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Socio-demographic Profile of Victims of Suicidal Hanging: A Study from Western Maharashtra, India

Bimbisar Mukherjee¹, Sanjay B. Buktar²

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ABSTRACT

A descriptive cross-sectional study was conducted on all cases of deaths due to hanging autopsied at the mortuary of Pravara Rural Hospital, Loni, a rural region in Western Maharashtra, India. The study was conducted over a period of 4 years, two years retrospective (record based) from September 2012 to August 2014 and two years prospective from September 2014 to August 2016. Data was being collected from medico-legal autopsy records of hanging victims. The cases were studied to know the socio-demographic profile of the victims. The cases represented approximately 3.73% of all autopsy cases. Majority of the victims were male (83.67%) belonging to the age group of 21-30 years (57.14%). Most of the victims (46.94%) were laborers by occupation. The most common motive behind suicidal hanging was financial crisis (34.69%) followed by family problems (16.33%). To reduce the rate of suicides, awareness has to be created by appropriate education and by influencing the media in their portrayal of suicidal news. Counselling centers should also be established in rural areas to help the victims.

KEYWORDS: Hanging; Suicide; Autopsy; Rural; Death.

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INTRODUCTION

Oxygen is essential to sustain life. A reduced concentration of oxygen in blood which reaches the brain can cause rapid loss of consciousness. The brain constitutes approximately 2% of the body weight, but utilizes 20% of the total available oxygen. The failure to deliver oxygen to the brain is commonly due to asphyxia.¹ Violent asphyxial deaths can be caused by different methods such as hanging, strangulation, drowning

and suffocation. Hanging is a common method of committing suicide and makes a major proportion of asphyxial deaths.

Suicide is a leading public health problem worldwide. It was the fourth leading cause of death in the young adult age group of 15-29 years globally in 2019.² People who are victims of some sort of violence including child abuse, bullying or sexual assault have a higher suicidal tendency.³ A prior suicide attempt is the single most important risk factor for committing suicide.² History of medical disorders such as depression, mood disorder and cognitive impairment are other important risk factors for suicide.⁴ The rate of suicide in young people increased dramatically over the last few decades. According to data collected from NCRB (2021), hanging was the most common method of committing suicide in India.⁵ An increasing trend of suicide by hanging was also observed in Japan, Australia and in 16 countries which participated in European Alliance Against Depression (EAAD).^{6,7,8} All cases of deaths due to hanging are considered to be suicidal until the contrary is proved. Accidental hanging may be possible in factories, by children during playing and in masochistic practice.^{9,10} Hanging is perhaps one of the most preferred methods used to commit suicide due to the anticipated nature of death it causes and easy accessibility of means.¹¹ It produces a painless death for the victims. A thin rope tied around the neck may cause unconsciousness in only 15 seconds.¹² Suicidal behaviour and rates vary in different populations and culture and also by age and sex.¹³ We have undertaken this study to evaluate the socio-demographic profile of hanging victims in Loni, a rural region of Western Maharashtra, situated 26 kilometers from Shirdi, holy place of Saibaba. This data can be used in future to identify individuals who are at risk of committing suicide by hanging and a suicide prevention program can be planned accordingly.

MATERIAL AND METHODS

The current study was a descriptive cross-sectional study carried out at Department of Forensic Medicine, Rural Medical College, Loni conducted over a period of 4 years, two years retrospective (record based) from September 2012 to August 2014 and two years prospective from September 2014 to August 2016.

Detailed analysis of the cases was based on medicolegal records and evaluation of postmortem reports of hanging victims autopsied at Pravara

Rural Hospital, a tertiary care hospital attached to Rural Medical College. Information regarding the deceased, circumstances of death were collected from the investigating officer and relatives. Data was analyzed with regard to socio-demographic profile of the victims, reason behind suicide, seasonal variation and place of occurrence of the cases. Putrefied bodies were excluded from this study. Approval for study was obtained from Institutional Ethics committee of Pravara Institute of Medical Sciences.

RESULTS

Overall, 1315 medicolegal autopsies were carried out during the study period, out of which 49 cases were deaths due to hanging which constitutes around 3.73% of total cases. In our study all the 49 cases of hanging were suicidal in nature. Majority of the victims (83.67%) were male as compared to female (16.33%) and the male/female ratio was 5.12:1. The age range of victims in the study period was 13 to 60 years and the commonest age group involved was 21-30 years (57.14%) (Table 1).

Table 1: Distribution in relation to age and gender of victims

	n	%
Total number of autopsies	1315	100
Total deaths due to hanging	49	3.73
Sex		
Male	41	83.67
Female	08	16.33
Age		
00 - 10	00	00
11 - 20	07	14.28
21 - 30	28	57.14
31 - 40	07	14.28
41 - 50	03	6.12
51 - 60	04	8.16
> 60	00	00

The incidence of hanging was more common in married persons (53.06%) as compared to unmarried ones (46.94%). In present study, the most common working group involved were laborers (46.94%), followed by farmers and students (12.24%, each) (Table 2).

Table 2: Distribution in relation to marital status and occupation of victims

	n	%
Marital Status		
Married	26	53.06
Unmarried	23	46.94
Occupation		
Laborer	23	46.94
Farmer	06	12.24
Student	06	12.24
Unemployed	05	10.20
Housewife	04	8.16
Service	03	6.12
Business	02	4.08

Most commonly known motive behind suicide was financial crisis (34.69%) followed by family problems (16.33%) and the reason was not known in 18.37% cases (Table 3).

Table 3: Distribution based on motive behind suicidal hanging

	n	%
Financial Problems	17	34.69
Family Problems	08	16.33
Love affair	06	12.24
Personal	05	10.20
Failure in Exam	04	8.16
Unknown	09	18.37

The peak incidence of suicidal hanging (42.86%) occurred in summer season in the months of March to June and June was the most commonly affected month in summer contributing to 16.33% cases. Most of the suicides (81.63%) occurred in the victim's home, 14.28% cases occurred at farm where the victims hanged themselves from the branches of trees, while in 4.08% cases the victims hanged themselves in their hostel room (Table 4).

Table 4: Distribution in relation to season and place of occurrence

	n	%
Season		
March-June (Summer)	21	42.86
July-October (Monsoon)	14	28.57
November-February (Winter)	14	28.57

Place		
Home	40	81.63
Farm	07	14.28
Hostel room	02	4.08

DISCUSSION

The pattern of suicidal hanging may highlight underlying social, economic, physical and psychological problems in a specific region or population. Out of the total number of autopsies carried out in the study period, deaths due to hanging constituted around 3.73% of cases. This is consistent with the studies of Smith Z (3.9%) and Kanchan *et al.* (4.50%).^{14,15} However, a relatively higher incidence rate (7.56%) was observed in the study of Nagar *et al.*¹⁶ The variation in hanging trends may be due to the difference in culture, ethnicity and genetic variations in different regions of the world. Also, different racial groups and geographical areas tend to adopt distinct methods to commit suicide.

Majority of the victims in our study were male and the male/female ratio was 5.12:1. A male preponderance was also seen in most of the studies by other researchers.¹³⁻²⁰ However in the study of Rao D, female victims were slightly more affected than males.²¹ There is an increase in stress and strain, tension, anxiety, poverty in daily life which leads to alcoholism and drug abuse, and ours being a male dominated society, males are more commonly affected than females.

The most common age group affected in our study was 21-30 years. An increasing trend of suicide by hanging in the age group of 21-30 years was also observed in the studies of other researchers in different parts of the world.^{14-16,18-20} However the fourth decade of life contributed to majority of cases in the studies of AI Madni OM *et al.* and Rao D.^{13,21} The third decade of life is probably the most active period of an individual's life. People are more vulnerable to the fast changing social trends and cultures in this period. Also there can be fluctuation of emotions as they are a bit immature at this stage. People are more exposed to stress, anxiety, failure in academics, financial problems, broken relationships, unemployment and unable to get married at this point of life.

