to patients as to whether doctors devote adequate time to a patient during diagonostic treatment and therepeutic perception Table 2 and Chart 2.

The patients interviewed while undergoing the treatment in SKIMS reveals that the majority of respondents 51% agree that doctors devote adequate time to a patient during the treatment and 28% opined that the doctors donot give their adequate time while treating patients. However 21% respondents are not very circumspect to doctors diligence.

Table 2 & Chart 2: Patient–Doctor relationship and health care

	Yes	%age	No	%age	Indiff-erent	%age
■ In patients 50	26	52	16	32	8	16
■ Our patients 50	25	50	12	24	13	26
■ Total 100	51	51	28	28	21	21
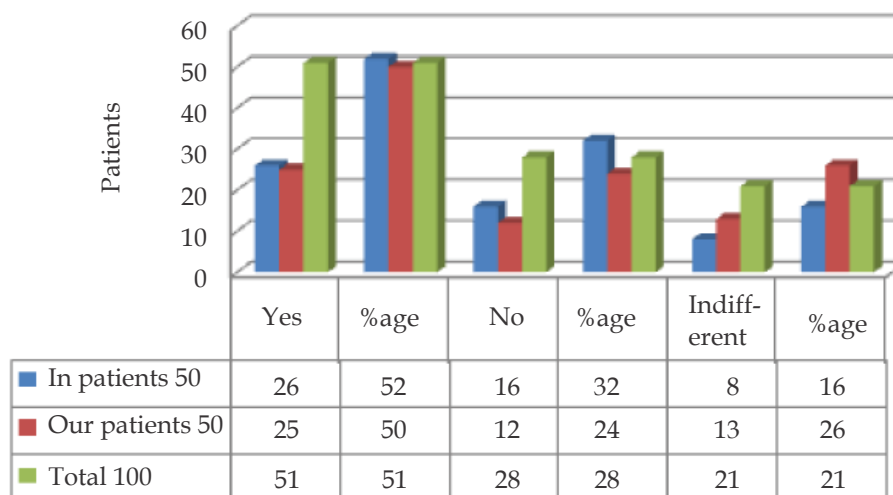
Source: *Field work*

Disease burden and doctors circumspection: The disease burden, appointment and availability with doctors represents uneven ratio in the hospitals. The enormity of outpatient department receiving medical services at appropriate time happens to be the central inquiry while getting treatment in SKIMS Table 3 and Chart 3.
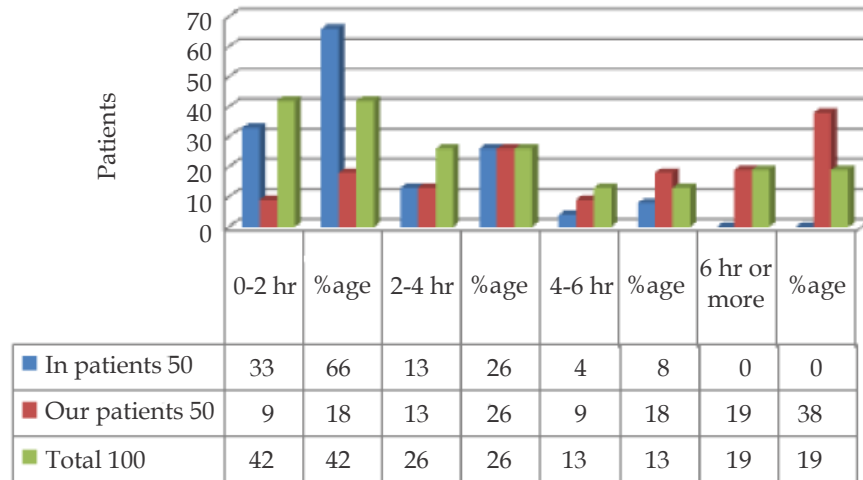
Nobody disagrees to the proposition that the time and health are two precious assets that we

don't recognize and appreciate until they have been depleted. The survey on this count reveals that 42% said that they had to wait for 2 hours for getting admitted in hospital to avail medical services, 26% respondents said that they waited for 2 to 4 hours for availing medical services, and 13% respondents waited for 4–6 hours. However, 19% respondents were waiting for more than 6 hr while receiving medical services in hospital.

Table 3 & Chart 3: Disease burden and doctors circumspection

	0-2 hr	%age	2-4 hr	%age	4-6 hr	%age	6 hr or more	%age
In patients 50	33	66	13	26	4	8	0	0
Our patients 50	9	18	13	26	9	18	19	38
Total 100	42	42	26	26	13	13	19	19

Source: Field work

Diagnostic and prognostic line of treatment: The enhancement of the accuracy of the diagnosis and prognosis are the key determinants of doctor–patient relationship. The patient's knowledge about the disease and medical tests are to be done in holistic health care framework. The simple question as to whether doctors are good in explaining the reason for conducting medical tests in auguring medication and treatment in their diagnosis

and prognosis Table 4 and Chart 4.

The patient interviewed regarding their response to medical tests revealed that the majority of respondents 53% are pretty satisfied with the doctors explaining the reason for conducting medical tests and 33% shows that the doctors don't explain reasons for medical tests. 14% respondents are either ignorant or indifferent about doctors explaining reasons for medical tests.

Table 4 & Chart 4: Diagnostic and prognostic line of treatment
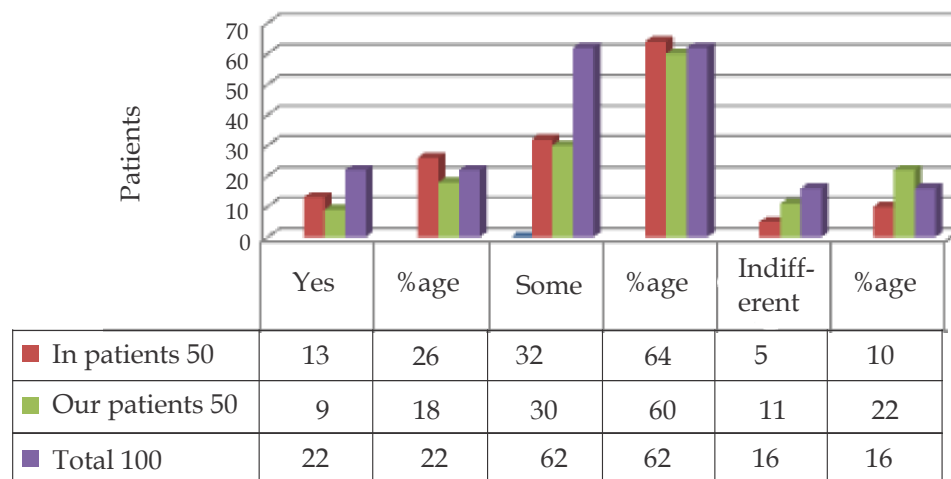
	Yes	%age	No	%age	Indifferent	%age
In patients 50	28	56	18	36	4	8
Our patients 50	25	50	15	30	10	20
Total 100	53	53	33	33	14	14

Source: Field work

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Access and availability of medicines: In the
entire process of health care system, the access
to medicine is pivotal to health and well being of
people. The medicines provided by government
hospitals on subsidized rates to needy and poor
people are an important benchmark. The aim and
objective of medicine delivery on subsidized rates
to poor patients is all the more important for health
right and equity because the exorbitant rates of
medicine and diagnostic treatment often pushes

them into destitution and misery Table 5 and Chart
5.

The question related to access to medicine by
patients at subsidized rates at government hospitals
revealed that 22% respondents received medicines
on subsidized rates whereas bulk of respondents
62% said that they are deprived of access to such
medicines. However, 16% respondents are either
ignorant or indifferent about their entitlement and
access to medicines.

Table & Chart 5: Access and availability of medicines

	Yes	%age	Some	%age	Indiff-erent	%age
■ In patients 50	13	26	32	64	5	10
■ Our patients 50	9	18	30	60	11	22
■ Total 100	22	22	62	62	16	16

Source: Field work

Discussion

The therapeutic perception of access to medicine
and health care UToJ&K discerns multiple
approaches and perspectives namely the biological
approach, biomedical approach, primary health care
approach and public health approach in advancing
equity, access, empowerment and preventing
epidemiology and biostatistics to health protection.

Access to medicines in J&K: According to the
World Health Organization (WHO), an estimated
649 million people in India do not have regular access
to essential medicines. The median availability of
30 essential medicines in six states in India varied
between 0 and 30%. Therefore, patients are forced
to buy medicines from the private market despite ill
affordability and sharing burdens of sickness and
healthcare costs.[18] The study has also documented
that of the rising out-of-pocket expenditures on
healthcare, which pushes an estimated 32–39 million
people below the poverty line annually more than
70% of expenditure was incurred on purchase
of medicines. Every year, UToJ&K consumes
medicines worth ₹. 600 cr, of which ₹ 400 cr is spent
in Kashmir alone. 63% of J&K's population do

not have purchasing power for medicines. 90.39%
purchase drugs through out-of-pocket payments.[19]
The study has shown that out-of-pocket costs were
lowered significantly among patients who were
prescribed generic medicines compared to patients
who were given branded drugs.

Jammu & Kashmir drug policy: It is under this
backdrop, the UToJ&K framed a policy to provide
free medicines in all government health facilities.
The State Administrative Council (SAC) formulated
the *Free Drug Policy*, 2012 mandating all government
hospitals to provide essential and generic medicines
free of cost to patients based on prescriptions by
government doctors.[20] The procurement of quality
drugs and timely supply by Health and Medical
Education Department and Drug and Food Control
Organization promote equitable, affordable and
quality health care. However constraints of capacity
and commitment need to be revamped because of
persistent disturbances and unrest since 1989.[21]

Health care delivery system: The health care
delivery system is one of the worst hit services. The
exodus of health care professionals from the valley
created a vacuum during early 1990s adversely
affecting the basic health services. The inadequate

health infrastructure, exodus of health care professionals coupled with lack of good governance has led to collapse of health care delivery systems.[22] The emergency care including trauma and disaster management service is available only in Srinagar and Jammu cities. Towns and rural areas have hardly any such facility and have to transport the patients to long distances which many times results in avoidable deaths on the way. The golden hour is lost in these long distances. Thus the improper implementations of national health programmes are highly discouraging because of accessibility of remote areas for communities like Gujjars and Bakarwals.[23]

Conclusion

The objectives of health for all enunciated the *National Health Policy* in 1983 in the context of UToJ&K thrust upon preventive and rehabilitative health care services at primary, secondary and tertiary level. The constraints in the improvement of health status of the people included lack of financial resources, dearth of technical staff, and inadequate health infrastructure. Recently the abrogation of Articles 370 and 35A of the *Constitution of India*, 1950 and Constitution of Jammu & Kashmir, respectively opened new vistas by for setting up of two medicities in Jammu and Kashmir. The facilities expected in the medicities includes medical colleges and hospitals, super specialty centres of excellence, nursing, pharmaceuticals, hospital management and dental colleges, ayurvedic colleges and hospitals and medical education hubs, AYUSH centers, research centers with residential areas, staff quarters and guest houses, etc. under these circumstances the private sector presence will increase manifold and patients will be forced to overpriced medicines. This makes public health system in UToJ&K more daunting in the context of health equity and governance. This is more glaring evidence of poor budgetary provision, lack of a comprehensive policy, and feeble regulatory framework to access to medicines and health care delivery in UToJ&K.

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Histopathological Study of Liver Lesions in Medicolegal Cases

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Abstract

Background: Autopsy study of liver aids in the knowledge of pathology by revealing lesions that were asymptomatic during life. Due to enormous functional reserve, many liver lesions are silent till late in the course of disease. Hence histopathological study of all liver specimens is necessary. *Material and methods:* The present study included 260 liver specimens from medicolegal autopsies received over a period of two years from August 2014 to July 2016. Thorough gross examination was done followed by microscopy. *Result:* The most common lesion was steatosis, accounting for 78 (30%) out of 260 liver specimens. There were 43 (16.5%) cases of congestion, 20 (7.8%) cases of hepatitis and 17 (6.5%) cases of steatohepatitis. Ten (4%) cases of cirrhosis were noted. Other lesions included microabscesses 7 (2.6%), granulomas 6 (2.3%), and one case (0.3%) each of bile duct hamartoma, sickle cell anemia, disseminated intravascular coagulation, extramedullary hematopoiesis, disseminated cryptococcosis and microfilarial infection. *Conclusion:* The present study showed that fatty change was the most common lesion encountered in liver specimens from medicolegal cases, followed by congestion and hepatitis. Hence histopathology of every liver specimen is must.

Keywords: Medicolegal autopsy; Fatty change; Cirrhosis.

Introduction

The liver is the largest organ of human body.[1] On the basis of blood flow, the functional hepatic acinus has three zones, peripheral zone one, perivenular zone three and intermediate zone two.[2] The hepatocytes perform numerous and vital functions like synthesis of serum proteins, production of bile, regulation of nutrients and metabolism of drugs.[1] Diseases affecting the liver are infectious diseases like viral hepatitis, tuberculosis, malaria, metabolic diseases like nonalcoholic fatty liver disease, Wilson, disease, cirrhosis and neoplasms.

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Received on 15.11.2019, **Accepted on** 03.01.2019

Cirrhosis is a progressive disease characterized histopathologically by diffuse fibrosis leading to formation of regenerative nodules. Etiologies of cirrhosis include alcoholism, chronic viral hepatitis, metabolic diseases, etc.

Primary benign neoplasms of liver include hemangioma, hepatic adenoma, hamartoma and others while primary malignant tumors include hepatocellular carcinoma, cholangiocarcinoma, angiosarcoma, hepatoblastoma, etc. Metastatic malignancy is more common than primary hepatic neoplasms. Common primary sources include colon, breast, lung and pancreas.[2] Hence a study was undertaken on liver specimens received from medicolegal autopsies to estimate the frequency and analyze the histopathological features of various liver diseases.

Materials and Methods

Total 294 liver specimens from medicolegal autopsies were received over a period of two years

from August 2014 to July 2016. Thirty-four specimens were excluded on account of extensive autolysis. Hence the study included 260 cases. Tissue was fixed in 10% formalin. Representative tissue bits were submitted for paraffin embedding. Sections were routinely stained by hematoxyline and eosin. Special stains were used wherever necessary. Sections were studied under light microscope and findings of the examination were recorded and analyzed.

Results

The age ranged from less than 1 year to 90 years. As depicted in (Fig. 1), majority of the cases were seen in the age group 21–30 years (80 cases, i.e. 28.6%). Number of males was 168 (64.6%) much higher than females (92 cases, 35.4%) with male : female ratio of 1.8 : 1. Various lesions encountered in the study and their prevalence is presented in Table 1. The most common lesion was steatosis (fatty change) seen in 78 (30%) cases affecting 61 males and 17 females. All cases showed macrovesicular steatosis. History of alcohol consumption was available in 17 males. Congestion was seen in 43 (16.5%) cases affecting 23 males and 20 females. We had total 20 (7.8%) cases of hepatitis, including 13 cases of nonspecific reactive hepatitis, five cases of ischemic hepatitis and two cases of acute hepatitis. There were 17 (6.5%) cases of steatohepatitis characterized by ballooning degeneration of hepatocytes, pericellular

Table 1: Showing histopathological findings of liver and their prevalence

Histopathological findings	Cases	Percentage
Steatosis	78	30%
Congestion	43	16.5%
Hepatitis	20	7.8%
Acute hepatitis	2	0.7%
Ischaemic hepatitis	5	1.9%
Nonspecific reactive hepatitis	13	5.2%
Steatohepatitis	17	6.5%
Cirrhosis	10	4.0%
Microabscesses	7	2.6%
Hepatic granulomas	6	2.33%
DIC	1	0.33%
Disseminated cryptococcosis	1	0.33%
Disseminated microfilarial infection	1	0.33%
Bile duct hamartoama	1	0.33%
Sickle cell anaemia	1	0.33%
Extramedullary hematopoiesis	1	0.33%
No specific lesion	73	28.0%
Total	260	100

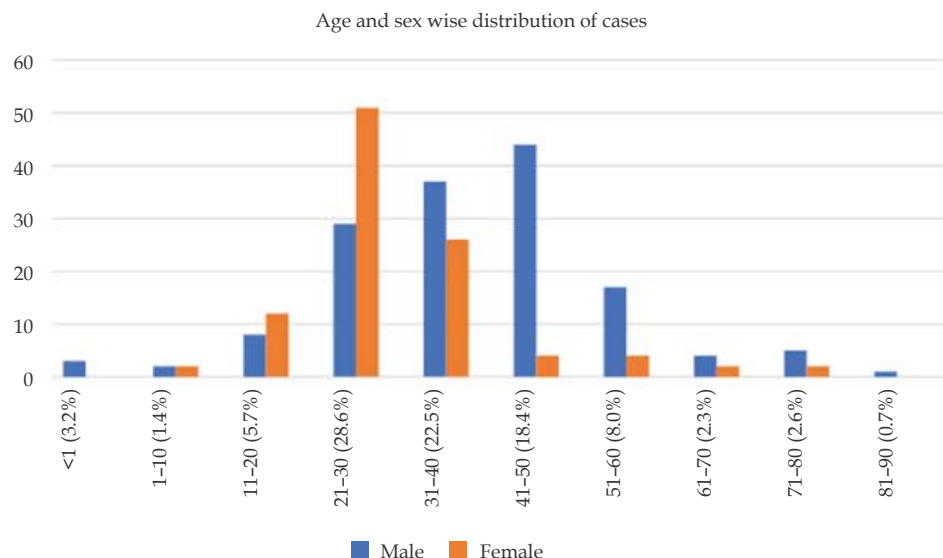Age and sex wise distribution of cases

Fig. 1: Showing age and sex distribution of medicolegal cases in the present study.

polymorphonuclear infiltrate and chickenwire fibrosis. Only two males with steatohepatitis had history of chronic alcoholism. Ten (4%) cases of cirrhosis were seen affecting 7 males and 3 females. History of alcohol consumption was present in two males. We had 7 (2.6%) cases of microabscesses and 6 (2.33%) cases of hepatic granulomas. There was one case each of disseminated intravascular coagulation, disseminated cryptococcosis, microfilarial infection, bile duct hamartoma, sickle cell anemia and extramedullary hematopoiesis.

Discussion

Liver diseases are an important cause of morbidity and mortality in both developed and developing countries. The incidence as well as pattern of liver disease varies from one region to another depending on the various etiological factors. Thus, a study of liver specimens from medicolegal autopsies was undertaken to evaluate the prevalence and relative frequency of various types of liver diseases. Excluding 34 specimens with extensive autolysis, 260 specimens were included in the study.

Steatosis (Fatty change) refers to accumulation of triglycerides in the cytoplasm of hepatocytes. Microvesicular steatosis is characterized by very small, fine fat globules that do not displace the nucleus and is a result of mitochondrial injury [3]. In macrovesicular steatosis the nucleus is displaced to the periphery. The degree of fat accumulation is variable. In the present study, 78 out of 260 medicolegal cases showed macrovesicular fatty change. Comparison of our findings with other authors is shown in Table 2.