In our study suicidal hanging was more commonly observed in married persons. Married victims also contributed to majority of cases in the study of Rao D.²¹ However in the study of

Smith Z in central South Africa, single men were more vulnerable to commit suicide by hanging.¹⁴ Prevalence of early marriages specially among rural people, increased familial responsibilities, limited source of income, maladjustment in married life, infidelity and unemployment are important causes of more deaths in married persons.

We observed in our study most of the victims affected were laborers by occupation. Male laborers also contributed around 75% cases of hanging in the study of AI Madni OM *et al.* while in the study of Smith Z majority of victims were unemployed.^{13,14} The daily laborers and working class people residing in rural areas are more exposed to poverty, heavy work load, alcohol and substance abuse, increased stress and subsequent frustration in life. Love affair, family problems and academic failures are the major risk factors in students and teenagers.

In our study, most commonly known motive behind suicide was financial problems followed by family problems. Whereas, in the study of Rao D, where female victims outnumbered males, most of the cases were due to domestic disputes (dowry related) followed by love and relationship issues.²¹

The highest number of hanging deaths (42.86%) in our study occurred in summer season (March–June). In the study of AI Madni OM *et al.* in Dammam, peak of the suicides was observed in June.¹³ Summer months also contributed to majority of cases in the studies of other researchers.^{15,16} Warm weather and elevated levels of heat is known to cause physiological and behavioral changes

in people that might lead to suicidal tendency.²² In present study, most of the suicidal hangings occurred in the victim's home. This is consistent with the studies of other researchers where the victims commonly preferred to hang themselves in their place of residence.^{14,17,21,23} The reason might be to maintain privacy and avoid interference while committing the act.

CONCLUSION

Though cases of suicide by hanging are increasing day by day, there is sufficient scope to reduce the number of fatalities through proper education and awareness. To reduce the rate of suicides, awareness has to be created by appropriate education and by influencing the media in their portrayal of suicidal news. Identified individuals must be involved in devotional, encouraging and motivational activities. Counselling, de-addiction and rehabilitation centres should also be established in rural areas to help the victims.

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Study on Epiphyseal Union at the Wrist Joint among the Adolescents of Eastern Odisha

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ABSTRACT

BACKGROUND: Age is regarded as an important factor in various medical examination related to criminal procedures such as assault, sexual offence, kidnapping and in civil matters like succession of property, marriage, employment and so on. Fusion of epiphyses of bones is considered as one of the methods for age estimation.

AIMS: To study the stages of epiphyseal union of radius and ulna at the wrist joint in males and females.

MATERIAL AND METHODS: The present radiological study was carried out in a tertiary health care centre in coastal Odisha over a period of two years. The epiphyseal union at the wrist joint among 124 subjects (70 males and 54 females) among the adolescent population between 13 to 19 years have been studied to assess the skeletal maturity.

RESULTS: Our study concluded that complete epiphyseal union of lower end of radius in case of males occurs at 18-19 yrs. The complete epiphyseal union of lower end of radius in case of females occurs at 17-18 yrs. The complete epiphyseal union of lower end of ulna in both the sexes occurs at 18-19 yrs. The epiphyseal union around the wrist joint occurs earlier in females than males among the adolescents.

CONCLUSION: It is recommended to have separate age chart for different geographic regions as many factors like nutrition, genetic, climatic conditions etc. may affect the process of epiphyseal union.

KEYWORDS: Radiological study; Epiphyseal union; Wrist joint.

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INTRODUCTION

Age is one of the primary data of identification. Determination of age is required not only for identification purpose but also in both civil as well as criminal cases like attainment of majority, marriage, inheritance of property, criminal responsibility, rape, kidnapping etc. Age is likewise significant

in sports mainly in the design of competitions according to age groups to guarantee equal chances.⁷ In criminal or civil cases age of a person is determined by medical officer on requisition from proper authority. Physical, dental and radiological examination are required to determine the age of an individual. Appearance and fusion of different secondary epiphyses follows a definite pattern which is utilized for estimation of age. The fusion of epiphyses varies in different individuals as it depends upon gender, health, hereditary factors, nutritional status, endocrinal status, physical activity, climatic conditions etc. Estimation of age from epiphyseal union of long bones is also possible in dead and decomposed bodies, as the bones resist put refaction and mutilation for long time. Ossification and the union of epiphyses gives relatively reliable results with less minimal error and is therefore more appropriate and acceptable to both medical and legal personnel.¹³ Now a days, the number of false age related documents have increased and therefore it becomes essential on the part of forensic expert to determine the actual age of the individual in medico legal as well as civil cases.

MATERIALS AND METHODS

The present cross sectional descriptive study was carried out in the department of Forensic Medicine and Toxicology of a tertiary care centre in coastal Odisha over a period of two years. A total of 124 subjects (70 males and 54 females) between the age group 13 to 19 years coming to department for medicolegal examination and age estimation purposes were selected for this study. Age was confirmed from history and other documents suggesting date of birth. Subjects showing evidence of nutritional and vitamin deficiency and endocrine disorder, congenital abnormalities, hereditary diseases and cases with trauma to the wrist joint showing gross physical deformity have been eliminated from the study. Radiological examination of wrist joints were done and different stages of epiphyseal union were studied.

Criteria for Union

The different phases of epiphyseal union will be graded into five stages according to McKern and Stewart.¹⁰

- i. **Non-union:** When the epiphyseal cartilage does not begin to decrease in thickness (0 degree union).

- ii. **Beginning union:** When the thickness of the epiphyseal cartilages is reduced (1st degree union).
- iii. **Incomplete union (Active union):** When the epiphyses begin to fuse with the shaft (2nd degree union).
- iv. **Recent union:** When the epiphyseal cartilage is bony in architecture and its density indistinguishable from the epiphyses and diaphysis in its neighbourhood, but the epiphyseal scar is still distinguished (3rd degree union).
- v. **Complete union with absence of epiphyseal scar (4th degree union).**

RESULTS

Stage-1 epiphyseal union of lower end of radius was observed at 14-15 years of age in 22.2% of cases. It is also observed that stage-4 epiphyseal union was seen in 64.7% of cases in the age group 17-18 years while 100% of males showed stage-4 epiphyseal union at 18-19 years (Table 1).

Table 1: Stages of epiphyseal union of lower end of radius in males

Age Group	Number of Cases	Stages of Epiphyseal union				
		0	1	2	3	4
13-14	2	2	0	0	0	0
		100%				
14-15	9	3	2	4	0	0
		33.4%	22.2%	44.4%		
15-16	12	0	2	6	4	0
			16.7%	50%	33.3%	
16-17	14	0	3	0	4	7
			21.4%		28.6%	50%
17-18	17	0	0	0	6	11
					35.3%	64.7%
18-19	16	0	0	0	0	16
						100%

Stage-1 epiphyseal union of lower end of radius was observed at 14-15 years of age in 15.4% of cases. It is also observed that stage-4 epiphyseal union was seen in 60% of cases in the age group 16-17 years while 100% of females showed stage-4 epiphyseal union at 17-18 years (Table 2).