In the study done by Selvi et al., 29 out of 108 (26.9%) cases showed fatty liver. Alagarsamy et al. reported 10 out of 50 cases (20%) while Umesh

reported 24 out of 105 (22.8%) cases of steatosis. Patel et al. observed 146 (35.69%) cases of steatosis out of total 450 cases. In the study conducted by Bal et al., steatosis was seen in 39 (46.9%) cases.[4–8]

Right sided cardiac decompensation leads to passive congestion of the liver. The liver is slightly enlarged, tense and cyanotic, with roughened edges.[9] Microscopically there is congestion of centrilobular sinusoids. In the present study 43 (16.5%) cases showed congestion. In the study done by Selvi et al., there were 18 out of 108 (16.7%) cases of congestion while Alagarsamy et al. observed congestion in 13 out of 50 cases (26%).[4,5]

We had total 20 cases of hepatitis, including 13 cases of nonspecific reactive hepatitis, five cases of ischaemic hepatitis and two cases of acute hepatitis. In nonspecific reactive hepatitis there is no uniform zonal distribution of the parenchymal changes and only some portal tracts are involved. The involved portal tracts contain variable chronic inflammatory cell infiltrate with predominance of lymphocytes. The limiting plate is intact. Parenchymal changes include foci of liver cell necrosis, which may involve only few hepatocytes or several liver cell plates. Surrounding these foci, there is accumulation of lymphocytes and macrophages.[10] We had 13 cases of nonspecific reactive hepatitis.

Ischemic hepatitis or shock liver is a manifestation of liver injury due to reduced blood flow. It is seen in acute myocardial infarction, circulatory shock due to sepsis, burns, severe trauma, vascular obstruction and other causes. We observed five cases of ischemic hepatitis characterized histologically by coagulative hepatocytic necrosis and marked perivenular sinusoidal congestion (Fig. 2.).

Table 2: Showing the comparison of liver diseases by various authors.

| Pathology	R.Thamil Selvi et al.[4] (2011)	Alagarsamy J et al.[5] (2012)	Umesh BR et al.[6] (2015)	Patel PR et al.[7] (2016)	Present Study (2016)
Total cases	108	50	105	450	260
Fatty change	29 (26.9)	10 (20%)	24 (22.8%)	146 (35.69)	78 (30.0%)
Congestion	18 (16.7%)	13 (26%)	10 (9.52%)	5 (1.22%)	43 (16.5%)
Hepatitis Nonspecific reactive Ischaemic Acute	15 (13.9%)	5 (10%)	22 (20.9%)	4 (0.98)	20 (7.8%)
Steatohepatitis	—	—	37 (32.2%)	—	17 (6.5%)
Cirrhosis	8 (7.4%)	8 (16%)	2 (1.9%)	10 (2.44%)	10 (4.0%)
Microabscesses	8 (7.4%)	—	—	—	7 (2.6%)
Hepatic granulomas	—	—	4 (3.8%)	2 (0.49%)	6 (2.3%)
Others	—	—	—	—	6 (2.3%)
No specific lesion	28 (25.9%)	3 (6%)	5 (4.76%)	233 (56.97%)	73 (28.0%)
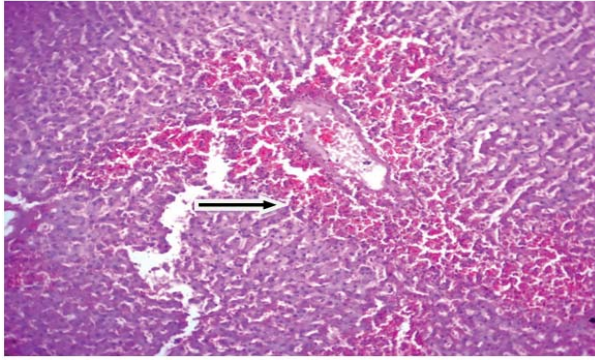
Fig. 2: Photomicrograph showing ischaemic hepatitis with centrilobular hemorrhagic necrosis of liver (H &E, x100)

Acute hepatitis is an inflammation of liver caused by infectious agents including hepatotropic viruses, certain medications or autoimmune etiology. We had two cases of acute hepatitis showing lobular disarray, ballooning of hepatocytes and mononuclear cell infiltrate in portal tracts and periportal parenchyma.[3] Prevalence of hepatitis in various studies ranged from 0.98% to 20.9%.[4-7] Selvi et al. reported 15 out of 108 (13.9%) cases, Algarsamy et al. reported 5 out of 50 (10%) cases, Umesh reported 22 out of 105 (20.9%) cases and Patel PR et al. reported only 4 (0.98%) out of 450 cases of hepatitis.

The essential features of steatohepatitis, i.e. ballooning degeneration of hepatocytes, inflammatory infiltrate and pericellular fibrosis were seen in 17 (6.5%) cases. Mallory–Denk bodies were however not seen in any of the cases. Common causes of steatohepatitis are alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD).[3,10] There is a significant difference in the prevalence of steatohepatitis in different studies. Selvi et al., Alagarsamy et al. and Patel et al. did not come across a single case of steatohepatitis in their study.[4,5,7] In the present study 17 (6.5%) cases of steatohepatitis were observed, while Umesh BR et al. reported steatohepatitis in 37 (32.2%) out of 105 cases.

Liver cirrhosis is a common end-stage liver disease characterized by diffuse hepatic fibrosis with replacement of normal lobular architecture by parenchymal nodules separated by bands of fibrous tissue. Morphologically liver cirrhosis is classified as Micronodular cirrhosis (nodules less than 3 mm), Macronodular cirrhosis (nodules more than 3 mm) and mixed cirrhosis.[3,11,10] In our study 10 cases of cirrhosis were observed out of which 7 were males and 3 were females. History of alcohol consumption was present in 2 males showing mixed nodular cirrhosis. Remaining eight

cases showed micronodular cirrhosis. However no specific cause could be identified in these cases. Selvi et al. and Algarsamy et al. observed cirrhosis in 8 cases each accounting for (7.4%) and (16%) cases respectively.[4,5] Umesh et al. reported 2 out of total 105 (1.9%) cases and Patel et al reported 10 out of 450 (2.2%) cases of cirrhosis.[4-7]

A multitude of organisms can infect the liver and biliary tree including bacteria, fungi, helminths and protozoa. In many cases of pyemic abscesses, the origin of the infection is not obvious.[10] We had 7 cases of microabscesses affecting one male and six females. There were multiple scattered 1–2 cm sized microabscesses in the liver along with mild to moderate cholestasis.All the females were postpartum with evidence of acute deciduitis and myometritis along with microabscesses in the spleen, kidney and lung in addition to liver suggesting puerperal sepsis. The single male patient had bronchopneumonia with microabscesses in liver, spleen and heart indicating sepsis. Selvi et al. observed microabscesses in 8 out of 108 cases (7.4%)[4]. Other studies have not reported microabscesses.

Hepatic granulomas may occur secondary to infections like tuberculosis or fungal infections, drugs, foreign bodies or immunologic, neoplastic and idiopathic causes.[12] We had 6 (2.3%) cases of hepatic granulomas. Microscopy showed numerous randomly distributed granulomas composed of occasional Langhan's type giant cells, epithelioid cells and lymphocytes. In 3 cases there was central caseous necrosis indicating tuberculosis as the most likely cause (Fig. 3).
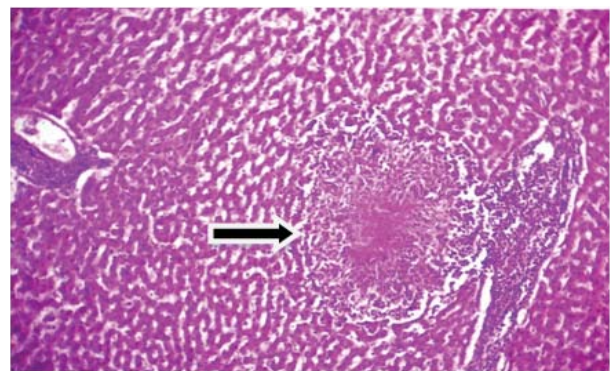

Fig. 3: Photomicrograph showing hepatic granuloma with central caseation suggestive of tuberculosis (H &E, x100)

However Ziehl Neelsen staining was negative for acid–fast bacilli. Liver may be involved in primary or reactivation tuberculosis, either alone or as a part of multiorgan involvement.[13] In remaining 3 cases definite cause of granuloma could not be identified. Patel et al. reported 2 cases of granulomas out of

total 450 cases (0.49%), while Umesh et al. observed 4 cases of granuloma out of 105 cases (3.8%).[6,7]

DIC is an acute, subacute or chronic thrombohemorrhagic disorder characterized by excessive activation of coagulation, which leads to the formation of thrombi in the microvasculature of the body. DIC is not a primary disease but occurs as a secondary complication of a variety of disorders such as septicemia, allergic reactions, liver cirrhosis, acute fatty liver, vasculitis, polytrauma, aortic aneurysm and obstetric complications.[2] In our study, we had a single case of DIC affecting a 25-year-old primigravida with history of nine months amenorrhea. Patient was admitted with shock, severe hypotension, and thrombocytopenia following cesarean section. Grossly, the external surface and cut surface of liver showed few blackish areas. On microscopy, there was subcapsular hemorrhage along with sinusoidal fibrin deposition in periportal areas. Capillary thrombi were seen in the lungs and kidney. Microhemorrhages were evident in the epicardium, myocardium and lung, features suggestive of DIC.

We came across a single case of disseminated cryptococcosis in a 42-year-old HIV positive male. Cryptococcosis more frequently presents as an opportunistic infection in immunocompromised people.[13,11] Grossly, the external and cut surface of liver was unremarkable. Microscopically, sections showed disseminated cryptococcal infection involving liver (Fig. 4.) and other organs like meninges, lung, spleen and kidney.
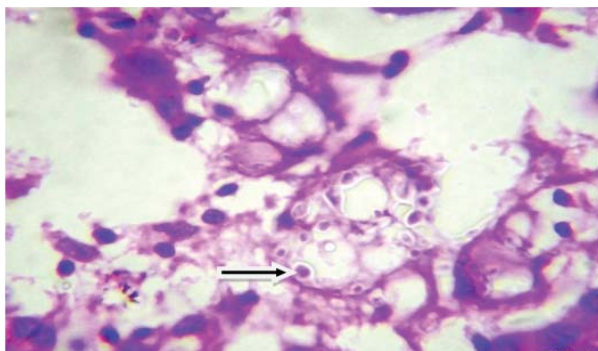

Fig. 4: Photomicrograph showing yeast forms of cryptococcus (H &E, x1000)

Nematode *W. Bancrofti* is a cause of lymphatic filariasis in tropics and subtropics. It is an adult worm which inhabits the lymphatics and produces lymphangitis and lymphadenitis. The best known clinical manifestation is tropical pulmonary eosinophilia.[11] We had a rare case of disseminated microfilarial infection. Patient was a 30-year-unknown male. Hence detailed history was not available. Grossly, all the organs were unremarkable. Microscopically, there were eosinophilic microabscesses and fibrin- rich inflammatory exudate containing microfilariae in the liver, spleen and kidneys.

Bile duct hamartomas also known as Von Meyenberg complexes or biliary microhamartomas are multiple biliary channels lined by bile duct epithelium set in a dense fibrous stroma. The lumen often contains inspissated bile. They are usually found incidentally and do not give rise to symptoms or abnormalities of liver function[3,13]. In the present study, we found a single case of biliary microhamartoma incidentally in a 70-years-old male (Fig. 5).
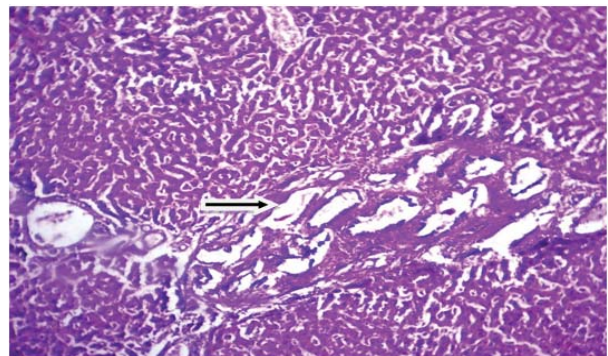

Fig. 5: Photomicrograph showing biliary microhamartoma of liver (H &E, x100)

We encountered a single case of sickle cell anemia in a 25-year-old pregnant lady with 32–33 week gestation with eclampsia and severely deranged liver function tests. Patient delivered a fresh still born female baby and died on the next day. Grossly, liver was dark brown to blackish in appearance. Microscopically, blood vessels of all organs as well as liver sinusoids revealed sickled RBCs.

Extramedullary hematopoiesis refers to hematopoiesis that occurs in organs other than bone marrow. Normal erythropoiesis occurs in fetal yolk sac, liver and spleen. We came across a single case of extramedullary hematopoiesis in the liver in a 3-day-old neonate.

Conclusion

Autopsy based studies are useful in estimating the prevalence of liver diseases which are often asymptomatic till late in the course of disease. Histopathological study of liver specimens enables to diagnose primary liver diseases like hepatitis,

steatohepatitis and cirrhosis as well as systemic diseases like DIC. Macrovesicular steatosis was the most frequently encountered lesion in this study.

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Carrea's Index: A Reliable Tool for Estimation of Stature?

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Abstract

Introduction: The teeth are one of the durable parts of our body which can withstand more assaults than any other part of the body and there exists a relation between tooth crown length and body height. The study aims to determine the possible correlation between tooth dimensions and stature estimation using Carrea's index. *Materials and methods:* The study group comprised about 67 subjects above 18 years. Alginate impressions were made for mandibular arch and height was assessed. The mesiodistal widths of mandibular anterior teeth were measured and substituted in the formula given by Carrea to obtain the minimum and the maximum estimated height of a person. *Results:* On comparison of the Carrea's index in right side, females showed 9.3% successful prediction, on left side 8.3% showed successful prediction in males than in females. The height was accurately predicted when average values of left and right side were considered. Females showed 55.8% accuracy and males showed 37.5% which was statistically significant. *Discussion:* Teeth could be used as a reliable source for stature estimation especially in those forensic cases where other body parts are not available. In the present study the average values from right and left side were considered for both genders to predict the height of the individual. Our findings could be explained on the basis that perfect bilateral symmetry seldom exists on right and left side arches which could be due to congenital or environmental factors or both. The Carrea´s index for stature estimation is a convenient, simple and inexpensive method, and can provide valuable information to the forensic investigation.

Keywords: Carrea's index; Stature; Forensic Odontology.

Introduction

The teeth are one of the most durable parts of our body which can withstand more assaults than any other part of the body. This is particularly useful in the identification of bodies in mass disasters and natural calamities. The principal advantage of dental evidence is that, like other hard tissues, it is often preserved after death. Forensic odontology is primarily concerned with the use of teeth and oral structures for identification in a legal context.

The four essential factors usually represented in determining personal identification are age, sex, stature and ethnicity. Among this 'big fours'[1] of the biological profile, determination of stature is considered as one of the main parameter of personal identification in forensic examinations. Stature is the height of a person in the upright posture and has a definite and proportional biological relationship with each and every part of the human body which helps the forensic experts to identify along with other evidences like dentition, footprints and hand dimensions.[1]

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There exists a possible relation between tooth crown length, especially of the anterior teeth, and the facial and body height. The study aims to determine the possible correlation between tooth dimensions and stature estimation using Carrea's index. Carrea has proposed an index to estimate the stature of an individual based on the measurements made from mandibular anterior teeth.[2]

Materials and Methods

The study group comprised about 67 subjects of age group above 18 years after their informed consent and after Institutional ethical committee approval. Subjects with intact mandibular dentition, with normal growth and development were included in the study. Subjects were excluded if they had restoration in mandibular anterior teeth, malocclusion, those who underwent orthodontic treatment and who were physically and mentally challenged.

Measurement of Height

The measurements of height was made using standard anthropometer by making the subject stand erect on the horizontal plane, barefooted, in the anatomical position according to the Frankfort plane, aligning the posterior surface of heels, pelvic girdle, scapular girdle, and occipital region to the vertical plane. The distance of the subject from the ground to the highest point of the vertex in the median sagittal plane were recorded.[3]

Odontometric measurements for Carrea's index

The patient plaster models mandibular arches of each subject were obtained using alginate impressions. For the Carrea's index, the mesiodistal widths of mandibular central incisor, lateral incisor and canine were recorded from the labial aspect and summed using a digital caliper. This is termed the 'ARCH'. Linear distance between the ends of the arch, represented by the mesial edge of central incisor and the distal edge of canine on the same side, measured on the lingual surface with a digital caliper. This is termed as 'CHORD' [Fig. 1]. The maximum and minimum statures of an individual were estimated according to Carrea's index as follows:

Formula:

$$\text{Maximum stature} = \frac{\text{arch (in mm)} \times 6 \times 3.1416 \times 100}{2}$$

$$\text{Minimum stature} = \frac{\text{chord (in mm)} \times 6 \times 3.1416 \times 100}{2}$$

Fig. 1: Arch and chord dimensions

The chord and arch values for each tooth were substituted in the formula given by Carrea to obtain the minimum and the maximum estimated height of a person. These values were compared with real stature.[2]

Successful prediction: The real stature measurements coincided with the interval between the minimum (chord) and maximum (arch) estimated stature measurements (tooth dimensions).

Unsuccessful prediction: The real stature measurements which does not coincide with the interval between the minimum (chord) and maximum (arch) estimated stature measurements (tooth dimensions).[3]

Statistical Analysis

The data was analyzed using the SPSS software (version 20). The accuracy of the height measurement was predicted if the actual height was between the minimum and maximum height predicted values. The frequencies of accurate height assessment were described in proportions and comparison according to gender was done using chi-square test. Pearson's correlation coefficient was used to assess the correlation of the predicted height (left, right and average) with the actual height. A *p*-value of <0.05 was taken as statistically significant.

Results

A total of 67 samples were selected which included 43 females and 24 males. Right side and left side hemi arches were measured separately. Figure 2 showed the distribution of successful and unsuccessful predictions of arches according to sex.
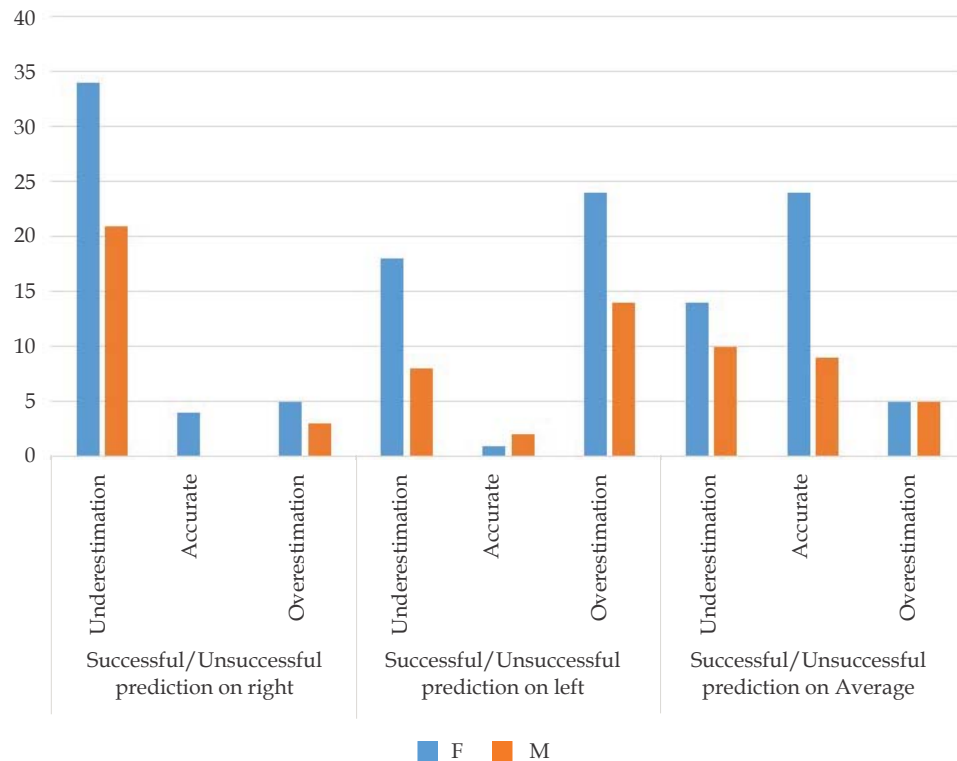
Fig. 2: Distribution of successful and unsuccessful predictions of arches according to sex.
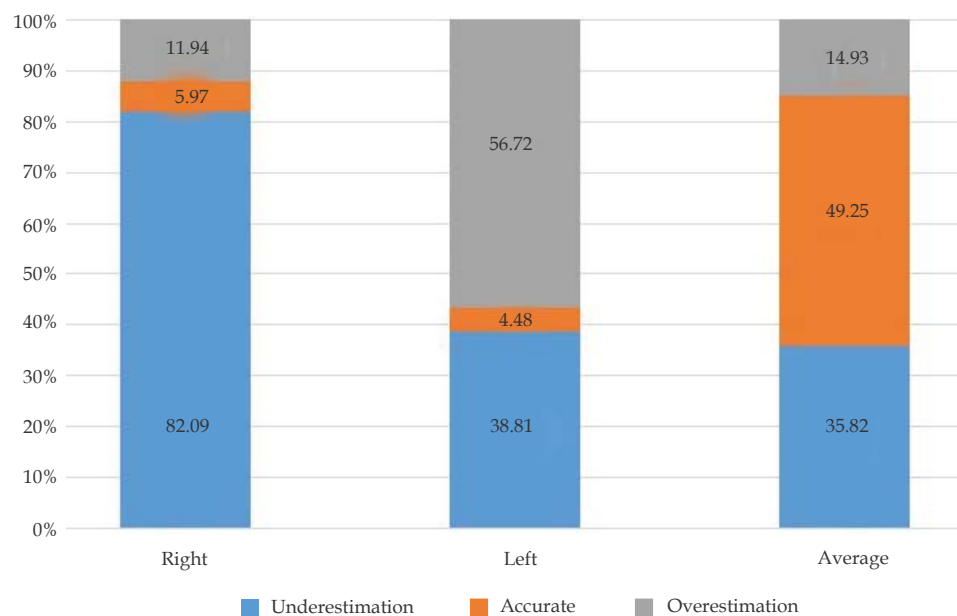

Fig. 3: Average values of left and right side arches

When the right sides were compared in males and females, females showed 9.3% successful prediction than males. Whereas on the left side 8.3% showed successful prediction that was seen in males than females (Fig. 2).

The height was accurately predicted when average values of left and right side were considered

females showed 55.8% accuracy and males showed 37.5% which was statistically significant (Fig. 3).

Discussion

Dental measurements and stature can be useful in anthropology and forensic identification. Teeth

could be used as are liable source for stature estimation especially in those forensic cases where other body parts are not available. Long bones are considered for stature estimation. In many investigations of human remains, not all the bones are present; possibly nothing but the skull and mandible may be recovered. Therefore examination of skull and teeth becomes very important, and the stature of the subject can still be estimated based on the proportionality with tooth dimensions.[4]

In this study subjects belonging above 18 years were considered. The growth spurt is almost completed by 18 years of age ensuing the completion of stature and mandibular growth, hence 18 years was selected as the lower limit whereas 30 years was taken as the upper limit in the study.[5]

In the present study right side and left side semi arches were measured separately in both males and females. Females showed 9.3% successful predilection on right side and 2.3% on left side, whereas males showed 8.3% successful predilection on left side than right side which was not considered to be significant. But when the average values from right and left side was considered the height of the individual could be predicted.

According to *Rayapureddy Sruthi,* she explained that there were a correlation between teeth and stature, as both dentin that forms the bulk of the tooth and bones that determines the height are derived from mesenchymal tissue (Dentin-Ectomesenchyme; Long bones-Mesoderm) there could be an embryological relationship between tooth formations and long bones and also have similarities in structural composition. Henceforth, it is presumable to accept the mere relationship between teeth and stature exists.[3]

Our findings could be explained on the basis that perfect bilateral asymmetry seldom exists in living organisms. Always right and left side differences are present in nature. Right-left differences occur everywhere in nature where two congruent types are present. According to *Maen Mahfouz* in humans there is functional as well as morphological asymmetries, e.g. right and left handedness as well as preference for one eye or one leg. Some of these asymmetries are embryonically rooted and are associated with asymmetry in the central nervous system.[6]

Asymmetry of tooth size in right and left side would be due to congenital or environmental factors or both of them; All the asymmetries are divided in two classes: quantitative asymmetry or difference in number of teeth in each half-arch and qualitative asymmetry, which is due to difference in size of teeth mesiodistal width or their location in the dental arch.[7]

Dental asymmetries can be caused by local factors such as early loss of primary teeth, congenitally missing teeth, and habits such as thumb sucking. Lack of exactness in genetic expression affects the teeth on the right and left sides, causing asymmetries in mesiodistal crown diameters.[2]

Conclusion

The Carrea´s index for stature estimation is a convenient, simple and inexpensive method, and can provide valuable information to the forensic investigation when dental remains are present.

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Assessment of c-erbB2 Expression by IHC and FISH in Invasive Breast Cancer— A Comparative Study: Experience from a Single Institute

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Abstract

Introduction: An accurate assessment of c-erbB2 expression in invasive breast cancer (IBC) has become crucial to precisely recognize the candidates to be treated with Trastuzumab. Presently, fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) are most commonly employed methods for evaluating c-erbB2 status. Recent literature has documented a strong correlation between the two c-erbB2 diagnostic analyzes. However, discordance between both the assays has been rarely reported. Therefore, we aimed to compare and correlate FISH and IHC results for c-erbB2 expression in Indian breast cancer patients. *Material and methods:* A total of 388 formalin fixed, paraffin embedded blocks of invasive breast cancer were retrospectively evaluated for c-erbB2 status by IHC (DAKO) and FISH (PathVysion dual-probe system) and results were compared. *Results:* 92.5% cases with IHC 3+ score showed significant concordance with the FISH results; while c-erbB2 gene amplification was noted in 48.3% of IHC 2+ cases. A large number of referral cases in the study group and variation in pre-analytical and analytical factors have attributed in escalating the number of indeterminate cases expressing c-erbB2 gene amplification. Additionally, an inverse correlation was revealed between ER/PR expression and c-erbB2 status. *Conclusion:* The results of the current study established a high degree of concordance between IHC and FISH in Indian breast cancer patients with 3+ immunoreactivity. However, reflex testing by FISH is recommended for IHC equivocal cases in order to avoid false results related to technical and interpretation errors, usually encountered while performing an immunohistochemical assessment.

Keywords: Breast cancer; c-erbB2; Concordance; Fluorescent in situ hybridization (FISH); Immunohistochemistry (IHC).

Introduction

Human epidermal growth factor-2 (HER-2) oncogene, also known as c-erbB2 or HER-2/neu and it's close relatives HER-1, HER-3 and HER-4 belong to the HER family of tyrosine kinase receptors.[1] It modulates cell growth, survival, and differentiation through multiple signal transduction pathways.[2] Acquired genetic defects lead to the aberrant functioning of the c-erbB2 gene and consequently protein overexpression in the cell membrane, which facilitates the acquisition of advantageous properties of a malignant cell. Therefore, it has been implicated in the pathogenesis of various human malignancies such as breast carcinoma, gastric carcinoma, esophageal carcinoma, ovarian carcinoma and others.[3] Amplification of c-erbB2 DNA has been stated in around 15–30% of invasive breast cancers (IBCs) and possesses both prognostic and predictive significance. Aforementioned alterations in the c-erbB2 oncogene are associated with an aggressive tumor phenotype, increased lymph node metastasis and reduction in disease-free and overall survival in breast carcinoma.[2,4]

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Received on 16.09.2019, **Accepted on** 28.11.2019

However, this endured a turnaround with the arrival of targeted therapy against HER-2 gene in the form of the humanized mouse anti-HER-2 monoclonal antibody Trastuzumab (Herceptin) and Lapatinib, dual receptor tyrosine kinase inhibitor. This breakthrough has significantly improved clinical outcome in c-erbB2 positive breast cancer patients. Unfortunately, Trastuzumab therapy is expensive and imports certain serious adverse effects like cardiac toxicity especially when used in combination with anthracyclines. Furthermore, Herceptin and Lapatinib have been found to be effective only in tumors possessing true c-erbB2 gene amplification.[5,6] Hence, it becomes a prerequisite to accurately identify the subset of patients who would benefit from this novel mode of therapy.

Currently, c-erbB2 expression in IBCs can be determined either by testing for gene amplification by polymerase chain reaction (PCR), Southern blot and fluorescence in situ hybridization (FISH); messanger RNA (mRNA) using Northern blot or protein overexpression via immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA) and Western blot on cytosols. In addition, recently described techniques for c-erbB2 detection include Chromogenic in situ hybridization (CISH) and Silver enhanced in situ hybridization (SISH). Among all the aforestated techniques, SISH is the most sensitive for recognizing c-erbB2 DNA amplification.[7,8] Nonetheless, the most commonly employed Food and Drug Administration (FDA) approved procedures for determining the c-erbB2 status include IHC and FISH.[9] Immunohistochemistry (IHC) is a semi-quantitative assay, which is economical, less labor intensive and more commonly available but is prone to exhibit reproducibility issues and disparity in test results due to technical differences and interobserver variability. On the other hand, FISH outweighs the drawbacks of IHC owing to its quantitative nature.[10,11] American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) recommends evaluation of patients with borderline IHC result by FISH to ascertain c-erbB2 status.[12] Several studies have attempted to draw a comparison between the results of these two techniques, so as to determine the "gold standard" for c-erbB2 testing, but, were unable to arrive at a conclusion.[13-16] Although the controversy continues over the most appropriate methodology to evaluate c-erbB2 status, there is clear consensus that a very strong correlation exists between IHC and FISH. However, discordance between both the assays have been rarely reported.[16,17]

Further, the status of steroid hormone receptors, primarily estrogen (ER) and progesterone (PR), is prognostically important as it plays a crucial role in the management of IBC. The development of anti-hormonal therapies such as Tamoxifen, have drastically improved the disease-free survival in women with ER/PR positive breast cancers. Apart from c-erbB2, ASCO/CAP has also approved IHC for determining steroid hormone receptor immunoreactivity. Despite the profound correlation, there exists a minor degree of disparity among the two hormonal receptors.[13,16,18]

To the best of our knowledge, there are not too many Indian studies correlating the IHC profiles of ER, PR, and c-erbB2 with each other and comparing the two c-erbB2 diagnostic analyses. With this impetus, the present study was undertaken to understand the relationship among the two hormone receptors and the results of IHC and FISH for c-erbB2 gene expression in patients with IBC at our institution.

Materials and Methods

The present retrospective cross-sectional study conducted on 775 patients with a histomorphologically confirmed diagnosis of invasive breast cancer (including both in-hospital and referral cases) registered during the period of January 2011 till December 2013 in the Department of Pathology, Kasturba Medical College, Manipal. The patients' information was retrieved from the pathology and hospital records according to the prepared checklist which included age, ER and PR status, c-erbB2 protein overexpression and c-erbB2 gene amplification by FISH. This study was approved by the Manipal Institutional Ethical committee (IEC no 15.49).

Case selection

All formalin fixed, paraffin embedded blocks from the abovementioned cases, which were scored as equivocal (2+)/positive (3+) by immunohistochemistry (IHC) were eligible for the study. Therefore, among the 775 patients, 388 were included and the remaining 387 cases were omitted from the study, as they were scored as negative (0/1+).

Immunohistochemical analysis

All the cases were immunohistochemically evaluated for ER and PR, and c-erbB2 protein overexpression. Sections of four micrometer

thickness were obtained from blocks with adequate and well preserved invasive breast carcinoma, carefully mounted on pre-treated poly-L-lysine-coated slides and incubated overnight at 37°C. Deparaffinization of the sections was done via two changes of xylene, then dehydrated using absolute alcohol followed by rehydration through a series of decreasing alcohol concentrations. Thereafter, endogenous peroxidase activity was blocked with 1% hydrogen peroxide in methanol and finally epitope retrieved by heating the slides at 125–127°C for 30 seconds, pressure 21–25 psi in 10 mmol/L citrate buffer (pH 6) using a water bath. Polyclonal anti-rabbit c-erbB2 primary antibody (DAKO, Glostrup, Denmark) was applied. The Envision Kit (DAKO) was employed for introduction of the secondary antibody and the reaction signals were recognized with 3,3'-diaminobenzidine (DAB) followed by light nuclear counter staining with Mayer's hematoxylin. The 2013 guidelines of the ASCO/CAP were used for the interpretation of staining and c-erbB2 protein overexpression was scored as 0 (negative), no stain or faint and incomplete membrane staining in ≤10% of the tumor cells; 1+ (negative), barely perceptible and incomplete membrane staining in >10% of the tumor cells; 2+ (equivocal/indeterminate/weakly positive), weak to moderate incomplete circumferential membrane staining observed in >10% of the tumor cells or intense and complete circumferential membrane staining in ≤10% of the tumor cells; 3+ (positive), strong and complete circumferential membrane staining in >10% of the tumor cells.[19,20] The application of this scoring system has varying interpretations that depend on the quality and quantity of reaction, the type of antibody used, and the observer evaluation.[8]

For immunohistochemical evaluation of estrogen/progesterone receptor, pre-staining and endogenous enzyme blocking processes were performed identical to HER-2 staining. Primary antibody clones utilized in our institute for ER and PR were DAKO EP1 and DAKO PgR636 respectively. The best-preserved and best-stained areas of the sections were assessed. Interpretation of nuclear intensity and proportion of invasive cancer cells that displayed staining was done as per Quick score.[21] For the current study, ER/PR positivity was defined as nuclear staining in >10% of tumor cells and all other results, i.e. nuclear staining observed in <10% of tumor cell nuclei was regarded as negative.[22] All the tests were interpreted in conjunction with positive and negative controls and if required, were repeated. The controls were previously tested positive and negative test samples.

FISH for c-erbB2 gene amplification

Fluorescence in situ hybridization was performed on 335 cases, which comprised of weakly positive (score 2+) and positive cases (score 3+) as 53 cases did not consent to undergo assessment for c-erbB2 gene amplification. The sample was outsourced to Oncoquest Laboratories Limited, Bangalore, India. FDA approved PathVysion HER-2 DNA probe test kits (Abbott Laboratories, Abbott Park, IL, USA) were employed for FISH. This kit comprises of a dual colored probe: the locus-specific identifier (LSI) HER-2 DNA probe, specific for c-erbB2 gene locus (17q11.2-q12) labeled in Spectrum Orange and chromosome enumeration probe (CEP) 17 DNA probe labeled in Spectrum Green, specific for the alpha satellite DNA sequence at the centromere of chromosome 17 (17p11.1-q11.1). FISH analysis was conducted on formalin fixed, paraffin embedded sections placed on acid treated, and double poly-L-lysine covered glass slides. Then, the cellular double-stranded DNA was denatured into single strands, which were later hybridized with the PathVysion probes. The unbound probes were removed by multiple washes and the nuclei were counterstained with DAPI (4,6 diamidino-2-phenylindole). Sections were scored instantaneously using an upright fluorescence microscope prepped with suitable excitation and emission filters to allow visualization of the signals. The interpretation was performed on interphase cells of the specimen. The determination of the presence of c-erbB2 gene amplification was based on the counting of immunofluorescent signals for HER-2 and CEP17 within the nuclei of the tumor cells. Latest 2018 ASCO/CAP recommendations for scoring HER-2 gene amplification by dual-colour FISH are as follows: a positive test result is indicated by HER2/CEP17 ratio $\geq$ 2.0 and an average of more than or equal to 4.0 HER-2 copy number signals per cell and the criteria for negativity is an average of less than 4.0 HER-2 signals per cell with an HER2/CEP17 ratio of < 2.0. The recent guidelines also propose that for all other results addition workup is required before rendering a definitive diagnosis.[20] In the current study, at least 20 interphase nuclei from cells in a homogeneous and contiguous malignant population; showing a minimum of one green and one orange signal, were enumerated for each of the cases. Results were scored as positive/amplified (HER2:CEP17 ratio ≥2.0 with an average HER2 copy number of either <4.0 or ≥4.0 signals/cell; HER2:CEP17 ratio < 2.0 with an average HER2 copy number ≥6.0 signals/cell), equivocal (HER2:CEP17 <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals/cell) and

negative/non-amplified (HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell) according to ASCO/CAP 2013 guidelines.[19]

Statistical evaluation

All statistical analyses were performed using Statistical package for social science (SPSS) version 24 for Windows (IBM Inc, NY). The concordance and correlation between the immunohistochemical silhouette of ER, PR & c-erbB2 and the two c-erbB2 diagnostic techniques (IHC & FISH) were evaluated by calculating percent agreements. In addition, contingency tables were also analyzed using the Pearson's chi-square test to identify significant associations between different variables. All statistical tests were two-sided and a p-value of <0.05 was considered significant.

Results

Clinical parameters

The analysis of 388 IBCs revealed that the patients belonged to the 27–82 age group with a median of 49 years and a standard deviation of ±10.5 years. All except two (2/388; 0.5%) were female (386/388; 99.5%) patients. 224 (57.73%) patients were ≤50 years while 164 (42.26%) were >50 years of age.

FISH evaluation

Of the 388 cases, FISH for detecting c-erbB2 gene amplification was performed on 335 cases as the remaining 53 cases did not approve for the investigation due to various reasons. C-erbB2 was amplified by FISH in more than half of the cases (214/335, 63.9%) whereas it was unamplified in 35.2% cases (118/335). Three cases (0.9%) were reported as inconclusive/equivocal and were excluded from statistical analysis. Therefore, the study group evaluated for correlating of c-erbB2 status with other parameters comprised of 332 patients.

IHC evaluation

All the 388 cases were immunohistochemically assessed for hormone receptor status and c-erbB2 protein overexpression. Fifty percent (194/388) cases expressed positivity for ER receptor while less than one-third (120/388, 30.92%) cases were PR-positive. Statistical evaluation disclosed a direct association ($p < 0.05$) between estrogen and progesterone receptors.

Furthermore, immunohistochemical staining for c-erbB2 revealed a substantial number of cases (255/388, 65.72%) exhibiting 2+ (equivocal/indeterminate) reactivity and 34.37% (133/388) cases showed 3+ (positive) reactivity.

Association of c-erbB2 expression with age and hormonal state

Statistical evaluation of the study group revealed 64.8% (127/196) patients with age ≤50 years were amplified with c-erbB2 gene and 63.9% (87/136) patients categorized above the age of 50 were also FISH-amplified. Although, no significant correlation ($p > 0.05$) between the two age groups and c-erbB2 gene amplification was noted.

With regard to the association of hormonal state with c-erbB2 status, a large subset of ER-negative cases (84.5%, 153/181) were FISH-amplified compared to 40.3% (61/151) among ER-positive cases with $p < 0.001$. 78.6% (191/243) of PR-negative cases were also amplified with c-erbB2 gene whereas a significant number of PR-positive (74.1%, 66/89) cases were non-amplified with a compelling statistical difference ($p < 0.001$). Additionally, 19.2% (64/332) cases were ER+ PR+ and FISH-negative whereas a greater part of the study population (151/332, 45.5%) was ER-PR- and FISH-positive. Thereby, signifying an inverse correlation ($p < 0.05$) between the hormonal receptors and c-erbB2 geneamplification. However, our study also unveiled a small number of cases (21/332, 6.32%) amplified for c-erbB2 DNA and expressing immuno-positivity for both ER and PR receptors and 7.8% (26/332) were triple negative (ER- PR- and FISH-negative) (Table 1).