Table 2: Stages of epiphyseal union of lower end of radius in females

Age group	Number of cases	Stages of epiphyseal union				
		0	1	2	3	4
13-14	5	5	0	0	0	0
		100%				
14-15	13	8	2	2	1	0
		61.5%	15.4%	15.4%	7.7%	
15-16	14	0	5	7	1	1
			35.8%	50%	7.1%	7.1%
16-17	10	0	0	2	2	6
				20%	20%	60%
17-18	7	0	0	0	0	7
						100%
18-19	5	0	0	0	0	5
						100%

Stage-1 epiphyseal union of lower end of ulna was observed at 14-15 years of age in 22.2% of cases. It is also observed that stage-4 epiphyseal union was seen in 64.7% of cases in the age group 17-18 years while 100% of males showed stage-4 epiphyseal union at 18-19 years (Table 3).

Table 3: Stages of epiphyseal union of lower end of ulna in males

Age group	Number of cases	Stages of epiphyseal union				
		0	1	2	3	4
13-14	2	2	0	0	0	0
		100%				
14-15	9	3	2	4	0	0
		33.3%	22.2%	44.5%		
15-16	12	0	2	6	4	0
			16.7%	50%	33.3%	
16-17	14	0	3	0	6	5
			21.4%		42.8%	35.8%
17-18	17	0	0	0	6	11
					35.3%	64.7%
18-19	16	0	0	0	0	16
						100%

Stage-1 epiphyseal union of lower end of ulna was observed at 14-15 years of age in 15.4% of cases. It is also observed that stage-4 epiphyseal union was seen in 71.4% of cases in the age group 17-18 years while 100% of females showed stage-4

epiphyseal union at 18-19 years (Table 4).

Table 4: Stages of epiphyseal union of lower end of ulna in females

Age group	Number of cases	Stages of epiphyseal union				
		0	1	2	3	4
13-14	5	5	0	0	0	0
		100%				
14-15	13	8	2	2	1	0
		61.5%	15.4%	15.4%	7.7%	
15-16	14	5	0	7	2	0
		35.8%		50%	14.2%	
16-17	10	4	0	0	4	2
		40%			40%	20%
17-18	7	0	0	0	2	5
					28.6%	71.4%
18-19	5	0	0	0	0	5
						100%

Table 5: Gender wise comparison of epiphyseal fusion of lower end of radius and ulna with its shaft with previous studies

Author	Distal end of radius		Distal end of ulna	
	Male	Female	Male	Female
Aiman Al-Qatait et al.	20-21	18-19	20-21	18-19
Arun M	18-19	-	17-18	-
Dere C et al.	-	17-18	-	17-18
Gaddewar R	19	18	19	18
Godswill OO et al.	18	18	19	19
Jaybhaye PL et al.	-	-	>16	-
Krishnamoorthy et al.	18-19	17-18	18-19	17-18
Kumar R et al.	18-20	18-20	18-20	18-20
Nemade KS	20-21	19-20	19-20	19-20
Ominde BS et al.	18-19	17-18	19-20	18-19
Shabnum N et al.	18-19	17-18	18-19	18-19
Singh M et al.	20-21	19-20	19-20	18-19
Singh OG	17	17	17	17
Present study	18-19	17-18	18-19	18-19

DISCUSSION

In the present study, epiphyseal fusion of the lower end of radius and ulna in the males and females of coastal region of Odisha were analysed and the results were compared with the previous

studies. Our study reveals that complete fusion of lower end of radius was seen in 100% of cases in the age group of 18-19 years in males while in case of females it was observed in the age group 17-18 years. Complete epiphyseal union of lower end of ulna (100% of cases) in case of both males and females were noticed in the age group 18-19 years. Our observation in relation to fusion of lower end of radius in males is in line with studies done by other researchers^{2,5,8,9,12,13} while in case of females it is similar with other authors.^{3,8,12,13} The result of this study in relation to fusion of lower end of ulna in males is in agreement with studies done by^{8,12} while in females it is in line with.^{1,12-14} Present study reveals early union of epiphyses of radius by one year in females than males which is similar with most of the authors^{4,8,11-14} except Godswill OO who observed the age of fusion of distal ends of radius is 18 years in both sex while in case of ulna it is 19 years in both the sex. Our study reveals complete epiphyseal union of lower end of ulna is 18-19 years in both males as well as females which is in

agreement with Shabnum N *et al.*

CONCLUSION

The current study is an effort to study the process of epiphyseal union at the wrist joint among the adolescents of eastern Odisha. The completion of epiphyseal union in this population for the lower end of radius is 18-19 years and 17-18 for males and females respectively. Epiphyseal union in case of lower end of ulna is 18-19 years in both the sexes. The union of epiphyses varies according to different geographic locations around the world. Therefore, it is recommended to gather more data from different parts of the country in future studies to have a standard chart for the age estimation. It is also suggested to include more number of epiphyses for accurate age estimation in both civil as well as criminal cases.

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Application of UV light as a Screening Tool in Gross Examination of Heart at Autopsy

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ABSTRACT

CONTEXT: The conditions causing sudden death may be inherited and requires screening and counselling of the next of kin. In cases of death due to myocardial ischemia, identifying the early stages of myocardial pathology becomes difficult in postmortem as the gross changes do not appear for 24 to 48 hours following the ischemic damage to the heart. Hence, there is a need for a screening tool to identify the diseased part of the heart.

AIMS: To determine the applicability of ultraviolet light for the detection of cardiac pathology at autopsy.

SETTINGS AND DESIGN: This cross-sectional study was conducted from 1st January 2021 to 30th June 2022.

METHODS AND MATERIALS: There were 200 cases in which there were 153 (76.5%) males and 47 (23.5%) females. All cases that were subjected to the medico-legal autopsy of more than 30 years old were included in our study. The cases were divided into 5-year intervals of age. Gross findings of the heart, if any were noted. The heart was dissected using the inflow-outflow method. The internal and external surfaces of the heart were seen under wood's lamp fluorescence. Sections from areas that showed fluorescence under ultraviolet light were taken and subjected to histopathology examination. The specimen was processed, and a histopathology slide was prepared and examined. Routine haematoxylin & eosin staining was done.

STATISTICAL ANALYSIS USED: After the collection and compilation of data, statistical tests were applied to analyze the data. The mean and standard deviation (SD) were calculated for quantitative data, and for qualitative data, the proportion was calculated. Data were analyzed using SPSS version 20.0 software.

RESULT: A significant relationship was observed between heart pathology in correlation to age and sex. The sensitivity

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of wood's lamp was also found to be significant in fluorescent positive cases. By identifying discrepancies between gross, fluorescence, and microscopic findings, our findings suggest that histopathology has a major impact on the interpretation of heart pathology and determining the cause of death at autopsy.

CONCLUSION: In this study, we have worked on a new aspect of the diagnostic property of ultraviolet light (UV) extending to recent and acute myocardial infarction besides old myocardial infarction that can aid pathologists in gross heart examination, especially in more guided sampling for histopathological examination. Histopathological studies provide the most accurate clues to better understand cardiac pathology.

KEYWORDS: Myocardial Infarction; Ultraviolet light; Screening; Gross pathology.

INTRODUCTION

As per the World Health Organization (WHO) around 17.9 million people died from cardiovascular diseases in 2019, representing 32% of all worldwide deaths. Of these deaths, 85% were attributed to myocardial infarction and stroke. Out of the 17 million premature deaths which are usually described under the age of 70 due to non-communicable diseases in 2019, 38% were caused by cardiovascular diseases (CVDs).¹ According to the American Heart Association, in 2019 approximately 121.5 million adults in the United States had some form of cardiovascular disease.² The World Health Organization (WHO) states that more than 85% of cardiovascular deaths occur in low and middle income countries.³ The World Heart Federation predicts more than 23 million cardiovascular disease related deaths per year by 2030.⁴ An estimated 1.3 million Indians died from coronary artery disease (CAD) in 2000. The projected death from coronary artery disease (CAD) by 2015 was 2.95 million, but overall, around 6.4 million cases of coronary artery disease (CAD) were reported in the year 2015.⁵

The role of autopsy in sudden death is to establish whether death is attributable to cardiac disease or to other causes of sudden death, the nature of the cardiac disease, whether the mechanism was arrhythmic or mechanical, and finally, whether the condition causing sudden death may be inherited, requires screening and counselling of the next of kin.⁶

However, unlike the experimental settings, it is impossible to determine the exact time of ischemia because conditions such as preinfarction angina and collateral blood flow often complicate determining the duration of ischemia.⁷ Though there is a

significant decline in mortality from coronary artery disease (CAD), this disease still remains the leading killer in adults of all ages. Though most prevalent in the elderly, coronary artery disease (CAD) also affects young adults. Cardiac death due to coronary artery disease (CAD) is the most common cause of sudden death in young adults aged between 35-55 years.⁸

Many times, in cases of death due to suspected cardiac pathology, findings are not detected in gross pathology and autopsy surgeons need to preserve heart specimens for further examination. In cases where sudden death occurs at a very early stage of infarction, the myocardial lesions cannot be easily detected by traditional macroscopic examination or routine histological stains. Hence, the opinion regarding the cause of death is delayed which often adds to the grievance of the relatives. Many times, normal parts of the heart are preserved instead of diseased parts which may result in non-conclusive things on histopathological examination and results in negative autopsy. Hence, there is a need for a screening tool to identify the diseased part of the heart. One such tool is the use of ultraviolet (UV) light. This study was conducted to explore the applicability of ultraviolet light as a screening tool in the gross examination of the heart at autopsy.

The application of ultraviolet light in forensic practice has wide range of applicability.⁹⁻¹⁴ M. Bohnert *et al* (2000)¹⁵ performed an ultraviolet fluorescence test on the hearts of 30 individuals who died from cardiac causes. Solhi Hassan *et al* (2012),¹⁶ also studied 204 cases suspected of myocardial infarction under UVB hand lamps and they found the sensitivity and specificity of wood's lamp was 100% and 93.5% respectively.

Fluorescence methods are attractive as they supply a rapid, and non-destructive way of screening

large and multiple objects. If the efficacy of fluorescence detection is enhanced, its effectiveness as a rapid screening tool would be increased.¹⁷ The use of a wood's lamp doesn't require great expertise. However, important points should be considered to avoid misapprehension of results. They are as follows:

1. The lamp should be kept to warm up for around 1 minute.
2. The room for the examination should be dark, preferably a room without windows.
3. The examiner should get dark-adapted in order to see the difference more accurately.
4. The wood's lamp should be 4 to 5 inches from the object/fluid.¹⁸

MATERIALS AND METHODS

The present cross-sectional observational study was carried out at the Department of Forensic Medicine and Toxicology, Shyam Shah Medical College, Rewa from 1st January 2020 to 30th August 2022, after obtaining ethical clearance from Institutional Ethics Committee (IEC). All cases that were subjected to the medico-legal autopsy of more than 30 years old were included in our study. All decomposed and charred bodies were excluded from the study.

In the study conducted, the heart was dissected out from the body and washed under tap water. The heart was not weighed at this point, as it contained blood clots which could have falsely elevated the weight, leading to mistaken impressions of hypertrophy.¹⁹ The gross findings if any were noted and the outer surface of the heart was seen under wood's lamp fluorescence in the dark room and any fluorescent area was noted. Coronary arteries were dissected and changes if any, were documented. The heart was dissected using the inflow-outflow method.²⁰ After the dissection of the heart, the internal surface of the heart was seen under wood's lamp fluorescence in the dark room and fluorescent area if any was noted. After that, the transverse slices of the heart were made and again seen under wood's lamp fluorescence for any fluorescent area.

Wood's lamp of 'Dermaindia', range 320-400 nm, 2X4 W was used in the present study. The gross findings if any were noted and the outer surface of the heart was seen under wood's lamp fluorescence in the dark room and any fluorescent area was noted. Coronary arteries were dissected and changes if any, were documented. After the dissection of

the heart, the internal surface of the heart was seen under wood's lamp fluorescence in the dark room and fluorescent area if any was noted. After that, the transverse slices of the heart were made and again seen under wood's lamp fluorescence for any fluorescent area. Gross pathology was considered in terms of an increase in weight of the heart >420 grams, occlusion of >50% in coronary arteries, infarct on the surface of the heart, signs of atherosclerosis, cardiac surgery/procedure, rupture of the heart, pathology of valves, increase in left ventricular wall thickness of >1.5 cm and increase in right ventricular wall thickness of >0.5 cm.^{20, 21, 22} Sections from areas that showed fluorescence under ultraviolet light were taken and subjected to histopathology examination and a control area from the same heart and chamber but from the site away from the fluorescent area was taken and preserved in a 10 percent formalin solution for fixation.

The specimen was then taken to the histopathology lab. Grossing of the samples was done, which included describing the specimen's size, shape, color, and overall general appearance, followed by placing samples of the tissue in processing cassettes. After staining the slides with haematoxylin and eosin stain it was viewed under an olympus microscope with magvision software and findings were observed and recorded.

After the collection and compilation of data, statistical tests were applied to analyze the data. The mean and standard deviation (SD) were calculated for quantitative data, and for qualitative data, the proportion was calculated. Data were analyzed using SPSS version 20.0 software. The data was tabulated as per guidelines for reporting statistics, JMIR Publication.²³

RESULT AND DISCUSSION

In our study, there were 200 cases in which there were 153 (76.5%) males and 47 (23.5%) females. We observed male preponderance in our study. This is due to the reason that, males are bread earners and females usually do household work, making males more vulnerable to accidents, violence, and stress. Hence, males are more frequently subjected to medicolegal autopsies due to their involvement in outdoor activities. We divided the cases into 5-year intervals of age, so as to correlate with the total number of cases in each group. In our study, the age of the cases ranged from as low as 30 years to as high as 90 years. The highest number of cases i.e., 37 (18.5%) were in the age group of 30-35 years.

It was followed by 32 (16%) cases in the age group of 36-40 years. There were 28 (14%) cases in the age group of 41-45 years and 22 (11%) cases in the age group of 46-50 years (Table 1). This is in concordance with the studies of other authors that the male population in 3rd and 4th decades of life is most commonly encountered in medicolegal autopsies.^{24,25,26}

Table 1: Distribution of cases according to age and sex

Age (years)	Males (%)	Females (%)	Total (%)
30-35 years	33 (16.5%)	04 (2%)	37 (18.5%)
36-40 years	29 (14.5%)	03 (1.5%)	32 (16%)
41-45 years	18 (9%)	10 (5%)	28 (14%)
46-50 years	19 (9.5%)	03 (1.5%)	22 (11%)
51-55 years	17 (8.5%)	09 (4.5%)	26 (13%)
56-60 years	12 (6%)	03 (1.5%)	15 (7.5%)
61-65 years	11 (5.5%)	04 (2%)	15 (7.5%)
66-70 years	02 (1%)	02 (1%)	04 (2%)
>70 years	12 (6%)	09 (4.5%)	21 (10.5%)

Table 2: Comparison of cases with gross pathology and positive fluorescence

Cases with norelevant gross pathology (n=128)		Cases with relevant gross pathology (n=72)		TOTAL (n=200)
Cases with no gross pathology and showing positive fluorescence	Cases with no gross pathology and not showing fluorescence	Cases with relevant gross pathology showing fluorescence	Cases with relevant gross pathology not showing fluorescence	
30 (15%)	98 (49%)	51 (25.5%)	21 (10.5%)	200 (100%)
128 (64%)		72 (36%)		

pathologies in 23.3% of the cases, which is lower than the present study. However, Garg M *et al*²⁹ and Vyas *et al*³¹ reported a higher number of cardiac pathologies, in 46.4% and 73.45% of the total cases in their studies. We found the maximum number of gross pathology of hearts in the age group of 36-40 years i.e., 13 cases. This can be due to a higher number of cases in the age group of 36-40 years, than in other age groups. However, our observation contrasts with observations made by Siddiqui *et al*²⁸ who found the maximum number of cardiac pathologies in the age group of 51-60 years.