Comparison between c-erbB2 expression by IHC and FISH

ASCO/CAP recommends FISH as the standardized method for detecting c-erbB2 DNA amplification. Hence, it was considered as a gold standard in our study while correlating both the diagnostic assays. The comparison between IHC scores and the FISH results have been tabulated (Table 2). Analysis of 332 cases revealed 121 with an IHC score of 3+ and 211 with an IHC score of 2+. Amongst the 121 positive IHC cases, 112 (92.5%) were FISH-amplified for c-erbB2 DNA and very few cases (9/121, 7.5%) were non-amplified. While the assessment of 211 indeterminate IHC cases, showed amplification in 102 (48.3%) cases whereas more than half of the cases (109/211, 51.6%) did not demonstrate c-erbB2 gene amplification (Table 2). Further, the

Table 1: Correlation of c-erbB2 expression with age, ER and PR status

Parameter		C-erbB2 status by FISH – number (%)	
		Amplified	Non-amplified
Age (in years)	<50 (*n* = 196)	127 (64.8%)	69 (35.2%)
	>50 (*n* = 136)	87 (63.9%)	49 (36.0%)
ER status	Positive (*n* = 151)	61 (40.4%)	90 (59.6%)
	Negative (*n* = 181)	153 (84.5%)	28 (15.4%)
PR status	Positive (*n* = 89)	23 (25.8%)	66 (74.1%)
	Negative (*n* = 243)	191 (78.6%)	52 (21.3%)

Table 2: Comparison of IHC and FISH results for detection of c-erbB2 expression

C-erbB2 protein overexpression by IHC	C-erbB2 FISH amplified	C-erbB2 FISH non-amplified	Concordance by IHC	Discordance by IHC
Positive (3+) (*n* = 121)	112	9	112/121 (92.5%)	9/121 (7.5%)
Equivocal (2+) (*n* = 211)	102	109	102/211 (48.3%)	109/211 (51.6%)

concordance and discordance between IHC and FISH results were evaluated. The concordance rate was defined as the number of FISH-amplified cases with an immunostaining score of 3+ or 2+ divided by the sum of immunohistochemically positive (3+) and equivocal (2+) cases. In addition, the discordance rate was the ratio of the number of immunohistochemically discrepant 3+ or 2+ cases (IHC positive or equivocal but non-amplified) and the sum of cases with immunostaining score 3+ and 2+.[23] In our study, the concordance between FISH results and IHC for scores of 3+ and 2+ was 92.5% and 48.3% respectively, while the discordance noted between the two assays for immunohistochemically positive (3+) and equivocal (2+) cases were 7.5% and 51.6% respectively ($p < 0.001$). We also analyzed the data by merging the samples with immunostaining score 2+ and 3+ and the rate of concordance and discordance observed were 64.4% (214/332) and 35.5% (118/332) respectively.

Discussion

Assessment of c-erbB2 gene status has become crucial while reporting of invasive breast cancer. IHC and FISH are the two FDA-approved methods commonly employed in clinical practice for testing of c-erbB2 expression. However, there exists a small degree of disparity between both the assays. Therefore, this study was undertaken to recognize and elucidate the agreement and disagreement among assays of c-erbB2 protein overexpression and c-erbB2 gene amplification (i.e. IHC and FISH).

In the present study, more than half of the sample population (63.9%) was found to express amplification for c-erbB2 DNA. This outcome was in concordance with the results of Payandeh et al.[23] However, the percentage of FISH-amplified patients reported in our study was indeed greater with regard to other Indian and western studies.[6,14,24-26] This could be considered a plausible referral bias as our institution is a tertiary care center catering to numerous cancer patients. Also, the omission of IHC negative (1+) cases from our study group, leads to a relative increase in the proportion of patients expressing c-erbB2 gene amplification. Very few cases (0.9%) were observed to be FISH-equivocal, which was within the range specified by ASCO/CAP guidelines.[14]

Notably, in our study a large fraction (64.8%) of younger patients were revealed to possess amplified c-erbB2 gene, which was also substantiated by other investigators.[6,24,28] In addition, a considerable number (63.9%) of patients with age >50 years were also FISH-positive. Nonetheless, a significant association was not established between the two age groups and c-erbB2 gene expression.

Regarding the relationship between hormonal receptors and c-erbB2 status, an inverse correlation was noted. These observations were consonant with those reported by Panjwani et al., Eswarachary et al., Mostafa et al., and Prati et al.[6,24,27,29] The rationale behind these observations may be attributed to an intricate network of cross-talk between estrogen and growth factor receptor tyrosine kinase, i.e. c-erbB2.[30] On the contrary, 18.3% cases did express co-positivity for ER and c-erbB2 receptors in our study. Massarweh et al., Shou et al., and Osborne et al. postulated that IBCs with amplified c-erbB2 gene are more likely to be exhibit de novo resistance to Tamoxifen, due a surge in ER co-activator AIB1 triggered by c-erbB2

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cross-talk with ER signaling pathways. AIB1 boosts the estrogen agonistic activity of the selective ER modulator, thus facilitating proliferation and survival of tumor cells.[30–32] Consequently, the subset of breast cancer harboring immuno-positivity for ER and high-level gene amplification are certain to display an unfavourable tumor phenotype and less liable to benefit from endocrine therapy.[33] We also identified a small percentage (7.8%) of triple negative patients. As per the existing literature, such patients are known to be associated with an aggressive clinical course, rapid metastatic spread and poor response to targeted therapies.[33]

A huge number (92.5%) of our cases with IHC score of 3+ were FISH-amplified. Thereby, implying a high level of concordance between IHC and FISH. This was incoherent with prior national and international studies as well as ASCO/CAP guidelines.[6,14,23–26,34,35] Alternatively, 7.5% of the cases with immunostaining score of 3+ were unamplified by FISH. This rate of discordance was similar to those reported by Eswarachary et al. and Owens et al.[24,35] The putative reasons for IHC false positivity include excess antigen retrieval, increase in receptor expression without genetic alterations due to transcriptional or post-translational activation, artifactually elevated sensitivity of immunohistochemical assays, single copy over-expression of the c-erbB2 gene at the mRNA transcription level and/or beyond and gene amplification below the detection level of the FISH assay.[6,24,34,36,37] According to the references cited in the current study, the range of disagreement rate between IHC and FISH in immunohistochemically positive cases lies between 0–16%.[6,23-26,35,38] However, an Indian study authored by Makroo et al. reported a high degree of non-conformance of 29.5% among the two diagnostic analyses assessing c-erbB2 expression.[37]

Gene amplification was noted in 48.3% of IHC equivocal cases. This was disproportionate to the results of previous studies, that revealed an amplification range of 6–25%.[6,27,39-41] Further, ASCO/CAP guidelines reported c-erbB2 gene amplification in 23.9% of IHC 2+ cases.[14] The possible justification for incongruity in our results may be attributed to a large number of referral cases and variation in pre-analytical and analytical factors such as type of surgical specimen, duration of fixation (recommended cold ischemia time is less than one hour), quality of tissue fixative (ideally 10% neutral buffered formalin with pH 7.4 should be used), method of tissue processing, magnitude of antibody dilution and interobserver variability in IHC interpretation.[6,24,27,38,42,43] In addition, Lewis et al. stated that IBCs with 2+ immunoreactivity are likely to undergo clonal evolution, thereby exhibiting intratumoral heterogeneity; which also accounts for gene amplification in IHC equivocal cases.[44] Therefore, the latest 2018 ASCO/CAP recommendations mandates reflex testing by FISH in IHC equivocal cases.[20]

Again, the congruency between IHC and FISH was computed by combining the samples with 2+ and 3+ immunoreactivity and we recorded a drastic drop in the concordance rate from 92.5% to 64.4%. Our observation was in accordance with the findings of Payandeh et al., Tsuda et al. and Yaziji et al.[23,41,45] The significant fall in the concordance rate was due to the fact that, a substantial number of immunohistochemically equivocal cases (51.6%) were unamplified by FISH. In contrast, Panjwani et al. recorded a rise in the agreement rate from 80.1 to 87.7%, when IHC 2+ cases were included as most of their equivocal cases (66.6%) showed amplification for c-erbB2 gene.[6]

Lastly, one of the main limitations of our study was the exclusion of immunohistochemically negative cases. We do not recommend FISH testing for c-erBb2 status in IHC 0/1+ cases and firmly abide by the guidelines laid down by ASCO/CAP; which clearly mandates reflex testing by FISH in IHC 2+ cases. Also, considering the financial burden on Indian patients with invasive breast cancer, an additional expensive investigation (i.e. FISH) would further trample their livelihood. Another limitation is that the authors were blinded by the pre-analytical variables like fixation time and tissue processing, which could play havoc with interpretation of IHC and FISH results.

Timely diagnosis of breast cancer is not only important from the treatment and prognosis perspective, but also important from medicolegal point of view. Delay in diagnosis provide an opportunity to the patients and relatives to seek redress through the courts. According to Ward CJ et al.[46] breast carcinoma is a leading source of medicolegal litigations and failure or delay in diagnosis were the reasons for those litigations. Though the newer diagnostic techniques like FISH are available for the diagnosis of breast cancer, appropriate processing techniques, interpretation ability as well as timely diagnosis are essential to avoid potential legal litigation.

Conclusion

To conclude, the results of the current study established a high degree of concordance between IHC and FISH in Indian breast cancer patients with 3+ immunoreactivity. However, reflex testing by FISH is recommended for IHC equivocal cases in order to avoid false results related to technical and interpretation errors, usually encountered while performing an immunohistochemical assessment. An accurate detection of c-erbB2 status would permit the patient to undergo appropriate treatment. Nonetheless, we advocate IHC as an economical and feasible initial step for HER-2 testing in patients with invasive breast cancer.

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Pattern of Cervical Cytology using Papanicolaou Stain: An Experience from a Tertiary Hospital

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Abstract

Introduction: Cervical cancer screening using Pap smear is the cornerstone of any cancer control program. The study aimed to know the burden of various cervical lesions which were assessed by conventional Pap smear study. *Methodology:* We included 500 referred symptomatic patients in the study. The history, deatiled clinical examination, per speculum examination and a vaginal examination were performed for all women. Pap smear was used to screen all women for cervical cancer. *Results:* Mean age of the study population was 44 years and the most common complaint was whitish discharge per vaginam (54%). Classifying patients according to the Bethesda System 2001 Guidelines, we observed 61% (n = 303) cases to be Negative for Intraepithelial Lesion or Malignancy (NILM), 36% (n = 182) as Atypical Squamous Cells (ASC), 2% (n = 10) as Atypical Endocervical Cells (AEC) and 1% (n = 05) as unsatisfactory. Of the 303 cases of NILM, non-specific inflammatory changes were seen in 63%, reactive cellular changes in 21%, atrophic changes in 10%, candidiasis in 3%, Gardnerella vaginalis in 2% and inflammation with Trichomonas in 1%. Of the 182 ASC, 30% had low-grade squamous intrapeithelial lesion, 26% atypical squamous cells of underdetermined significance, 24% with high-grade squamous intraepithelial lesion and 21% with squamous cell carcinoma. Of the 10 AEC cases, 1 case had adenocarcinoma. *Conclusions:* Pap smear is less invasive, cost-effective and simple procedure which can be used to detect dysplasia in the cervix.

Keywords: Cervical malignancy; HSIL; LSIL; Pap smear.

Introduction

Cervical cancer is a leading cause of cancer mortality in Indian women aged above 15. More than two-thirds of the Indian cases present at later stages. Around one-fifth of women who develop cervical cancer die within the first year of diagnosis and the 5-year survival rate is 50%.[1] In high-income countries, early detection of precancerous lesions by regular screening programs has resulted in prompt diagnosis and early treatment, before they progress to invasive cancer. This has led to a reduction in incidence of cervical cancer and decreased the mortality due to the same.[2] The international standard of screening is the Pap smear, an examination of cells on the surface of the cervix for precancerous lesions. Another investigation which involves detecting the DNA of the human papillomavirus (HPV) costs substantially more than the Pap smear. Unfortunately, cervical cancer affects women of lower socioeconomic status more commonly and therefore they are more likely to develop invasive cancer.[3] Since 2001, the inclusion of Pap smear in the government's cancer control program has been recommended as it is cheap and

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easily available. In 2006, the Indian government and WHO developed guidelines to advocate the use of the Pap smear at district level, along with cheaper, simpler screening methods like visual inspection with acetic acid/Lugol's iodine at the primary health center level. The aim of the study is to know the burden of various cervical lesions by conventional Pap smear study.

Materials and Methods

Study design and sampling

The present cross-sectional study was conducted in the Department of Pathology, Vijayanagar Institute of Medical Sciences, Bellary in which referred patients from the outpatients clinic of the Department of Obstetrics and Gynecology were included. Women with symptoms like vaginal discharge, postcoital bleeding, postmenopausal bleeding, intermenstrual bleeding and persistent leucorrhea not responding to antibiotics, with normal looking but symptomatic cervix; and women with cervical lesions like polyps, erosion, hypertrophied cervix, cervix with nabothian cyst or with clinical evidence of acute pelvic infection were included in the study. Women who were bleeding at the time of examination, pregnant women and the ones with a history of hysterectomy/any cervical surgeries/radiotherapy/chemotherapy were excluded from the study. During the study period of 18 months, 500 cases were enrolled in the study. The study was approved by the Institutional Ethics Committee. Eligible patients were approached, the purpose of the study was explained to them and an informed written consent was taken before being included in the study.

Pap smear

Included patients were subjected to per vaginam and per speculum examination. Pap smears were collected using an extended-tip/Ayer's spatula to sample the transformation zone and adjacent squamous epithelium and an endocervical brush device was used to sample the endocervix. The scrapings were evenly spread onto the glassslide, and immediately fixed by dipping the slide in the jar containing equal parts of 95% alcohol and ether. The smear was stained with Papanicolaou stain and cytological interpretation was done by senior Pathology consultants. Reporting of the slide was done according to Bethesda classification[4] which is as follows: NILM (Negative for intraepithelial lesions or malignancy), ASCUS (Atypical squamous cells of undetermined significance), LSIL (Low-grade squamous intraepithelial lesions) and HSIL (High grade squamous intraepithelial lesions). Satisfactory cervical cytology was defined by the number of squamous cells in the sample. Criteria for "satisfactory for evaluation" included smears having at least 8000 to 12,000 well-visualized squamous cells and labelled specimen.

Data Collection and Data Analysis

Data were collected on a pre-designed semi-structured questionnaire. The data were compiled and described with the help of percentages.

Results

The age group of the patients ranged from 20 to 65 years with the mean age of 44 years (Table 1). Majority of the patients were in the age group of 40 to 49 years (31%) followed by 30 to 39 years (30.4%). The most common symptom at presentation was whitish discharge per vaginum (54%). Classifying patients according to the Bethesda System 2001 Guidelines, we observed 61% (n = 303) cases to be negative for intraepithelial lesion or malignancy (NILM), 36% (n = 182) as atypical squamous cells (ASC), 2% (n = 10) as atypical endocervical cells (AEC) and 1% (n = 05) as unsatisfactory. Figure 1 describes the distribution of patients interpreted as NILM. Of the 303 cases of NILM, 106 cases were in the age group between 40 and 49 years and the commonest mode of presentation was whitish discharge per vaginam (n = 184). Non-specific inflammation was the commonest subtype. Reactive cellular changes associated with inflammation was seen in 65 cases (21.45%). Mild nuclear enlargement, binucleation/multinucleation showing prominent nucleoli with cytoplasmic vacuoles and polychromasia with surrounding severe inflammation were considered reactive cellular changes with inflammation. Atrophic smear was interpreted in 10% of the cases. Atrophic smears were considered when predominantly small, round or oval parabasal cells which were scattered singly or in large sheets with scant basophilic or cyanophilic cytoplasm, increased N:C ratio, centrally located round to oval nuclei with were seen in a background of degenerated cellular debris and chronic inflammatory cells.

Atypical squamous cells were interpreted in 182 cases in cytology (Fig. 2). Among them, 47 cases were diagnosed as ASCUS. ASCUS was described when cells showed atypia in the form of nuclear

enlargement (2.5–3 times the normal cell size), mild increase in N:C ratio and mild hyperchromasia with nuclear membrane abnormality. There were 54 cases interpreted as LSIL. Pap smear in these patients showed superficial or intermediate atypical squamous cells with nuclear enlargement (>3 times the normal superficial or intermediate cell), nuclear pleomorphism, hyperchromatism

and binucleation/multinucleation. Some of the cells showed koilocytic change with perinuclear halo and peripheral dense rim of cytoplasm in an inflammatory background. HSIL was interpreted in 43 cases, in which the Pap smear in these patients showed small, less mature, basal or para basal atypical squamous cells present either singly or in small aggregates with scant cytoplasm,

Table 1: Baseline characteristics of the patients included in the study

| Variables	n (%)
Age distribution (in years)	
20 to 29	61 (12%)
30 to 39	152 (30%)
40 to 49	156 (31%)
50 to 59	80 (16%)
≥ 60	51 (11%)
Presenting complaints	
Whitish discharge per vaginam	270 (54%)
Lower abdominal pain	50 (10%)
Irregular menstruation	50 (10%)
Postcoital pain	45 (09%)
Cervical growth	40 (08%)
Pruritus	30 (06%)
Burning micturition	20 (04%)
Cytological diagnosis based on Pap smear	
Negative for intraepithelial lesion or malignancy (NILM)	303 (61%)
Atypical squamous cells	182 (36%)
Atypical endocervical cells	10 (2%)
Unsatisfactory	05 (01%)
Others	00 (00)
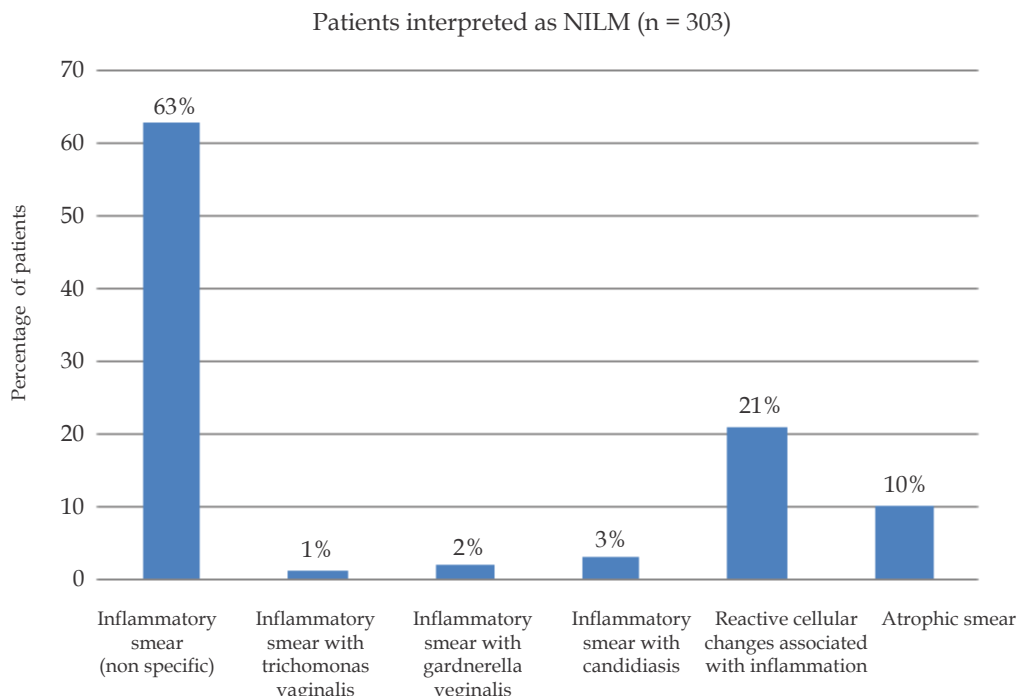
Fig. 1: Distribution of patients interpreted as negative for intraepithelial lesion or malignancy (NILM)

increased N:C ratio, hyperchromatism and nuclear membrane abnormality in an inflammatory background. SCC was interpreted in 38 cases, twenty-three of whom had chief presenting complaint of bleeding per vaginam. Pap smear showed cellular pleomorphism in the form of flat, round, polygonal, tadpole, spindle-shape cells in a background of nonspecific inflammatory cells. The nuclei were usually large and hyperchromatic with coarse chromatin. Mitotic figures were usually seen in the less well-differentiated cells.