In the present study, coronary occlusion was seen most commonly in the age groups of 51-55 years and 61-65 years i.e., 7 (15.5%) cases in each group. Coronary artery occlusions of >50% were taken into account in our study. Coronary occlusion can be classified as slight narrowing, 30%; moderate narrowing 50%; and severe narrowing that is 70% and above.³² Occlusions of >50% are considered significant.^{33,34} The age groups that were affected by

Total	153(76.5%)	47 (23.5%)	200 (100%)
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In the present study, the average age of total cases was 49.7 years. Jhaji *et al*²⁴ observed that the average age of cases in their study was 39±2 years, which is almost similar to our study. However, Yazdi *et al*²⁶ observed the mean age in their study to be 30 years, which is lower than the present study. We observed that the mean age of cases in cardiac pathology was 54.85 years. Similarly, Dhruva *et al*²⁷ and Siddiqui *et al*²⁸ also observed the mean age of cases with cardiac pathology on histopathological examination to be 55±15 years and 55.1±17.16 years respectively. Garg M *et al*²⁹ and Sudha *et al*³⁰ also observed the average age in cardiac pathology to be 52 ± 14 years and 54 years respectively, which is almost similar to our study.

In the present study, on gross examination of 200 samples of the heart, we observed that 128 (64%) cases showed no remarkable findings and 72 (36%) cases showed significant gross pathology (Table 2). Yazdi *et al*²⁶ observed cardiac pathologies in 40% of the total cases, which is almost similar to the present study. Dhruva *et al*²⁷ observed cardiac

coronary artery disease ranged from 32-90 years. Similar to the present study, Siddiqui *et al*²⁸ and Garg M *et al*²⁹ also found coronary artery disease in the age range of 22-85 years and 20-70 years, respectively. Although calcification is found more frequently in advanced lesions, it may also occur in small amounts in early lesions, which appear in the 2nd and 3rd decades of life. It is due to ageing which is associated with structural and functional changes in the vessel wall, which result in decreased vascular distensibility and elevated arterial stiffness.³⁵

We observed that the left anterior descending coronary artery (LAD) was the most common artery to be involved i.e., in 42 (21%) cases. Left circumflex artery (LCX) and right coronary artery (RCA) were affected in similar percentages i.e., in 19 (9.5%) cases each. Vyas *et al*,³¹ Dhruva *et al*,²⁷ Siddiqui *et al*,²⁸ Garg *et al*,³⁶ Sudha *et al*,³⁰ and Yazdi *et al*,²⁶ also found the most frequent involvement of the left anterior descending coronary artery (LAD), which is similar to our study. However, they found

the right coronary artery (RCA) to be the least commonly involved which is in contrast with our study. Gradwhol quoted the range of left anterior descending coronary artery (LAD) to be 45-64%, while right coronary artery (RCA) comes next in frequency, 24-46%; followed by left circumflex (LCX) 3-10%; and least affected is the left main coronary artery.³⁷

In the present study, we observed single vessels to be most affected i.e., in 19 (42.1%) cases. The isolated involvement of the left anterior descending coronary artery (LAD) was seen in 16 (35.5%) cases, and the right coronary artery was seen in 03 (6.6%) cases. Frequent involvement of two and three vessels was also seen in the present study. Incidence of involvement of double vessels was seen in 17 (37.7%). In double vessels, we observed the left anterior descending coronary artery (LAD) and left circumflex artery (LCX) to be involved in 10 (22.2%) cases, the left anterior descending coronary artery, and right coronary artery in 07 (15.5%). Involvement of double vessels was followed by the involvement of triple vessels i.e., the left anterior descending coronary artery (LAD), left circumflex artery (LCX), and right coronary artery (RCA) in 09 (20%) cases. Hence, in the present study, the involvement of single vessels was more frequent than the involvement of double and triple vessels. This can be due to the reason that in our study there were maximum number of young patients. Piyush J *et al*³⁸ also observed the single vessels to be more frequently involved, which is similar to our study. In contrast to our observations, Vyas *et al*,³¹ Dhruva *et al*,²⁷ Garg M *et al*,²⁹ and Yazdi *et al*²⁶ observed the triple vessels to be more frequently involved than single vessels.

We observed infarction on macroscopic examination in 14 cases (7%). Our findings were almost comparable with Maru³⁹ in 6.5% of cases, and Dhruva *et al*²⁷ in 9.72%. However, Garg M *et al*²⁹ observed evidence of myocardial infarction in 26.8% of cases which is higher than our study. According to the age of infarcts, they are classified as acute or recent, upto 4 weeks. In 4 to 6 weeks, an acute infarct turns into an old or healed infarct.⁴⁰ We observed that acute myocardial infarction (4 weeks) was present in 4 cases (2%). In one case, we observed an old healed myocardial infarction in form of a whitish patch and an acute myocardial infarction in form of a hyperemic border with central yellow-tan softening. Marwah *et al*⁴¹ observed acute myocardial infarction in 7% of cases and old myocardial infarction in 3.5% of cases, which is almost similar to the present study.

Vyas *et al*³¹ observed a slightly higher percentage of acute myocardial infarction in 10.8% of cases and old myocardial infarction in 13.25% of cases. In the present study, we observed 10 male cases and 4 female cases of myocardial infarction at autopsy. Out of 4 females, 3 females were of the postmenopausal age group. Premenopausal women have a lower risk and incidence of hypertension and cardiovascular disease (CVD) compared to age-matched men and this sex advantage for women gradually disappears after menopause, suggesting that female hormones play a cardioprotective role in women. In the present study, cardiac rupture was seen in 03 (1.5%) cases, and in all the cases, we observed occlusion of the coronary artery (LAD, RCA, LCX) of more than >75%, which is considered a severe occlusion. Rao *et al*,⁴² observed cardiac rupture with coronary occlusion in 31.07% of cases, which is higher than our study. This may be due to the reason that they have included cardiac deaths in their study. In all 03 cases, the rupture was seen on the postero-lateral surface of the left ventricle. Our findings were similar to those observed by Tas *et al*,⁴³ who observed the most common sites of cardiac wall rupture being the lateral wall and posterior wall of the left ventricle. Rupture of the left ventricular (LVW) wall during acute myocardial infarction (AMI) is nearly always fatal, and a higher frequency has been reported in hypertensive compared with normotensive patients.⁴⁴ On histopathological examination, the torn area also showed disintegration of dead myofibers, vacuolar degeneration surrounded by neutrophils, abundant mononuclear infiltration, and the aorta showed atherosclerotic changes. An increase in right ventricular wall (RVW) thickness of >0.5cm is considered significant.¹⁹ We observed an increase in right ventricular wall (RVW) thickness was seen in 5 (2.5%) cases. Siddiqui *et al*²⁸ observed an increase in right ventricular wall (RVW) thickness in 16.6% of cases, which is higher than our study. In the present study, we observed that all 05 cases of an increase in right ventricular wall (RVW) thickness, were associated with an increase in left ventricular wall (LVW) thickness and coronary occlusion in 04 cases. Anna S *et al*⁴⁵ found a predominance of right ventricular hypertrophy and left ventricular hypertrophy in men aged 60-79 years and obese individuals.