Out of 10 cases which showed glandular cell abnormality (Fig. 3), 9 cases were reported as AEC, in which Pap smear showed sheets and strips of endocervical cells with scant cytoplasm, enlarged and hyperchromatic nuclei. One case was reported as adenocarcinoma, with the Pap smear showing sheets of columnar glandular cells with large, round, hyperchromatic nucleus, tumor diathesis, prominent nucleolus, abundant cytoplasm. Few cells showed gland formation and strips with pseudostratification.
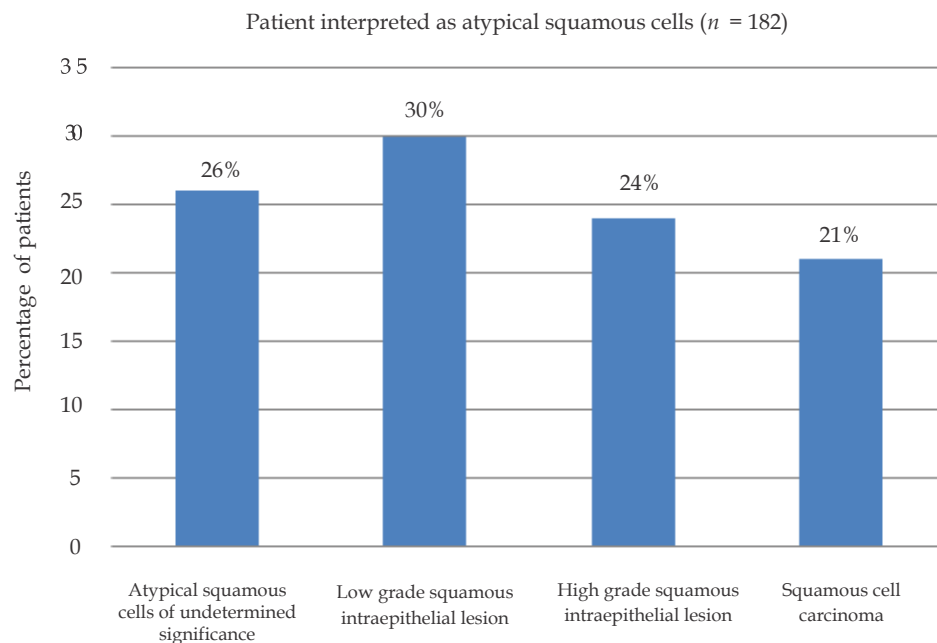

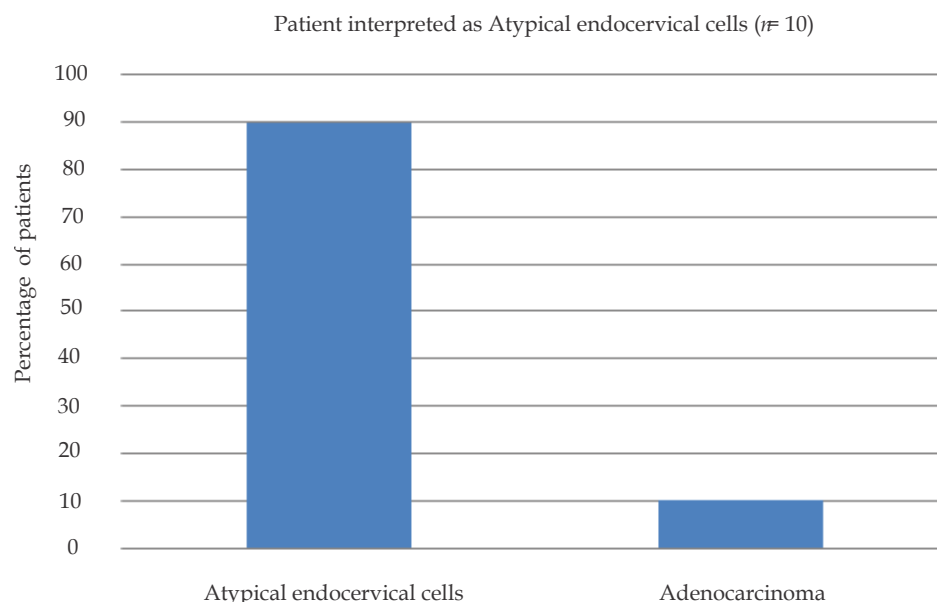**Fig. 2:** Distribution of patients diagnosed with Atypical squamous cells

Fig. 3: Distribution of patients diagnosed with atypical endocervical cells

Discussion

In our study, mean age of the patients was 44 years. It is well established that unhealthy cervix is more common in women of reproductive age group, who are sexually active. Bamanikar et al.[5] and Kaveri et al.[6] in their study, also found the majority of their cases in the similar age group. Whitish discharge was the most common presenting complaint (54%). Lower abdominal pain, irregular menstruation and postcoital pain were present in approximately 10% of the patients. Similarly, Bamanikar et al.[5] and Kaveri et al.[6] reported that whitish discharge per vaginum was the most common symptom (23.95%) in their study, other common symptoms being pain in the lower abdomen, intermenstrual bleeding and dyspareunia. Verma and colleagues[7] in a similar study, found the commonest presenting complaint to be abnormal vaginal discharge which was 54.5% followed by inter menstrual bleed in 19.5%.

NILM was interpreted among 61% of our patients. Atla et al. found 69% of the Pap smears to be NILM ($n = 248/356$).[8] In 248 cases of NILM, nonspecific inflammation was seen in 100 cases, reactive cellular changes, squamous metaplasia and atrophy constituted the others. Sixty-four cases showed specific infections in smears. *Candida* species infection was most common, followed by *Trichomonas vaginalis*. Verma et al.[7] reported 56% NILM with 32.5% inflammatory smears. Similarly, Sharma et al. found 45.3% cases of inflammatory smears.[9]

We interpreted 36% of the smears as atypical squamous cells and 2% with atypical endocervical cells. Of these, LSIL were the most common, followed by ASCUS. LSIL cervical cytologic specimens occasionally contain a few cells that are suspicious for, but not diagnostic of, a high-grade squamous intraepithelial lesion. Retrospective studies have found that these women have a significantly higher likelihood of a high-grade lesion on biopsy than other women with LSIL (approximately 30 versus 15%).[10] Although this is not included in the Bethesda classification, some experts report such cytology as LSIL with a statement regarding the presence of a possible high-grade abnormality. These women should undergo colposcopy and endocervical sampling. Alta et al. found 27% to have epithelial abnormalities, approximately half of which has ASCUS.[8] Bal et al. observed 3% cases of squamous intraepithelial abnormalities.[11] Nayir et al. observed 1.7%, 0.2%, 0.5% & 0.1% ASCUS, ASC-H, LSIL & HSIL respectively.[12] Sachan et al. detected in 8.48% with epithelial cell abnormalities.[13] Padmini et al. found 16% of their smears to have epithelial abnormalities, 8% with ASCUS, 5% LSIL, 3% HSIL and 1% SCC.[14] The high prevalence of epithelial abnormality observed in our study might be due to cultural differences, age of study participants, incidence of related infections and the variability of cervical screening programs in different parts of the country.

Conclusion

Pap smear is a less invasive, cost-effective and simple procedure which can be used to detect common problems of infection in the cervix. The present study was done to describe the distribution of cervical lesions in symptomatic women referred to our department. Of the 500 smears, 61% were negative for intraepithelial lesion or malignancy. As the epidemiology of cervical lesions vary with the geographical location, socioeconomic status and cultural practices, similar studies are needed from different parts of India.

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To Evaluate the Epidemiological Factors Affecting the Severity of Scorpion Envenomation in Pediatric Age Group

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Abstract

Background and objectives: Scorpion sting is a frequent, life-threatening medical emergency in children. They constitute a significant public health problem in many underdeveloped countries, including India. This study was done to study the epidemiological factors responsible for high prevalence of scorpion sting in our community. *Methodology:* This is an observational study of 35 cases of scorpion sting, admitted at our institute. An epidemiological study was done to determine the factors predisposing to prevalence of scorpion sting in the community. *Results:* Scorpion sting is a common, pediatric emergency in our area. Rural male children, from lower socioeconomic groups, aged between 1–3 years (28%) and 3–10 years, (57%) were most commonly affected. Maximum admissions in May, June. *Conclusion:* Scorpion sting is a serious, potentially fatal emergency in our area. Cardiovascular manifestations are most common and life-threatening. Scorpion stings constitute a "occupational hazard" for children employed as agricultural laborers. The epidemiological factors affecting the severity of scorpion envenomation are studies in the present study. The various factors are season summer being 49% cases, rural area common being 64%, scorpion sting common in lower socioeconomic strata residing in kaccha house. Sting found more in night time and 44% were in outdoor. Sixty eight percent scorpion stings were on exposed part of body.

Keywords: Scorpion sting; Prazosin; Occupational hazard.

Introduction

Scorpion envenomation is an important public health hazard in tropical and subtropical regions. Envenomation by scorpions can result in a wide range of clinical effects, including, cardiotoxicity, neurotoxicity and respiratory dysfunction. Out of 1500 scorpion species known to exist, about 30 are of medical importance. India is a country where agriculture forms the infrastructure of the nations economy.[1] The majority of land is under green belts for cultivation or is occupied by dense forests. Increased deforestation in recent years have increased the exposure of the tribals and other people living in rural areas to various forms of wildlife. This has led to increased incidences of various bites and stings.

Scorpions are found commonly in our country. Hence, scorpion stings constitute an important health hazard. They are specially quite common in the rural and coastal areas.[2]

In lndia, about 86 species of scorpions are found of which are only two are known to be poisonous.

These are:

1. Mesobuthus tamulus (the red scorpion)
2. Palamneus swammerdami (the black scorpion)[3]

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Received on 05.12.2019, **Accepted on** 14.01.2020

In Maharashtra, stings by the red scorpion are quite common in Konkan area and the dry districts of Ahmednagar and Aurangabad. Scorpion stings are relatively less hazardous in adults, but may lead to serious toxicity in children. Hence, it assumes so much clinical importance in children.

Various epidemiological factors play a major role in the incidence of scorpion sting, like the type of house in which the victim reside, as "kuchcha" houses, which provide good hiding places for the scorpions, record more instances of stings.[4]

Environmental factor like summer season also play in important role in the epidemiology.

Other factors, which may determine the severity of envenomation, include:

* Age of the victim

* Size of the victim

* Breeding time of the scorpions

* Number of stings

* Time interval between sting and initiation of treatment

* Season[1,5]

Materials and Methods

Method of collection of data

Study group:

All the children admitted for scorpion sting in 2 hospitals: Anand Rishiji hospital & Siddhivinayak Children's hospital during the period of 11 month formed the study group.

Inclusion criteria:

1. All cases of definite scorpion sting in children up to 18 years of age in which a scorpion was seen in the vicinity either by the patient or by the parents, immediately after the sting.

2. Children with history of bite coupled with classic clinical manifestations of scorpion sting were also included in the study.

Exclusion criteria:

1. Cases of scorpion sting in patients > 18 year of age.

2. Unknown bites and cases where the clinical manifestation was not compatible with scorpion sting envenomation were excluded.

Study design

Thirty-five cases of scorpion sting, admitted at our institute from 15 July 2011 to 15 June 2012 were included in the study. On admission, a detailed clinical history, including the time of sting, symptomatology, details of treatment received before admission was taken. Further description of the scorpion and details about the circumstances leading up to the sting were obtained.

All the patients were subjected to a detailed clinical examination at admission and at frequent intervals thereafter, as was necessary in each case. Hourly monitoring of heart rate, respiratory rate, blood pressure, urine output, cardiovascular and respiratory status was done.

Results

Season

• Maximum admissions were in May or June.

• Admissions in winter were mainly in mild groups.

• Admissions in summer were mainly in moderate to severe envenomation groups.
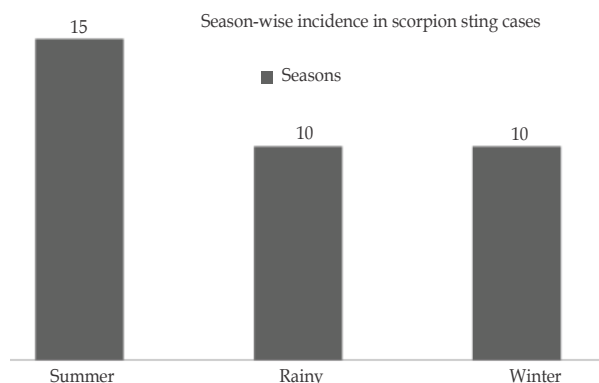
Graph 1: Season-wise incidence in scorpion sting cases

Age & Sex

• Maximum admissions were in the 3–10 age group (56%) followed by 28% in 1–3 age group.

• Prevalence was very low in children less than 1 year of age group.

• Males were affected more than females, M:F ratio 5:2.

• Mortality was mainly in 1-3 age group and 3–10 age group. Percentage wise mortality more in the 1–3 age group (28.5%) than the 3–10 age group (14.28%).

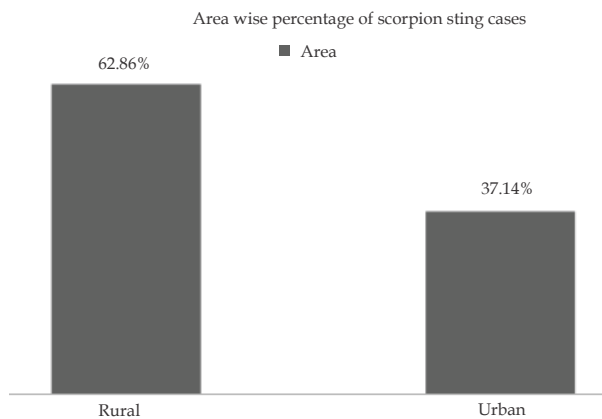**Table 1:** Age & Sex wise Distribution of The Cases of Scorpion Sting

Age group	Male	Female	Total (%)
Less than 1 year	1	0	1 (2.86%)
1–3 years	9	1	10 (28.57%)
3–10 years	11	9	20 (57.14%)
More than 10 years	4	0	4 (11.43%)
Total (%)	25 (71.43%)	10 (28.57%)	35 (100%)

Graph 2: Age and Sex-wise cases of scorpion sting

Area: (Urban / Rural)

Area wise distribution of various cases, whether rural or urban, was as shown in

Graph 3: Area-wise percentage of scorpion cases

- The incidents occurred much more in rural areas (62.86%) as against 37.14% in urban areas.

Type of House

The type of house in which victim resided was also a major factor in the study as shown in the diagram Graph 4.
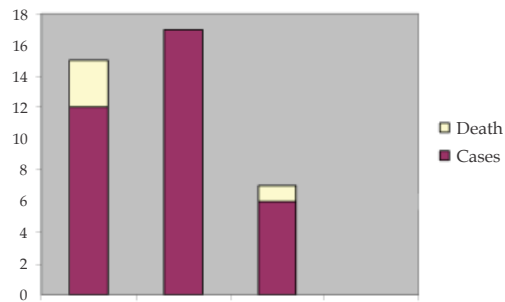
- Eighty percent of the cases occurred in "kuchcha" type of the house, i.e. either huts or old stone building or "wadas".
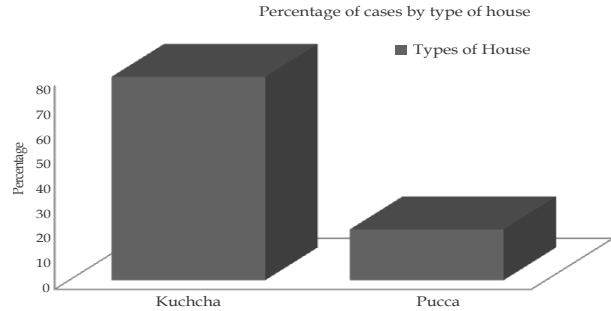
Graph 4: Percentages of cases by type of House

Type of Scorpion

The type of scorpion was identified in most of the cases and the prevalence of the red versus black scorpion stings was as shown in Graph 5.

Graph 5: Mortality in scorpion sting cases by type of scorpion

- 17 cases (48%) were due to black scorpion sting, while 12 (32%) due to red scorpion sting.
- There were 3 deaths in red scorpion stings and 1 death in unknown group. But no death was reported in Black scorpion stings.

Site of Sting

The site of sting was a major determination of the severity of envenomation and an indicator of the mode of causation. Sixty percent of the cases were due to stings on the feet, mostly due to accidental stepping on the scorpion.

Time of Presentation Since sting and Mortality

Following table shows correlation between time of presentation and mortality due to scorpion sting.

Table 2: Correlation between time of presentation and mortality due to scorpion sting.

Time (Hr)	No. of cases	Deaths	Mortality %
less than 1 hr.	6	0	0
1–5 hr	18	1	5.56
5–12 hr	7	1	14.2
More than 12 hr	4	3	75

- Mean time of presentation was 6 +/- 1.3 hr.
- A delay in presentation was associated with a significant increase in mortality.

Severity of Envenomation

Table 3: Severity of Envenomation in Cases and its Relation with Mortality.

Severity	Cases	Deaths	Mortality %
Mild	10	0	0
Moderate	21	1	4.76
Severe	4	4	100

- A significant increase in mortality was associated with increasing severity of envenomation.
- Severe envenomation is associated with 100% mortality.

Discussion

- Scorpion sting is an acute life-threatening, time-limiting medical emergency of villages. Numerous envenomations go unreported and the true incidence is not known.[5] Dominant clinical effects vary from species to species and from one geographical location to another.[1] Case fatality rates vary widely among different regions from 3% and over the years, with improvement in management protocols, there has been a dramatic reduction in mortality.

- We studied 35 cases of scorpion sting, admitted to 2 hospitals under Anand Rishiji Hospital and Siddhivinayak Children's Hospital from 15 July 2011 to 15 June 2012, and our observations are discussed below.

- Clustering of cases was noted in the summer months (49%) and in the early winter months (28%). No study has documented the seasonal pattern of scorpion sting, but it is widely observed that cases of scorpion stings increase dramatically in summer and are lowest in winter. This is in keeping with the hibematory behavior of scorpions in winter. Scorpions tend to creep out of the Burrows in summer, thus increasing the risk of accidental human contact and thus leading to an increased incidence of stings.[6]

- A majority of cases (64%) were from rural areas. Scorpion sting is mainly a rural emergency, with habitats of scorpions being primarily, paddy fields, sugarcane, coconut and banana plantations. Thus, children from rural areas are at highest risk for accidental contact with scorpions.

- The proportion of scorpion stings, sustained indoor was almost equal to that sustained outdoors. However, female children and children from urban areas were more likely to be stung indoors, when compared to male children from rural population. Rural male children, are more often involved in agricultural activities and hence are more at risk of accidental contacts with scorpions in the fields. This could explain the high incidence of stings sustained outdoors in them.[7]

- The incidence of scorpion sting is higher in children living in Kuchcha houses. Kuchcha houses have mud floors and walls and thatched roofs. Scorpions inhabit the crevices and underground burrows in dwellings and these houses provide a safe haven for them. In contrast, Pukka houses with tiled floors and cemented walls and roofs are safer.[8]

- A higher incidence of sting was noted in lower socioeconomic groups. The high incidence of stings in this group, is probably due to the type of housing and to their predominantly agricultural presents.

- Most of the stings sustained outdoors were in the fields (44%), when children accidentally trod over or handled the scorpion and were stung. Barefoot walking also increased the risk of sustaining a sting. Stings sustained indoor were mostly when children were sleeping on the floor. Infants were stung, while sleeping in a cradle or a swing made of cloth and hung on the roof (Hammock). Stings also occurred when scorpions were hidden in clothes and in poorly lighted rooms. Outdoor stings are more common than indoor stings in all parts of the world.[10] However we noted a significant number of indoor stings especially in the urban areas and in females. Further, a number of stings in infants were related to the cradles and hammocks used to put babies to sleep. This should be considered when suggesting appropriate measures for prevention of scorpion stings. Stings due to Mesobuthus species (Red scorpion) were slightly less than those due to Palamneus species (Black scorpion). This could be because of an increased prevalence of scorpions of the

Mesobuthus species, scorpions of this species being more venomous, could result in increased rates of hospitalization in children with stings due to this species.[10]

• Night-time stings were more common in our study. This is similar to earlier studies, which showed a preponderance of stings sustained during night-time due to nocturnal habits of the scorpion.[11] This could be because a significant proportion of stings in our study were sustained outdoors while engaged in agriculture-related activities.

• Although any part of the body can be exposed to sting, in 68% of cases in our study, the sting was sustained on the extremities. This is comparable to many studies in the past which showed an increased incidence of stings on the peripheries of 60–80%.[8] Most of the cases in our study were stung when accidentally stepping over or handling scorpion in fields or in poorly lighted rooms. Thus, most of the stings were sustained on extremities.

• The epidemiological factors affecting the severity of scorpion envenomation are studies in the present study. The various factors are season summer being 49% cases, rural area common being 64%, scorpion sting common in lower socioeconomic strata residing in kaccha house. Sting found more in night time and 44% were in outdoor. Sixty-eight percent scorpion sting were on exposed part of body.

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Estimation of Stature from Footprints Measurements by Linear Regression Analysis in South India Population

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Abstract

Background: Human identification using footprint is an emerging biometric technique, and footprints as valuable physical evidence in crime scenes are used to link the crime to the perpetrator. Footprints can be collected from almost all types of crime scenes and the possibility of their recovery at the scenes of sexual offenses and homicide is relatively more. *Aim and Objective:* To estimate stature from footprint length measurements in Telugu people of Nalgonda district at Narketpally State Telangana South India. *Place of Study:* Department of forensic medicine and toxicology on the consenting adult males of Nalgonda District of Telangana State. *Type of Study:* Descriptive cross-sectional study with analytical and comparative components. *Material and method:* The subjects were confirmed to be descent from Nalgonda district and were specifically selected with residence of Nalgonda district only, irrespective of their caste, religion, dietary habits and socioeconomic status. The footprint measurements collected in 150 adult males' volunteers with age of 18 to 40 years. *Observation and Discussion:* In footprint first toe - heel footprint length measurement, i.e. PRT1 and PLT1 was found to be longest, i.e. 24.789 cm and 24.795 cm respectively. In our study it was observed that the footprint length from left foot was larger in comparison to footprints from right foot. In both right and left footprint the first toe length was highest and it was observed that after the great toe length both left and right footprint length measurements from toe 2 to toe 5 till hill length gradually declined, i.e. 24.795 to 20.832 respectively. Simple linear regression equations accuracy in our study verified by comparing the estimated stature with actual stature revealed that both regression equations and scatter graphs indicated the existence of statistically significant positive correlation between footprint lengths and stature of Nalgonda populations. *Conclusion:* The result of this investigation provided regression equations for stature estimation from footprints in Nalgonda populations. The regression equations derived for this pooled sample can be used to estimate stature, as in real crime scenarios.

Keywords: Stature estimation; Footprints; Linear regression.

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Introduction

Each and every part of the body in its own way is different, not only within a particular body but also from one body to another. Each part of the body has a relationship with the whole body, nothing exemplifies the very truth that more than the relationship that various parts of the body have to the stature of an individual.[1] Bertillon system was invented by French anthropologist based

on anthropometry for the purpose of Human identification, various body parts can be used to estimate stature since there is a strong relationship between each part of the body and whole body.[2–6]

With these points it can be considered that an individual's footprint may represent his or her identity, Human identification using footprint is an emerging biometric technique, and footprints as valuable physical evidence in crime scenes are used to link the crime to the perpetrator. Footprints can be collected from almost all types of crime scenes and the possibility of their recovery at the scenes of sexual offenses and homicide is relatively more.[7–8] In various Asian countries like Malaysia, Sri Lanka, Thailand, Indonesia and India people have a habit of walking barefoot, there is a tendency that majority of the rural population walk barefoot, it may be due to socioeconomic and climatic conditions. Foot impressions are found at crime scenes as accused often tend to remove their footwear either to avoid noise or have a better grip while climbing walls, etc., while entering or making an exit.[9–10]

It was Gayer who was probably the first researcher to conduct an in-depth study of footprints while working in united province of India and published his observations in his book. Various other studies were being conducted on the individualization and stature estimation from foot and footprints, and they all suggest different ways of utilization of footprints in forensic examinations.[11–13] Most of the researchers in their studies for stature estimation from foot and footprints were on mixed population and they have specifically cautioned that the people from different regions of a country bear different morphological features based on their different geographical distribution and primary racial characteristics and that is the reason a single formula cannot represent all and various parts of that particular country or world.

Foot and footprints parameters used for stature estimation, various investigators concluded that toes to heel length measurements from foot and footprints are more reliable and had more accuracy in comparison to other measurements of foot. In this present cross-sectional prospective study we aim to estimate stature from footprint length measurements in Telugu people of Nalgonda district at Narketpally State Telangana South India.

Materials and Methods

In the present study was conducted at Kamineni Institute of Medical Sciences and Research Center, at Narketpally District Nalgonda by the Department of forensic medicine and Toxicology on the consenting adult males of Nalgonda District of Telangana State. The research was with the aim of estimation of stature from foot print measurements collected in 150 adult males' volunteers with age of 18 to 40.

The subjects were confirmed to be descent from Nalgonda district and were specifically selected with residence of Nalgonda district only, irrespective of their caste, religion, dietary habits and socioeconomic status. The study was a predominantly descriptive cross-sectional study with analytical and comparative components. Sufficient permissions and consents are procured before the measurements of the volunteers are taken and clearance from the Institutional Ethical committee is obtained in advance.

During data collection, volunteers were advised to clean their feet with water, Kores quick drying blue/black duplicating ink was used with the help of a footprint roller. Left foot was inked first with minimal pressure and volunteers was asked to place his foot on A4 size plain white paper on a uniform surface, anatomical landmarks of the feet was marked on the paper at mid-rear heel point, anterior point of all toes. Designated longitudinal axis (DLA) and the base line (BL) were made on the footprints. 90° on the footprint placed on the DLA and the midpoint of the protractor base at Pternion, perpendicular baseline by drawing a line through Pternion along the base of protractor, five diagonal footprint length measurements were taken from mid - rear heel point to most anterior point of each left toe. Same procedure was repeated for right footprint length measurements. Stature; using the stadiometer, the subject was made to stand barefoot in the standard standing position on its baseboard. Both feet are in close contact with each other and head oriented in Frankfurt's plane. The height was then recorded in centimeter from the standing surface to the vertex in the weight-bearing position of foot.

Footprint length measurements: PLT: Pternion to the most anterior point of Left Toe 1 on footprint of left leg and similarly for PRT: Pternion to the most anterior point of Right Toe 1 on footprint of Right leg and so on for every toe on right and left footprints.

Exclusion criterion: Those with any apparent disease, orthopedic deformity, morphologically showing the congenital malformations, dwarfism/achondroplasia, features of nutritional deficiencies and injuries to extremities, using medication thought to alter growth, neuromuscular weakness

or abnormal tone or with any other major medical illnesses or growth disturbance were excluded from the study.

Results

Descriptive statistics like minimum, maximum mean and SD, etc. of stature and all footprint length of right and left foot was done. Association between stature and footprint length including great toe was present by scatter diagram. All association positively exists. All toes including great toe are positively correlated with stature. So, on the basis of that we calculate the simple and multiple regression equations on both footprints length, by using this equation we can predict the stature value by using footprint lengths. The whole statistics was done by MS-Excel.
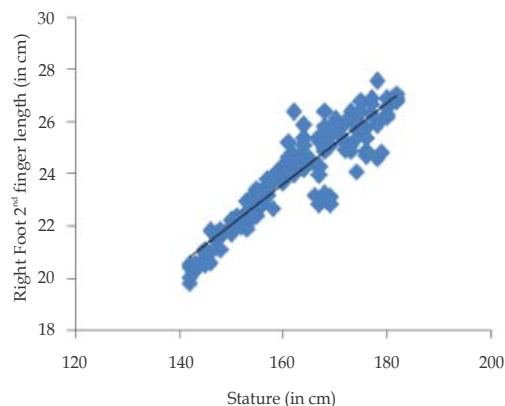
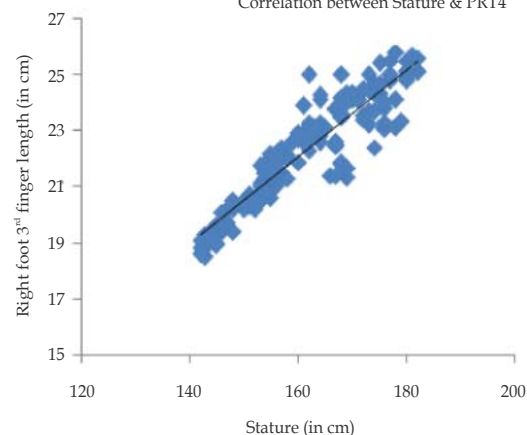Correlation between Stature & PRT2
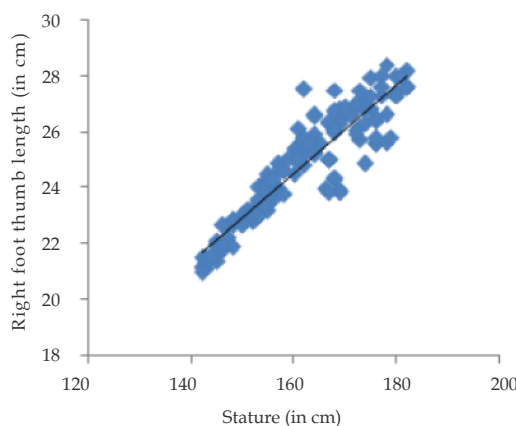
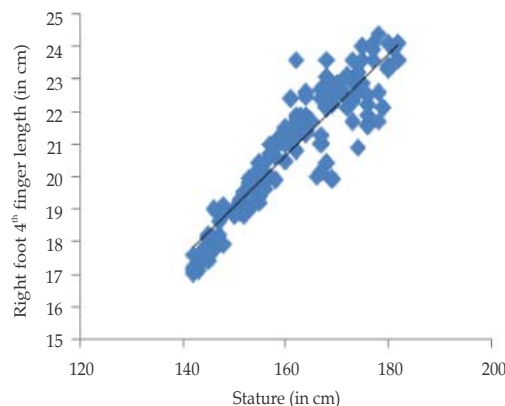
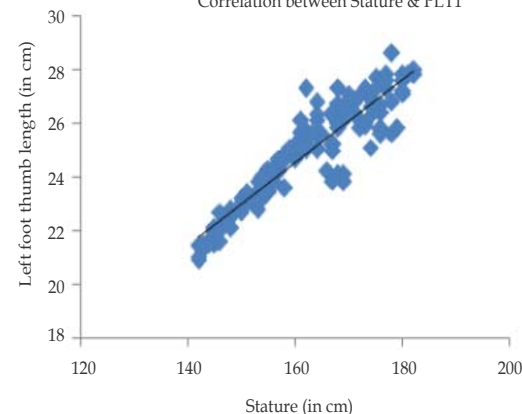Correlation between Stature & PRT2
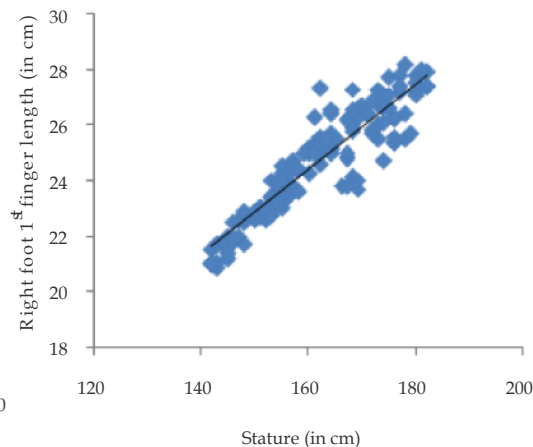
Correlation between Stature & PRT3

Correlation between Stature & PRT4

Correlation between Stature & PRT5
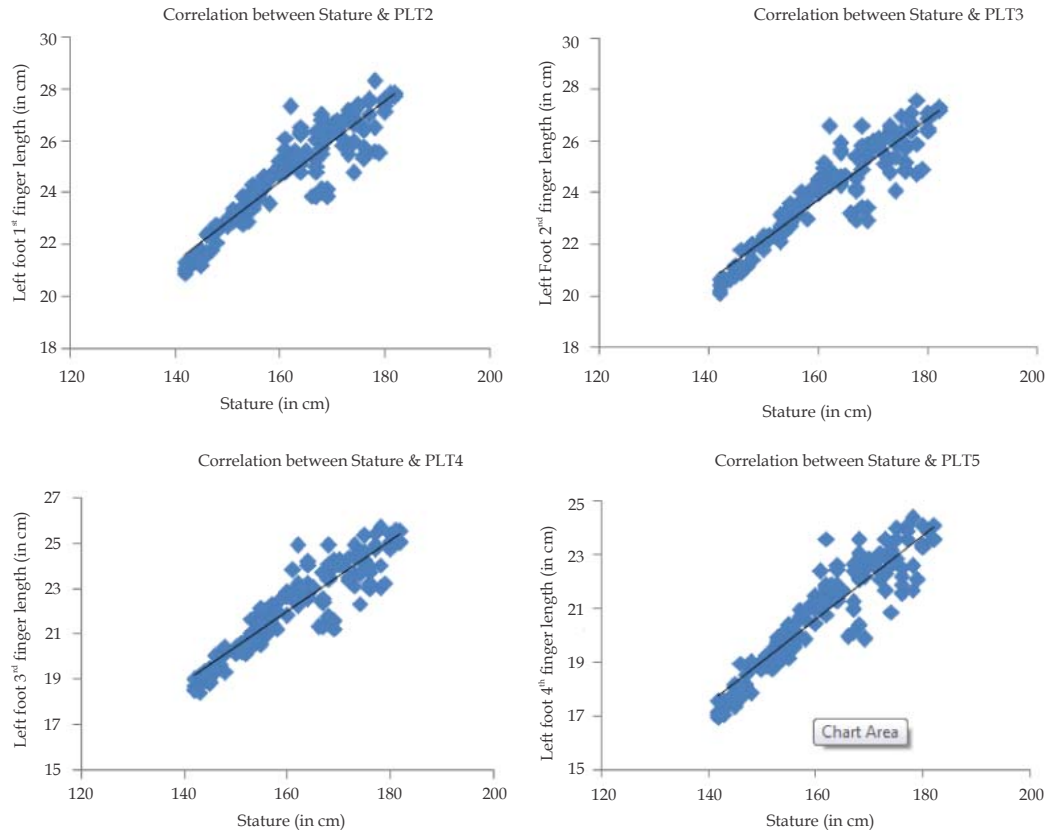

Correlation between Stature & PLT1

Fig. 1: Positive correlation reflected by scatter graph between footprints length measurements PRT1-5 and PLT1-PLT5 and stature.

Table 1: Basic statistics of all footprint length

Variables	N	Min (CM)	Max (CM)	Range Max-Min (CM)	Mean (CM)	SD
Stature	150	142	182	40	161.61	11.06
PLT1	150	20.9	28.6	7.7	24.789	1.858
PLT2	150	20.9	28.3	7.4	24.674	1.85
PLT3	150	20.1	27.6	7.5	23.984	1.876
PLT4	150	18.44	25.74	7.3	22.249	1.866
PLT5	143	16.96	24.36	7.4	20.832	1.887
PRT1	150	21	28.5	7.5	24.795	1.87
PRT2	150	20.9	28.2	7.3	24.658	1.857
PRT3	150	19.8	27.6	7.8	23.839	1.869
PRT4	150	18.5	25.8	7.3	22.31	1.865
PRT5	144	17	24.4	7.4	20.872	1.847

As per our observation in Table 1 the descriptive basic statistics of stature and both right and left footprints length measurements is being highlighted. In our study stature ranges from 142 cm to 182 cm (mean 161.61 cm). In foot print first toe- heel footprint length measurement i.e. PRT1 and PLT1 was found to be longest, i.e. 24.789 cm and 24.795 cm respectively. In our study it was observed that the footprint length from left foot was larger in comparison to footprints from right foot. In both right and left foot print the first toe length was highest and it was observed that after the great toe length both left and right foot print length measurements from toe 2 to toe 5 till hill length gradually declined i.e. 24.795 to 20.832 respectively. There had been a significant degree of decrease between T4 and T5 of both foot print length compared to the difference from T1 to T3. The reason attributed to this significant decrease was due to T5 of around 13 prints did not make a contact with the ground during the process of imparting of footprints.

In our observation as per Table 2 and 3, it was observed that all length measurements were statistically significant asymmetry and T1 and T2 lengths were found to be significantly more asymmetric in our study. The Table 2 and 3 reflects means, bilateral differences in footprints, standard deviations, p - value, t – values. The highest t - value was found for T4 (0.8616) and the lowest t value for T3 (0.6698). In the Table 5 our study represents the simple linear regression equations to estimate stature from footprint length measurements in both right and left footprints. Table 4 represents the correlation coefficient with stature and shows that there is positive relationship and statistically significant correlation and all are highly significant. The coefficient of determination R^2 the predictive accuracy in our study was found to be statistically highly significant for estimation of stature. Fig 1 represents the regression line, it is crossing through the center of the data in the scatter diagram. The standard error of estimate predicts the deviations of the estimated stature from the actual stature, it is considered that if the SEE is zero then there is no variation about the regression line and correlation is perfect, as shown in Table 5 in our observation. Standard error estimate ranged in between from 18.12 to 19.68 cm in our study. PLT1 shows the least SEE and PRT2 exhibits the highest SEE in our study. Scatter diagram as shown in Fig 1 reflects an elliptical pattern of distribution of values and its analysis strongly indicated the positive correlation between footprint length measurements and the stature.