However, we observed a predominance of right ventricular hypertrophy much earlier in men aged between 35-87 years old. In our study, we also observed that in 02 cases, an increase in right ventricular wall thickness was associated with a history of cardiac procedures (bypass grafting and

ventricular septal defect repair). Bhattacharya *et al*⁴⁶ documented right ventricular hypertrophy in response to pressure overload, most commonly due to pulmonary hypertension and conditions affecting the tricuspid valve. Moreover, Attoh *et al*⁴⁷ have reported right ventricular hypertrophy to be due to an increase in COVID-19 infections due to pathological effects of COVID-19 infection in the lungs as adult respiratory distress syndrome/diffuse alveolar damage with thromboembolic phenomena. Hence, an unexplained increase in right ventricular wall thickness (RVW) can be, due to COVID-19 infection which was on a surge, at a point of time in our study.

Many times, it is seen that when gross pathology could not help to evaluate the cause of death, microscopic examination can conclusively opine the involved cardiac pathology.⁴⁸ Thus, the histopathological examination was considered to be the gold standard in our study. In our study, we observed waviness of muscle fibers in 20 cases, coagulative necrosis in 05 cases, waviness and neutrophilic infiltration in 04 cases, chronic myocarditis and necrosis in 03 cases, and waviness of muscle fibers and coagulative necrosis in 01 case. The histopathological findings we observed in our study, of myocardial infarction were similar to those given by Kumar *et al*,⁴⁹ Bouchard and Majno,⁵⁰ and Smilowitz NR⁵¹ which include, wavy myofibrils, coagulation necrosis, hemorrhage, and neutrophilic infiltrate. Siddiqui *et al*²⁸ and Garg *et al*³⁶ also reported similar findings of myocardial infarction in their studies.

Among 200 cases, 81 (40.5%) fluorescent positive cases were subjected to histopathological examination along with a control sample from the same heart and chamber, but away from the fluorescent area i.e., nonfluorescent area. We found that 33 (16.5%) cases showed significant histopathological findings and 48 (24%) cases were histopathologically unremarkable. In our study, the waviness of muscle fibers was the most predominant finding on histopathological examination and was seen in 20 (24.6%) cases. It is often the earliest change of myocardial infarction observed in a microscope and has been a characteristic and diagnostic feature of myocardial infarction.⁵¹ However, Derias *et al*⁵² have raised considerable suspicion about the specificity and reliability of the wavy fiber as a histopathological index of myocardial infarction as they were seen in about half the normal and half of the infarcted human hearts and were even present in heart of the 6-week-old infant and was prominent in older

subjects. Such fibers were also indistinguishable from those around areas of myocardial infarction. In the present study, chronic myocarditis with necrosis was seen in 03 (3.7%) cases. In 02 cases, it was associated with a history of coronary artery bypass grafting (CABG) and angioplasty. In 01 case, it was found as an incidental finding. Myocarditis is an inflammatory condition of the heart muscle and its causes are extremely varied and include infectious and non-infectious agents.⁵³ In our study all the cases of myocarditis were males. Our findings were consistent with those of Okoda *et al*,⁵⁴ who reported male preponderance in chronic myocarditis in their study. However, we reported a lesser percentage of myocarditis than Joshi C *et al*⁵⁵ (9%), Basso C *et al*⁵⁶ (10%), Ozdemir *et al*⁵⁷ (7%), Dory *et al*⁵⁸ (25%), and Karmer *et al*⁵⁹ (29%). Although congestion was considered an insignificant finding in our study, we observed congestion of the myocardium in 47 (58%) cases which was higher than observed by Jhajj *et al*²⁴ in 24% of cases in their study.

In our study, the maximum number of significant histopathological findings (waviness of muscle fibers, coagulative necrosis, neutrophilic infiltration, chronic myocarditis, and necrosis) were observed in the age group of 46-70 years. Siddiqui *et al*²⁸ observed maximum cases of myocardial infarction in the age groups of 51-60 years which is almost similar to our study. Dhruva *et al*²⁷ observed the maximum number of cases with findings of myocardial infarction in age groups of 32-80 years. Garg M *et al*²⁹ also observed a maximum number of cases of myocardial infarction in the age group of 29-80 years. In the present study, we observed a maximum number of histopathologically significant findings i.e., 17 fluorescent positive areas in the posterior wall of the left ventricle, and 02 fluorescent positive areas on the anterior wall of the left ventricle, which showed histopathologically significant findings. The posterior wall of the right ventricle showed 10 fluorescent areas. Verma *et al*⁶⁰ and Mortensen *et al*⁶¹ also observed that the posterior ventricular wall is most commonly involved in ischemic heart disease.

However, Hassan *et al*,¹⁶ observed the sensitivity and specificity of wood's lamp was 100% and 93.5% respectively. The sensitivity observed by them is much higher than the sensitivity observed in our study. This can be due to the reason that they had included cases of suspected myocardial infarction in their study. The areas of infarction varied in their study and they sent only one sample from any of the fluorescent areas observed by them. But in our study, we had sent all the fluorescent areas for

histopathological examination. In our study, 40.5% of the hearts showed bluish-white fluorescence

under wood's lamp and when they were subjected to histopathological examination, 16.5% showed

Table 3: Results of the wood's lamp fluorescence

Wood's lamp fluorescence-positive cases (n=81)		Wood's lamp fluorescence -negative cases (n=119)	Total (n=200)
HPE Significant	HPE Unremarkable		
33 (16.5%)	48 (24%)	119 (59.5%)	200 (100%)

Table 4: Distribution of cases according to gross findings in fluorescence positive and its correlation with histopathology results

Gross findings observed in fluorescence-positive cases (n=81)	Number of cases	HPE significant (%)	HPE negative (%)
Coronary artery disease	34	23 (67.6%)	11 (32.3%)
Increase in cardiac weight	28	14 (50%)	14 (50%)
Left ventricular hypertrophy	22	16 (72.7%)	6 (27.2%)
Fibrotic areas/ Infarcts	9	8 (88.8%)	0 (11.1%)
Right ventricular hypertrophy	04	04 (100%)	00 (0%)
Cardiac procedure (Bypass surgery, angioplasty and ventricular septal defect repair)	04	04 (100%)	00 (0%)
Rupture of heart	03	02 (66.6%)	01 (33.3%)
No gross pathology	28	03 (10.7%)	25 (89.2%)

evidence of myocardial pathology (Table 3). However, Bohnert *et al*¹⁵ observed that 33% of the hearts showed bluish-white fluorescence under wood's lamp and when these were correlated with

104 microscopic examination 9% of cases showed patches of myocardial infarction. So, we reported higher sensitivity of wood's lamp examination of cardiac pathology than observed by Bohnert *et al*.¹⁵

Table 5: Sensitivity of wood's lamp

Wood's lamp fluorescence – positive cases (n=81)		Wood's lamp fluorescence - negative cases (n=119)	Total (n=200)
Cases with significant HPE finding	Cases with no significant HPE findings		
33(A)	48(C)	119(D)	200
81 (A+C)			



Fig. 1: Wood's lamp

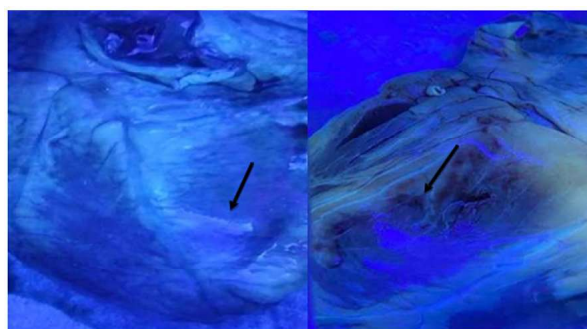


Fig. 2: Fluorescent areas under wood's lamp illumination

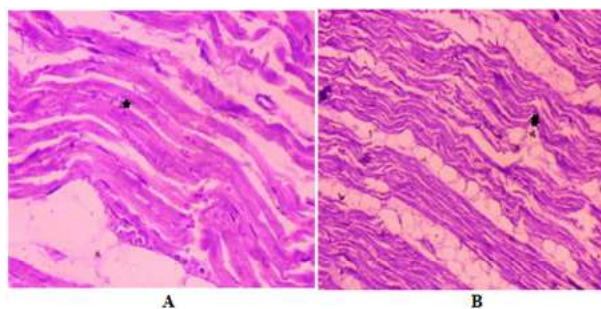


Fig. 3: Histology of myocardium showing

A. Waviness of muscle fibers (10x)

B. Section showing neutrophilic infiltration (40x)

(*waviness of muscle fibres, #neutrophilic infiltration)

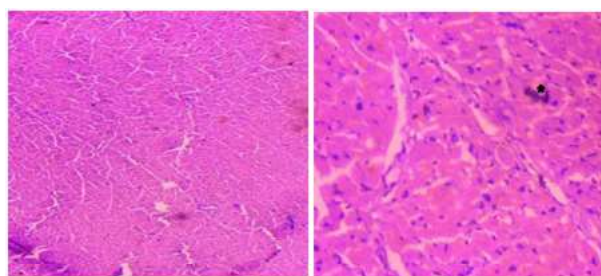


Fig. 5: Histology of unremarkable myocardium

A. Section showing unremarkable myocardium (4x)

B. Section showing congested myocardium. (40x)

(*congestion of myocardium)

(Table 5)

CONCLUSION

World Health Organization (WHO) scientific group once said ‘Since many cases of sudden death from acute ischemic heart disease become the subject of medico-legal autopsy, it is essential that forensic pathologists should be well acquainted with the most suitable techniques and be able to put them into practice.’⁶² Postmortem diagnosis of early myocardial infarction is an ever recurrent problem in forensic pathology due to the lack of good conventional techniques for the diagnosis of myocardial ischemia. In the present study, we observed that gross examination could alone identify 71% of cardiac pathologies as confirmed by subsequent

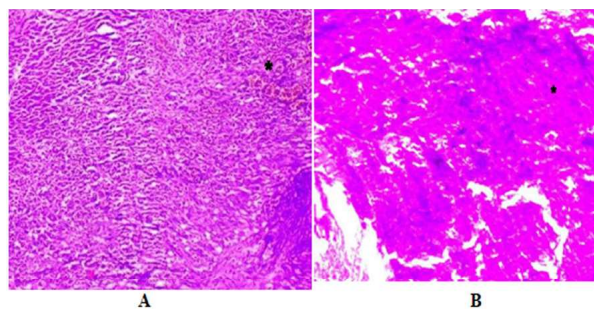


Fig. 4: Histopathology of myocardium

A. Section showing chronic inflammatory infiltrate consisting of lymphocytes and plasma cells. (10x)

B. Areas showing coagulative necrosis. (40x)

histopathological examination, which is far more than the wood’s examination. Hence, we can conclude that if a gross examination of the heart is done properly at autopsy, it can detect most of the cardiac pathologies.

RECOMMENDATION

To determine the better efficacy of wood’s lamp fluorescence, cases of known cardiac pathology should have been taken into account. We did not perform the histopathological examination on all the cases. All hearts i.e., fluorescent and non-fluorescent should have been sent for the histopathological examination. The waviness of fibers was the most predominant finding in wood’s lamp fluorescence positive samples and it has been discussed earlier that the waviness of muscle fibers is not specific to myocardial infarction. Failure to access the clinical history of autopsy cases was another restriction of our study. Although sampling was not conducted systematically, there was no bias of age or gender within our sample. The study was conducted on autopsy cases from the Vindhya region only. As sample size is less and number of reference studies are very few, further studies with greater sample size is recommended.

Conflict of Interest: Nil

Source of Funding: Nil

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A Orthopantomography Study of Age Estimation Based on Periodontal Ligament Visibility of Mandibular Third Molars in Chennai Population: An Aid in Forensics

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ABSTRACT

BACKGROUND: Forensic age determination has evolved to be intriguingly significant and relates to the challenges originating from globalisation as a result of a growing amount of foreign nationals with questionable birth date information and to identify victims who are unknown.

AIM: Aim of this research was to evaluate the estimation of age through visualisation of periodontal ligament (PDL) around fully mineralised lower third molars in the Chennai population, and also to assess the 18-year age threshold using PDL visibility.

MATERIALS AND METHOD: The research was a retrospective analysis that involved 100 OPGs (orthopantomographs) from the archives of the Department of Oral and Maxillofacial Radiology from 2021 to 2022 were analysed to determine PDL present around the mandibular third molars. Grading each mandibular third molar was done by two observers. IBM SPSS version 23 was used to statistically evaluate the gathered data.

RESULTS: Among 100 study subjects, the mean age group was found to be 24.2 years. The frequency distribution of age was inferred from that of the study participants, 15% were under the age of 18, and 85% were above the age of 18. Males and girls with both lower third molars on the left and right had grades of stage 1 (34%), stage 2 (31%), and stage 3 (20%) at the age of 18. The 18-year threshold can be established by using periodontal ligament visibility, according to our findings, starting from Stage 1. The relationship between the variables was examined using the Chi square test, which produced a very significant p value of 0.00.

CONCLUSION: Radiographic age evaluation was a simple, repeatable, non-invasive technique that can be applied to both living and deceased people. Our research indicated that less than 18 years old was the minimal age for Stage 0 in both males and females. At the onset of stages

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1, 2, and 3, all males and females were at least eighteen years old. Stages 1, 2, or 3 indicate that the person is older than 18. The populace of Chennai would benefit from this innovative method of age estimation for use in forensics.

KEYWORDS: Age estimation; Periodontal ligament; Mandibular third molar; Forensic.

INTRODUCTION

Forensic age determination has evolved to be intriguingly significant and relates to the challenges originating from globalisation as a result of a growing amount of non-national individuals with uncertain information about their birth date and to identify unknown victims, calculating the age at death, identifying cluster victims, figuring out who qualifies for social benefits, and helping immigration services in the processing of undocumented immigrants.¹ Accurately determining the person's age is crucial when attempting to identify a deceased person.² Third-molar mineralization has frequently been employed as a technique for forensic age determination. Estimating age is required throughout both criminal and civil processes.³ Teeth eruption or formation can be used to gauge dental development. In contrast to eruption, which is a discontinuous and variable measurement influenced by a number of factors including dental caries, malnutrition, early loss of deciduous teeth, and malalignment, root formation is thought to be a more robust measure with a low coefficient of variation, high heritability, and resistance to environmental effects.⁴ Research on the growth and development of teeth benefits from knowledge of the time and sequence of tooth eruption. To determine a child's or adult's chronological age, several techniques are available.⁵