Multiple regression equation of stature on left & right foot thumb and all fingers.

1. Stature = 39.37 – 1.83* PRT1 – 2.54* PRT2 + 6.81* PRT3 + 3.38* PRT4 – 0.36* PRT5

2. Stature = 24.07 + 2.93* PLT1 + 0.89* PLT2 + 2.98* PLT3 – 0.85* PLT4 – 0.46* PLT5

Table 2: Descriptive statistics of footprints length

Variables	N	Mean diff.	SD	t - value	p - Value
T-1 (PLT1-PRT1)	150	0.1367	0.092	0.6936	0.489
T-2 (PLT2-PRT2)	150	0.1804	0.1364	0.7227	0.471
T-3 (PLT3-PRT3)	150	0.1625	0.1084	0.6698	0.504
T-4 (PLT4-PRT4)	150	0.061	0.1754	0.8616	0.3903
T-5 (PLT5-PRT5)	143	0.039	0.1271	0.7965	0.427

Table 3: Descriptive statistics and comparison of footprint length of both foot

Variables	Left Foot (in cm)		Right Foot (in cm)	
	Mean	Mean ± S.D.	Mean	Mean ± S.D.
T1	24.789	24.789 ± 1.858	24.795	24.795 ± 1.87
T2	24.674	24.674 ± 1.85	24.658	24.658 ± 1.857
T3	23.984	23.984 ± 1.876	23.839	23.839 ± 1.869
T4	22.249	22.249 ± 1.866	22.31	22.31 ± 1.865
T5	20.832	20.832 ± 1.887	20.872	20.872 ± 1.847

Table 4: Correlation between stature (in cm) and all footPrint length (in cm)

Variables	Correlation with Stature	Z - test	p - Value	Significance
PRT1	0.902	25.4167	0.0000	All are highly significant
PRT2	0.91	26.7014	0.0000	
PRT3	0.8946	24.3546	0.0000	
PRT4	0.8671	21.1765	0.0000	
PRT5	0.8838	22.9808	0.0000	
PLT1	0.9	25.1187	0.0000	
PLT2	0.9008	25.2369	0.0000	
PLT3	0.8846	23.0763	0.0000	
PLT4	0.8734	21.8178	0.0000	
PLT5	0.8691	21.3754	0.0000	

Table 5: Simple Linear regression equation of stature on different footprint length of both foot.

Variables	Linear Regression line of stature on different foot print Length	R^2	SEE	p - Value	Significance
PRT1	Stature = 27.03 + 5.43 * PRT1	0.845	18.98	0.0000	All are highly significant
PRT2	Stature = 26.963 + 5.46 * PRT2	0.84	19.68	0.0000	
PRT3	Stature = 31.308 + 5.466 * PRT3	0.853	18.04	0.0000	
PRT4	Stature = 40.316 + 5.437 * PRT4	0.841	19.59	0.0000	
PRT5	Stature = 49.36 + 5.378 * PRT5	0.848	19.62	0.0000	
PLT1	Stature = 25.216 + 5.502 * PLT1	0.854	18.12	0.0000	
PLT2	Stature = 25.517 + 5.516 * PLT2	0.852	18.13	0.0000	
PLT3	Stature = 30.897 + 5.45 * PLT3	0.855	18.17	0.0000	
PLT4	Stature = 40.643 + 5.437 * PLT4	0.846	19.59	0.0000	
PLT5	Stature = 49.58 + 5.378 * PLT5	0.851	18.24	0.0000	

Discussion

India is a multi-racial, multi-ethnic and multicultural land of great diversity. The stature estimation is considered as important parameters in identification of a person, the human body parts has biological correlation with stature and this very fact had been utilized by many investigators and had used body parts or skeletal remains to determine stature. Stature estimation by measuring various long bones has been attempted by several researchers with variable degree of success in past. Each investigator has derived their own formula for determining the stature from long bones, while few had used body parts like, forearm length, head length, etc.[14,15]

Limited investigators had conducted studies on footprint to determine stature in India, the observation of our study had provided linear regression equations to determine stature from various bilateral footprint length measurements among Nalgonda district of Telangana state south India when footprints found at scene of crimes to determine the identity of suspects. In our study the sample size of the volunteers taking part in this study was 150, with the age in a range of 18 to 40. Stature at around 18 years is usually accepted as adult even though there are small increments in stature even after this age.[16] Few researchers highlighted that foot in males grows to its adult size by 16 years of age.[17] So the volunteers were preferred with minimum age of 18 to conduct the study. Even though, loss of stature seen with the increase in age is not accompanied by diminution of foot size and it is not possible to see how much variability could be incorporated into predictive calculation in the study.[18] Investigator Friedlaender et al.[19] in his study suggested that a decline in stature does not commence until the fifth decade of life.

The study reveals that the left footprint length measurements found to be larger than the right footprint length and this indicate the existence of statistically significant bilateral asymmetry and such bilateral asymmetry in lower limbs of Nalgonda population of Telangana state is in consistent with the study made by Irene's on Egyptian population.[20] Krishan in his study found asymmetry in T2, T4 and T5 in Indian population on Gujjars of North India.[21] While Kanchan found asymmetry in T1-T3 in Indian population. Similarly Philip and Robbins also did not find significant bilateral asymmetry in their study on footprints and various measurements of feet in south Indian population and U.S. population respectively. Both the investigators commented that measurements of most variables in person's left and right bare footprints are similar enough to permit either right or left foot being used for height and weight analysis. The mean footprint length measurements of Nalgonda population showed an appreciable size of variation, it can be compared to the mean footprint length of other studies from North of India population.

Sarah[22], Kanchan[23] and Nataraja Moorthy et al.[24] all in their research study highlighted an important observation during the development process of footprint, fifth toe of few subjects was found to be missing that is it did not made any contact with the ground and was reflected as missing toe in prints of foot. In our study 4.76% of the subjects T5 did not made the contact while producing the footprints. The comparative percentage of non contact of T5 is considerable low in Nalgonda population, Kanchan et al. 8%, Natraja Moorthy et al. 8.8% and Sarah et al. with the highest 16.1%. This non contact of fifth toe comes out to be an important and valuable clue in crime scene investigation and perpetrator identification could be possible in this scientific way. In our study also we found the observation in similar pattern unlike with other researchers.

Simple linear regression equations accuracy in our study verified by comparing the estimated stature with actual stature revealed that both regression equations and scatter graphs indicated the existence of statistically significant positive correlation between footprint lengths and stature of Nalgonda populations. In the regular trend in crime scene investigation wherein most of the crime scenes are disturbed by the general public and team members by imparting footprints at the scene of crime, hence it is the duty of the investigating officer to recognize and locate the appropriate footprints for stature estimation and so as to link effectively the crime scene and the perpetrator forensically.

Conclusion

The present study concludes that footprint length measurements have a strong relationship to stature of adult male in Nalgonda population of south India. This investigation revealed that the footprints of Nalgonda populations are different from other populations in India and outside India. It is clear evident that people from different regions of a country and world have different morphological features which depend upon geographical distribution and racial characteristics. The result of this investigation provided regression equations for stature estimation from footprints in Nalgonda populations. The regression equations derived for this pooled sample can be used to estimate stature, as in real crime scenarios. It is improper to utilize these population specific equations to estimate stature from footprints for any other populations either in India or elsewhere in the world. It is also suggested that similar study with larger subjects living in various other parts of India and the world need to be conducted for the meaningful forensic investigation.

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Renal Failure Associated with Animal Toxins

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Abstract

Venomous and poisonous animals are a major cause of global morbidity and mortality with cardiovascular and renal toxicity as common presentation. Renal functional impairment as a result of their toxicity is manifested in form of specific histopathological changes. Regardless of vast mentioning of renal toxicity in literature previously, only few studies are currently available with an integrated approach. This paper mentions about various such animals with nephrotoxic potential describing the toxic principles in their venom and inflicted changes in renal pathology.

Keywords: Animal; Poisoning; Nephrotoxicity; Renal failure; Acute tubular necrosis

Introduction

Fatalities in human are caused by various venomous and non-venomous animals.[1] Animal toxins and venom are well acknowledged for their hazards to mankind. Animal toxins consists of enzymes, peptides and proteins that can cause cellular injury with a broad range of systemic manifestations such as cardiovascular and renal system.[2–4]

Prevalence of human exposure to such poisonous and venomous animals and recent awareness of their nephrotoxic manifestations has led to the recognition of toxic induced nephropathy. The kidney is susceptible to injury by due to its high vascularity, either by hemodynamic changes induced by toxin effects on ion channels, or by peptides and enzymes causing ischemia or by direct injury.[4]

Hemodynamic alterations, vasoactive and inflammatory mediators and direct nephrotoxicity are closely integrated to cause renal failure.[5]

Nephrotoxicity results directly from action of a toxin on the kidney from secondary acute tubular necrosis due to hypotension or rhabdomyolysis. Knowledge about these medically important venomous and poisonous animals will help in prevention, diagnosis and clinical management of their nephrotoxicity.[6]

We conducted a comprehensive search of literature for nephrotoxic animals in PubMed, ProQuest, Science Direct, Springer, ClinicalKey, Scopemed and Google Scholar. Most of the included studies were focused on nephrotoxic effects on human.

This paper by an integrated approach presents an overview various venomous and poisonous animals that have been recognized in the literature causing nephrotoxicity.

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Literature

Various pathological changes can occur in the kidney after exposure to animal toxins, which has a broad spectrum, and all renal structures can be involved. Envenomation and poisoning by these toxins from various animals is well documented. The toxic components of the venom, bites, stings, contact and ingestion of various such animals along with their nephrotoxic effect in form of renal pathological changes have been described here.

Reptile

Snakes

The World Health Organization (WHO) estimates there are between 81,410 and 1,37,880 deaths out of about 5.4 million snakebites occur annually.[7] Acute renal failure (ARF) is a common presentation of venomous snakebite like Viperidae and Elapidae bites and nonvenomous bites like sea snakes.[8]

Family Elapidae

Krait

The greater black krait, *Bungarus niger* which is commonly seen in India, Nepal, Bhutan and Burma, and *Bungarus candidus* and *Bungarus multicinctus* in Thailand and Vietnam have reported to cause rhabdomyolysis and consequent ARF. Main toxins present in the venom are bungaro toxins, phospholipases A2.[9]

Pseudonaja

Pseudonaja textilis, Eastern or Australian brown snake envenoming causes consumptive coagulopathy due to a potent prothrombin activator. Thrombotic microangiopathy characterized by thrombocytopenia with fibrin thrombi and red cell sludging in glomerular capillaries, microangiopathic hemolytic anemia, some apoptotic cellular debris and possible segmental early necrosis, resulting in ARF was also reported.[10]

Mulga snakes

Pseudechis australis has wide distribution in Australia. Venom includes mulgotoxin and myotoxic phospholipase which produce rhabdomyolysis and myoglobin cast nephropathy. Tubulopathy with tubular epithelial cell degeneration mostly involving proximal convoluted tubular necrosis,

focal glomerular changes, including dilatation of Bowman's space and decline in number of glomerular tufts were noted. The proximal convoluted tubules showed features of tubular necrosis.[11]

Red-bellied Black snake

Pseudechis porphyriacus commonly seen in Australia envenomation resulted renal failure which revealed rhabdomyolyis and marked tubular necrosis with intraluminal occlusion subsequent to pigmentary casts. The major toxic component (pseudexin) takes the form of a mixture of three Phospholipase A2 isoenzymes, a factor Xa-like prothrombin activator and myotoxin.[12]

Tiger snake

Notechis scutatus (mainland tiger snake) and *Notechis ater* (black tiger snake) commonly occur in Australia. Thrombotic microangiopathy, which occurred with venom induced consumption coagulopathy, was characterized by ARF. Notexin is a neurotoxic and myotoxic phospholipase A2 derived from venom.[13,14]

Coral Snakes

Coral snakes are the American members of the family Elapidae represented by the genera Micrurus, Leptomicrurus and Micruroides. Micrurus snakes can be found in North America. Venom exhibited phospholipase and myotoxic activity. Kidneys presented with extensive tubular necrosis with fragmentation of nucleus, the brush border destruction, basal membrane rupture and tubular epithelial cells exfoliation, granular cast and tubular thickening. The histological features of the lesions suggest an important role of deposition of myoglobin in indirect damage to glomerulus.[15]

Taipan snake

Oxyuranus scutellatus, coastal taipan venom, contains mixture of toxins including taipoxin, a phospholipase A2 presynaptic toxin and myotoxin; taicatoxin, a calcium channel blocker and a prothrombin activator. It commonly exists in Australia. Renal failure exhibits as hemolytic uraemic syndrome with rhabdomyolysis and myoglobinuria.[16]

Family Viperidae

Adder

Vipera berus, common European adder, is the most widely distributed species of viper in Europe. It is a common venomous snake existing in England, Wales, and Scotland, envenomation of which results in ARF.[17] The venom of *V. berus* contains a complex mixture of high molecular weight proteins, predominantly proteases, hyaluronidase, peptide hydrolases, and phospholipases with predominantly hemorrhagic and cytotoxic effects.[18] *Bitis arietans* (Puff adder) found in savannah and grasslands from Morocco and western Arabia in Africa reported glomerulonephritis.[19] *Vipera raddei* (Armenian adder) is widely distributed in Armenia. The venom contains potent toxins and phospholipases. Light microscopy revealed thin capsule and weak disruption of the histological structure of kidney. Congestion of capillaries of the glomerular apparatus and vesicles of the middle and cortical layers were noted. Several nuclei in the lymphocytes between capillaries' loops and glomerular apparatus were found. The cytoplasm of proximal channels epitheliocytes was homogeneous. The channels were sporadically filled with homogeneous mass.[20] *Venom of Acanthophis antarcticus* (common death adder) from New Guinea contains myotoxic phospholipase A_2. Toxicity showed features of renal failure.[21,22]

Desert horned vipers

Cerastes gasperettii and *Cerastes cerastes* are the most common snakes of the Middle East, including Iraq and North Africa. Toxins present in venom includes serine proteases and other thrombinlike enzymes, fibrinogenases (IVa, Cerastocytin, Cerastotin, RP3 4, Afaaˆcytin and Cerastase F-4), which causes hypofibrinogenemia; platelet aggregation/ agglutination activators (Cerastocytin, Cerastotin); platelet aggregation inhibitors (IVa, Cerastatin, Cerastin), activators of Factor X (calcium-dependent and independent serine proteases, Afaaˆcytin), haemorrhagic protease (Cerastase F-4), protein C activator and an alpha-beta fibrinogenase (Afaaˆcytin); a phosphodiesterase exonuclease and a weakly toxic phospholipase A2. Envenomation by direct nephrotoxicity and ischemia results in mesangial proliferative glomerulonephritis resulting in acute tubular necrosis (ATN). Cortical necrosis resulting from thrombosis and bleeding was also noticed.[23]

Pit viper

Bothrops snakes, lance-headed pit viper belonging to genus Bothrops especially *Bothrops asper*/*B. atrox* cause nephrotoxicity. They are commonly seen in Central and South America mostly in Columbia, Mexico, Venezuela, etc. *Porthidium nasutum*, *B. puntacus* and *B. schlegelii* are other species. ARF occurs as a result of hypovolemia, or by the presence of nephrotoxic components in venoms or by the occurrence of disseminated intravascular coagulation (DIC) causing ischemic damage. Renal pathology demonstrates acute glomerulonephritis, ATN or cortical necrosis.[24] *Bothrops jararaca* is the most common species of Brazil. Its venom include metalloproteinases, serine-proteinases, C-type lectins and bradykinin-potentiating peptides; and *B. insularis* contains transcriptome.[25,26] *B. moojeni*, commonly seen in Brazil has potent phospholipase A2 and proteolytic activities which reported ATN and glomerulonephritis with mesangiolysis, glomerular microaneurysms, and glomerular basement membrane abnormalities.[27] *Hypnale hypnale*, hump-nosed pit viper bites causing ARF have been reported in India and Srilanka.[28]

Russell's viper

Daboia russelii russelii is widely distributed in India, Pakistan, Sri Lanka, Myanmar, Cambodia, Thailand, Indonesia, Southern China and Taiwan. Toxins commonly reported in venoms are the acidic and basic phospholipases A2, serine proteinase and metalloproteinase, phosphodiesterase, snaclec protein and L-amino acid oxidase. Procoagulant toxins such as Factor X activating enzyme induce intravascular clotting in the renal microcirculation, compromising the delicate renal perfusion.[29] Pathogenesis of ARF is associated to intravascular hemolysis, DIC, and also direct nephrotoxicity. Histopathology findings also reported necrotic changes in the tubular area.[30]

Saw scale viper

E. carinatus (carpet viper) is the most common snake in India. It also occurs commonly in Nigeria, Israel and Thailand. Hypotension in *E. carinatus* envenomation occurs due to bleeding either into tissues or externally. It can also occur due to release of bradykinin. The hypotension and circulatory collapse lead to ischemic ARF. Its venom directly activates prothrombin to thrombin. Viper venom produces Factor V activation with

fibrinolysis leading to DIC. This can result in hemorrhage, hypovolemia and thrombin in the microvasculature and capillaries of glomerulus and a microangiopathic hemolytic anemia with subsequent ARF. DIC plays a main pathogenetic role in snakebite induced cortical necrosis. Tubulointerstitial lesions, principally ATN were observed. Acute cortical necrosis occurs and can be patchy or diffuse.[31]

Rattle snake

Crotalus durissus, South American rattlesnake is commonly found in Brazil. Its venom is a complex mixture of toxins, enzymes, and peptides. The main identified toxins are crotoxin, crotamine, giroxin, convulxin, and kininogenases, phospholipases and hydrolases. Crotoxin is accountable for the high toxicity and has myotoxic, neurotoxic and nephrotoxic activity. Crotalid-induced ARF is connected to renal vasoconstriction, rhabdomyolysis, and a direct nephrotoxic effect of the venom. Crotoxin administration resulted in an increase in glomerular filtration rate attributed to direct effect on the glomeruli and further a rise in urinary flow rate by venom natriuretic peptides.[32,33]

Family Colubridae

Boomslang snake

Dispholidus typus, African tree snake is found throughout southern Africa. ARF with hematuria and hemoglobinuria often occurs due to envenomation. The boomslang venom is a potent procoagulant causing a consumption coagulopathy with resultant profuse hemorrhage.[34] Venom contains metalloproteinases. Renal pathogenicity is attributed to DIC caused by fibrinogen consumption and subsequent in coagulable blood with hemorrhage into muscle and brain tissues. Renal failure in form of ATN may also occur from pigment nephropathy.[35]

Keelback snake

Rhabdophis subminiatus, red-necked keelback snake belonging is common in Singapore and Netherlands. It reported acute kidney injury. Factor X activator in the venom induce severe hemorrhagic diathesis.[36,37]

Family Hydrophiinae and Laticaudinae

Sea snake

Sea snakes are widely distributed in the tropical Pacific and Indian Ocean. A toxic phospholipase A2

(PLA2-H1), in the venom of *Hydrophis cyanocinctus* cause myonecrosis and mild nephritis.[38] Proliferative glomerulonephritis and acute tubular degeneration in mice by the venom of *Apiysurus laevis* was also reported.[39]

Fish

Fresh water fish

Danio rerio, fresh water fish belong to the minnow family, Cyprinidae. Zebrafish larvae can develop cystic kidney disease. Lesions in genes involved in cilia formation and function result in the formation of cysts in the glomerular-tubular region.[40] Icthyotoxic acute kidney injury was observed after fish gall-bladder or raw bile ingestion. Toxin cyprinol sulphate causes ischemic ATN or acute tubule-interstitial nephritis. Renal failure by gall-bladder consumption includes fresh water fishes like grass carp, Ctenopharyngodon idellus in India and Pennsylvania, and Labeo rohita, other freshwater fish common in India, the black shark (minnow) fish, Morulius chrysophekadion and bony-lipped barb fish, Ostechilus melanopi in Vietnam.[41–43]

Phylum cnidarians

Sea Anemone

Night sea anemone, *Phyllodiscus semoni* is found commonly in Western Pacific ocean in Japan. Stings by these demonstrated in the renal pathology, mild ischemic changes in glomeruli, glomerular endothelial damage, thrombus formation, mesangiolysis, and partial rupture of glomerular basement membrane. Dilation of tubules or tubular degeneration and detachment of epithelial cells in the outer media were prominent suggestive of ATN. The venom extracted from the nematocysts (PsTX-T) and 115-kd protein toxin (PsTX-115) was nephrotoxic.[44,45]

Jelly fish

Cyanea capillata are common in China. Its tentacles cause marked renal morphological changes. Renal pathology reported partially destroyed glomerular capillaries or withdrawal of the capillary tufts, deposition of fibrin microthrombi in glomerular capillaries, and hyaline casts along with vacuolations in Bowman's capsule. In addition, severe proximal tubular degenerative changes occur characterized by cytoplasmic vacuolation, nuclear pyknosis and loss of proximal brush border. Few completely necrotic renal epitheliums in some

tubules, along with hyaline casts and detached cellular debris deposition in the collecting ducts and distal tubules was noted. Further, in some areas, diffuse congestion of peritubular capillaries and erythrocytes extravasation were also seen. Pore-forming toxins in the venom act by disrupting normal transmembrane ion concentration gradients in vulnerable cells. The Portuguese man-of-war (*Physalia physalis*) also reported acute tubular necrosis.[46]

Box jelly fish, *Chironex fleckeri* (sea wasps) occur commonly in Australia and Thailand, stings of which cause acute renal failure. The toxins are composed of a complex mixture of proteins and polypeptides, including cardiotoxic, hemolytic and dermatonecrotic toxins.[47] Jelly fish, *Stomolophus meleagris* or *Nemopilema nomurai* often seen in the China Sea showed renal failure with features of swelling of renal glomerulus, stricture of renal vesicle and dilatation of renal tubules. The several toxins in venom includes hemolysin, C-type lectin, phospholipase A2, metalloprotease, protease inhibitor and potassium channel inhibitor.[48]

Arthropod

Fire ants

Common group of ants seen in the United States of America is fire ants of Solenopsis species like the red fire ant, *Solenopsis invicta* or *Solenopsis wagneri*. Large doses of formic acid in these ants acts as mitochondrial cytochrome oxidase complex inhibitor causing tissue asphyxia, and subsequently cell death; resulting in rhabdomyolysis. The venom also constitutes nonproteinaceous alkaloid which cause local swelling and induce hemolysis. The pathogenesis of ARF is due to constriction of renal vasculature, formation of intraluminal cast, and direct tubular toxicity by heme proteins like myoglobin.[49]

Wasps and bees

Hymenoptera insects include Apidae (bees) and Vespidae (wasps and hornets). Stings by insect of order Hymenoptera like wasps (*Vespa orientalis*, *V. gnifica*), bees (*Apis mellifera*) and hornet have been reported in Australia, Sweden and India. ARF is infrequent with wasp bites and present as acute interstitial nephritis directly associated to the venom or tubular injury induced indirectly by immense hemolysis and rhabdomyolysis. Renal histopathology reported ATN, interstitial and glomerulonephritis.[50–52] Renal biopsy also revealed thrombotic microangiopathy with mild diffuse ischemic shrinkage, and interstitial ischemic tubular nephropathy with positive immunohistochemical staining of tubular granular casts with hemoglobin and myoglobin. Acute interstitial nephritis with infiltration of mononuclear cells with polymorphonuclear cells and eosinophils was also reported.[53] In ARF due to wasp and bees, toxic principles in venom include active amines such as serotonin, histamine, phospholipase A2, kinins, mastoparan, hyaluronidase, toxic surface-active polypeptides (apamine and mellitin). Phospholipase A2 triggers the release of arachidonic acid from lipid in the cell membrane which initiates production of inflammatory eicosanoids. Spread of venom is facilitated by action of hyaluronidase which causes breakdown of hyaluronic acid and chondroitins in the connective tissues.[50,51] The venom of *Vespa orientalis*, oriental hornet has a proteolytic activity on 14C-globin,which is inhibited partially by ethylenediamine-tetracetic acid and trasylol. Thus, the plasma coagulation factors activity is affected by both metaloprotease and serine activities.[53,54]

Brown Spider bites

Loxoscelism results from bites by spiders belonging to family Sicariidae, commonly known as brown spiders, recluse or fiddle-back spiders. In South America, it is caused by *Loxosceles intermedia and Loxosceles laeta* in Brazil and Argentina; and *Loxosceles gaucho* in Brazil, and in North America and Mexico, it is caused by *Loxosceles deserta and Loxosceles reclusa*. And in South Africa, Europe and South Australia, *Loxosceles rufescens* rarely reported cases. The principal components are phospholipase D, which cause dermonecrosis. Injection of venom triggers a complex inflammatory response, including the release of lipid mediators and pro-inflammatory cytokines. Additionally, the venom can result in complement activation and platelet aggregation by direct hemolytic effect on erythrocytes, and also increases the size of the tissue lesion attributed to hyaluronidase activity, which is a hallmark feature of loxoscelism. Systemic loxoscelism is characterized by renal failure with intravascular hemolysis. Rhabdomyolysis results in raise in creatine kinase which contribute to the acute renal injury.[55–57] *Loxosceles intermedia* venom contains sphingomyelinase D, Metalloproteases, hyaluronidase, lipase and alkaline phosphatase. Renal biopsy specimens on light microscopic analysis showed proximal and distal tubular hyalinization, interstitial edema, blebs and vacuoles in tubule epithelial cells, glomerular collapse,

erythrocytes in Bowman's space, and eosinophilic material deposition in the tubular lumen. Electron microscopic findings revealed disorders of the basement membrane and endothelial and glomerular epithelial cell cytotoxicity. Tubular epithelial cell cytotoxicity with increase in smooth endoplasmic reticulum, mitochondrial changes, cytoplasmic membrane blebs, autophagosomes along with tubular deposition of amorphous material was noted.[58]

Centipede

Bite of the giant desert centipede *Scolopendra heros* causes tubular necrosis. It is found in Arizona, southern California, Texas, Georgia, Alabama, Louisiana, Kansas and Mexico. ARF occur due to muscle injury and myoglobinuria. Hyaluronidase, Hemolytic phospholipase A, cardiotoxic protein and serotonin have been described in Scolopendromorph venoms. Furthermore, the toxin is referred cytolysin due to its ability to lyse cells. Some centipede venoms are complex mixtures containing histamine, 5-hydroxytryptamine, polysaccharides, lipids, and various enzymes such as proteinases.[59,60]

Caterpillars

A hemorrhagic syndrome caused by cutaneous contact with caterpillars of the species *Lonomia obliqua* has been observed in Brazil and other parts of South America. The toxin in the bristles contains mediators that initiate Factor XIII activation and cause intense intravascular coagulation. Enzyme serine-protease called Lopap in the extract of bristles is capable of thrombin activation, and result in micro-coagules formation which efficiently consume coagulation factors. Its venom also contains phospholipase A2, in addition to procoagulant serine and cysteine proteases. Acute renal failure with acute tubular necrosis is reported.[61]

Scorpion

Hemiscorpius lepturus is the most important scorpion in Iran. The venom from *H. lepturus* is primarily cytotoxic and has nephrotoxic, hemolytic, and hepatotoxic effect to some extent. Its venom has gelatinase, caseinase and hyaluronidase. Toxicity demonstrated ARF with hemolytic uremic syndrome with variable degrees of congestion and hemorrhage in kidney tissues.[62,63] *Tityus serrulatus*, yellow scorpion, is the most studied species in Brazil and other parts of South America. The histopathology revealed renal tubular protein deposit and perfusion of kidney urinary spaces with venom. Its venom contain pore-forming peptides which promote renal alterations with rise in perfusion pressure from increased vascular resistance resulting in decreased renal flow.[64] Venom of the Buthidae scorpion, *Buthus occitanus tunetanus* induce ARF in patients following severe scorpion accidents presenting peritubular congestion.[65]

Beetle

Cantharis Q is a crude alcoholic extract of *Lytta vesicatoria*, commonly known as the Spanish fly or blister beetle which carries venomous substance cantharidin in its hemolymph. It is commonly used as aphrodisiac. Cantharidin from *Mylabris cichorii* or *Lytta vesicatoria* or *Mylabris pustulata* causes tubular necrosis and glomerulonephritis. The histopathology revealed glomerular shrinkage with widening of Bowman's space, vacuolation, macrophages infiltration in the peripheral areas of glomerulus, and degenerative changes in the proximal and distal convoluted tubules.[66]

Conclusion

The renal pathology effected by animal toxins and venom has a wide spectrum, with involvement of all renal structures. This review by providing information about various animal envenomation and poisoning that exhibits nephrotoxic effects enables the health care providers to manage the morbidity and mortality due to renal failure. We expect that this review will further encourage the researchers to identify the specific nephrotoxic principles resulting in envenomation or poisoning. Further we hope this paper will enable toxicologist, pathologist, and health care providers in emergency medicine department in management of such cases.

Disclosure of Potential Conflicts of Interest: No potential conflict of interest relevant to this paper was reported.

Ethical clearance: None required

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A Planned Complex Suicide: Cut Injury to the Wrist with Hanging

Brijesh Tatwal¹, Sachin Kumar Meena², Amit Joshi³

Abstract

A planned complex suicide is the complex action mechanism, formerly planned to protect the victim of suicide from failure. A 22-year-old female body was brought by the relative to the hospital with cut injury in the both forearms. Autopsy findings revealed cut injury in both forearms with ligature mark around the neck. Death scene investigation, Forensic science lab reports and different observations confirmed, it is a case of planned complex suicide. To the best of our knowledge combination of methods used in this case is unique and has not been reported. The sequence of events in this case was difficult to determine as both the methods used, viz. cut wrist and hanging were sufficient to cause death individually. Also the sequence of event made it more difficult to determine.

Keyword: Planned complex suicide; Cut wrist; Hanging; Death scene investigation.

Introduction

A complex suicide is defined as the use of more than one method to induce death, either simultaneously chronically. The term has been widely accepted in the forensic medicine literature.[1-4] In 1974, Marcinkowski et al. had considered a general division of methods of suicide. In this classification, suicides are divided into simple versus complex, the complex one submitting to suicide by a combination of more than one method[1,2,4,7-9] planned complex suicide or primary combined suicide is the complex action mechanism formley planned, to protect victim for failure. This manner of suicides is used by the victim so as to prevent failure of one of the mechanisms. On the contrary, the characteristics of complex unplanned suicide, or "second combined suicide", is that the victim, after the failure of an attempt, continues try by the using one or moreself —destruction modalities to achieve death. A few cases of planned complex suicide.

Case Report

One day during postmortem duty I have received a police request with *panchnaama* for a postmortem of a 26-year-old lady by board. Lady was the wife of a constable belongs to a middle class family in Kota. We, members of board, have done all the pre-PM formalities before starting the postmortem like identification; etc. as per *panchayatnama* panchas are not sure about the cause of death. During pre-postmortem formalities, police and others told that it was a case of suicidal hanging with injuries on both forearms. As per police her husband was out of Kota from last 2–3 days and he reached to home on incidence day about 10 am and knocked the door many time, when there was no response, he opened the gate by the iron rod *sabbal* and taken down the body and dragged out and called to deceased. She was brought to the hospital about 11 am but was declared as brought dead. Father of the deceased told that *sahib meri ladki ko mara ha, wo suicide nahi karsakti,* and also tell that *lekin sab, mahmanji to aso karhi koni sake wo to bahut seeda ha, ghar ke liyan ladwada ko kam hai sab.*

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External examination

During postmortem examination, it is found that deceased was a Hindu female wearing *Sari, Blouse* and *Petticoat* with undergarments. Body was placed on postmortem table in supine position. Lower parts of all the cloths are stained with blood which was partially clotted. During the removing the cloths from the body, we found a piece of *chudi,* not matching with the dead body's *chudi.* All the cloths and chudi was sealed and handed to police for cross matching. She was an averagely built and nourished adult female. Rigor mortis was present about whole the body. Postmortem staining was present over the back and dependent parts in the patches but not appreciated on hands and feet like hands and gloves appearance. Eyes are slightly open, pupils dilated and fixed. There was NO cyanosis seen at the lips and finger tips (Figs. 1 & 2). There was no sign of dribbling of saliva or saliva stain on face or cloths. Then we have examined the external injuries on the body. Spindle shaped incised wounds of size 4 cm × 0.25 cm × tendon deep on lower third of the left forearm and 3 cm × 0.25 cm × tendon deep on the lower third of the right forearm. The margins of both wounds were clean cut with tailing laterally and infiltrated with blood (Figs. 3 & 4). On further examination we find that ulnar artery of both hands and the tendons were cut. Bones were healthy.

Ligature mark: Ligature mark of size 25 cm of maximum width 3 cm present on the anterior and both lateral of the neck above the thyroid cartilage level (Figure 3 & 4) with a gape of 5 cm on the occipital region then both sides it goes backwards and slightly upwards. On further examination during layer-by-layer neck dissection, skin and sub mucosa beneath the ligature is redish brown parchment like, inner aspect of skin beneath ligature mark os showing contusion at places. Both the margins of ligature mark shown slight contusion with veins and artery wall. Collection of blood around the artery and veins seen. Esophagus and tracheal wall healthy. Thyroid cartilage and hyoid bone intact. Trachea contains minimal froth.

Internal examination

Head: Sub scalp and skull are normal but brain and membranes are pallor otherwise normal.

Chest: Pleura and lungs are pale and trachea shows minimal froth. Heart grossly normal, right side chambers contain little blood and left sides empty.

Abdomen: In abdomen wall was intact, peritoneum was pallor, gastric mucosa is slightly congested with 100 ml food mixed material.

Other internal organs liver spleen kidney and uterus are grossly normal but pallor.

During postmortem examination following samples have been preserved and handed to police for FSL:

1. One sealed glass jar stomach with one loop of small intestine with contents in saturated solution of common salt.
2. One sealed glass jar pieces of liver, spleen and both kidneys in saturated solution of common salt.
3. One sealed glass bottle 20 ml blood.
4. One sealed glass bottle saturated solution of common salt as preservative.

Opinion regarding cause of death kept pending till toxicological analysis report.

Crime scene

I have requested to police to visit crime scene with forensic team after postmortem so 3–day later visit of crime scene was arranged by police. During visit the crime scene it revealed a bedroom of size 397 cm × 280 cm of height 281 cm with single gate without attached toilet. There were no marks of forceful opening by *sabbal* on the outer part of door and adjacent area with kundi but inner area shows the same. As per forensic expert the marks of sabbal was from inward to outward on the adjacent area of wall with inner kundi with blood stains (Fig. 2 & 3). There were blood stains also present on switch board of same room light and the inner handle of the gate (Fig. 2 & 3). The floor of the room was formed by simple white tiles. There were stains of blood with dragging signs over the floor up to the door. Room contain a 4*6 sq.feet

Fig. 1:

bed and a iron box (*Baksa*) of dimensions (L*W*H) 173*81*80 cm. Height of ceiling fan hanging hock was 294 cm and of ceiling fan was 257 cm from the floor. So the height of fan from Baksa is 177 cm. A suicide note of 4 pages was also found at the crime scene below the thin handmade mattress placed on box. Suicide note does not contain date, time and signature, it was sent to FSL for writing expert report. No weapon was observed during this crime scene visit (Figs. 5-11).

Final opinion

After passing about one year police came with FSL reports of writing expert (writing of suicide note and control is of same candidate), blood grouping reports of blood stains of crime scene and cloths (blood groups of the all stains and sent for chemical analysis are of same candidate), crime scene FSL expert report, IO investigation reports (all in the favor of planned suicide) with toxicological reports (all viscera gave negative test for metallic poisons, ethyl and methyl alcohol, cyanide, alkaloids, barbiturates, tranquilizers and insecticides) for finalize the opinion regarding cause of death.

After considering all the above reports, *panchayatnama* postmortem report, we found that "The Death has been Due to Asphyxia as a Result of Ante-Mortem Hanging with Cumulative Effect of Ante-Mortem Injuries to Both Forearms and Hemorrhage Which are Sufficient to Cause Death in Ordinary Course of Nature."

Fig. 3:

Fig. 4:

Fig. 2:

Fig. 5:

Fig. 6:

Fig. 7:

Fig. 8:

Fig. 9:

Fig. 10:

Fig. 11:

Discussion

In the forensic literature, complex suicides account for about 1.5–5.0% of all suicides.[2,12] Use of forearms has been earlier reported as one of the most preferred methods employed in complex suicide.[7] Demirci et al. in their study have found that most common methods of complex suicide were wrist cutting combined with self-strangulation, insecticide ingestion with shotgun injury, and insecticide ingestion with jumping from a height.[2] Palmiere et al. have reported a complex suicide by self-strangulation associated with multiple sharp force injuries.[3] In the literature, the use of maximum up to 5 suicidal methods applied one after the other has been illustrated.[10] Victims prefers to use methods of lesser lethality before choosing to use more lethal techniques. The adaptation from lesser to greater methods of lethality is most likely concerned with pain, anguish, and frustration experienced by the person.[8] Bohnert[12] and Pollak[5] have accounted that self-inflicted injuries by sharp force, especially cuts of the wrists, are often preferred as the primary act of suicide in complex suicides.[5] Demirci et al. reported that wrist and/or flexor surface of the elbow cutting was chosen in seven out of 16 cases in their study.[2] In these cases, subsequent method was applied because the first method takes much time as well as gives pain and uneasiness. Hence, the victim had selected the second and more lethal method due to the reasons of pain, ache, and taking too much time. Cingolani et al. have reported that even if hanging and shooting are frequently used alone in a planned suicide, their use at the same time is rare.[4]

In the present case, a combination of methods was found as cut injury to the both wrist with hanging. In most of the cases of complex suicide, wrist cutting was found with other combinations.[2] To the best of our knowledge, a combination of cut injury to the wrist with hanging has not been reported previously. Commenting on the sequence of events in the present case is difficult. It can be just guessed on the basis of previous studies that cut wrist injury might have taken place earlier than ingestion of poison.[2]

Most of the questions may remain unanswered if the scene of death is not investigated. The scene may disclose features about suicide, like a suicide note or any material used as a means of suicide. Relatives or friends of the decedent also may reveal background information such as history of depression, previous suicide attempts, social, marital or economic problems.[11]

Conclusion

A planned complex suicide represents a tricky medicolegal case, because the combination of mechanisms concerned in such cases may be complex and homicide could be suspected. Homicide should be carefully ruled out in every case of sharp weapon injury. Only a careful assessment of all the elements, including examination of the scene and postmortem findings, can reconstruct the lethal chain of events and elucidate the time, manner, and cause and of death.

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The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http://www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/l 7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35:540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003;61:347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www. statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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Tables should be self-explanatory and should not duplicate textual material.

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Explain in footnotes all non-standard abbreviations that are used in each table.

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