The primary radiographs of specific structures, such as the fusion of long bones, the epiphyseal union of the anterior iliac crest, the epiphyseal head, the fusion of the sphenoid bone with the basilar part of the occipital bone, and the medial extremity of the clavicle, are used to estimate age using skeletal maturity.^{6,7} The most frequently used techniques for determining chronological age are those that use an orthopantomograph or cephalometric radiograph to visualise dental development.⁸ The presence or absence of wisdom teeth, the age at which they erupt, the eruption's position and direction, and its timing are important factors, especially in forensic dentistry.⁹ With the exception of the third molars, most teeth have calcified and erupted after the early teen years. Therefore, the most important factor in determining age between the late teens and early twenties is the third molar's development.¹⁰ Thus, the evaluation of dental radiographs is capable of being easy and non-invasively used to staging a third molar. To describe the dental maturation process, several staging systems have been developed.¹¹ Olze *et al.* presented a technique for estimating dental age in the German population by visualizing the

PDL of the mandibular third molars from dental radiographs taken of patients between the ages of 15 and 40. The PDL visibility was classified into four stages from 0 (entirely visible in both roots) to 3 (visible in only part of one root). The chronological age for each grade of periodontal ligament visibility (PLV), along with the minimum age and suggested PLV, was reported in this study, which was useful in identifying individuals who were younger than 18 or who were between the ages of 18 and 21 years.¹² In another study, Timme *et al.* found that while the minimum age of periodontal ligament visibility stages was appropriate for males under the age of 21, but not for females, it was unable to distinguish between individuals under the age of 18.¹³

As a result, age estimation varied across different population groups and location, previous studies are reported in Germany¹², Chinese¹⁴, Portuguese¹⁵ and in India Mumbai.¹⁶ Studies were not available for the Chennai population. Therefore, the purpose of this study was to determine the 18-year age threshold using PDL visibility and to evaluate the estimation of age using visualizing the periodontal ligament around fully mineralized lower third molars in the Chennai population.

MATERIALS AND METHOD

100 orthopantomographs from the archives of Saveetha Dental College and Hospital's Department of Oral Medicine and Radiology in Chennai were screened for the visibility of PDL surrounding the mandibular third molars between the years of 2021 and 2022. This is a retrospective study.

Inclusion Criteria: Patients with fully mineralized mandibular third molars, ranging in age from 17 to 40 were included.

Exclusion Criteria: This study did not include any pathological conditions that could impede PDL visibility, radiographs that were faulty, or periapical pathology related to mandibular third molars, such as cysts and tumors.

Radiological evaluation of the third molars:

One hundred OPGs were assessed for the mandibular third molars on the left or right side. Two selected observers examined the radiographs. Scores were assigned to every mandibular third molar by observers. Olze *et al.* assigned the PDL visibility of third molars with full root formation including apical closure wherein four scores were given.¹² (Fig. 1)

Stages:

Stage 0: PDL is visible along the full length of all roots

Stage 1: PDL is invisible in one root from apex to more than half root.

Stage 2: PDL is invisible along almost the full length of one root or along part of the root in 2 roots or both.

Stage 3: PDL is invisible along almost the full length of 2 roots.

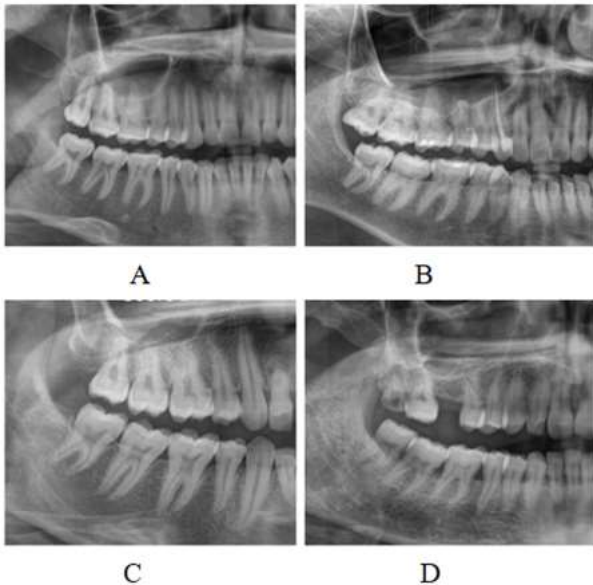


Fig. 1: The radiographic visibility in lower third molars is displayed by the OPG as follows: (A) Stage 0 (B) Stage 1 (C) Stage 2 (D) Stage 3.

STATISTICAL ANALYSIS

Orthopantomographs was assessed and cross-checked by 2 oral radiologists. Data was collected and compiled in an excel spreadsheet. IBM SPSS v23 software was used to perform statistical analysis on the data. To investigate the relationship between the various research variables, the chi square test was used. ANOVA test was performed to identify the mean of age in different populations.

RESULTS

Among 100 study subjects, the mean age group was found to be 24.2 years. 15% of the study participants were under the age of 18, and 85% were over the age of 18, according to the frequency distribution of age shown in Fig. 1. Hence, the above 18 years age group was reported higher in number. The gender distribution of the patients was reported as 59% were males and 41% were females shown

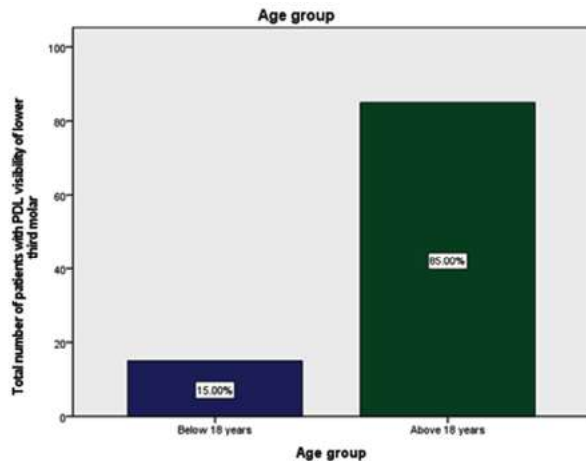


Fig. 1: The frequency distribution of age groups is displayed in a bar graph. The X-axis indicates the age group above or below 18 years. The percentage of people who reported to this study is shown on the Y-axis. Highest prevalence was among above 18 years of age group (green) and below 18 years were 15%.

in Fig. 2, Fig. 3 shows the periodontal ligament visibility frequency distribution. The vast majority of the study participants belonged to Stage 1 (34%) followed by Stage 2 (31%), Stage 3 (20%) and Stage 0 (15%). Fig. 4 shows the frequency distribution of the age group with PDL visibility stage. It was inferred participants below 18 years belong to stage 0 and stage 1, 2 and 3 was seen in above 18 years age group. Using the chi square test, the relationship between the variables was assessed, and a p value of 0.00 indicated that it was highly significant. Fig. 5 shows tooth number and PDL visibility stage. The most common third molars in stage 1 (18%) were lower left (38) and most common in stages 1 and 2 (16%) were lower right (38) molars. Chi-square was

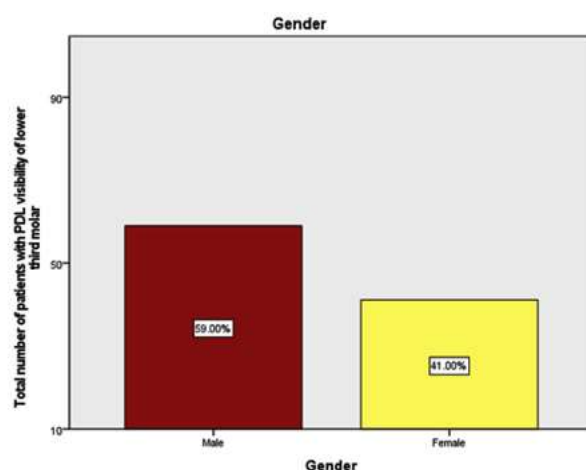


Fig. 2: The gender distribution is shown in a bar graph. Males are indicated by red, and females by yellow. Males (59%) made up the majority of the patients reported for the study, compared to females (41%).

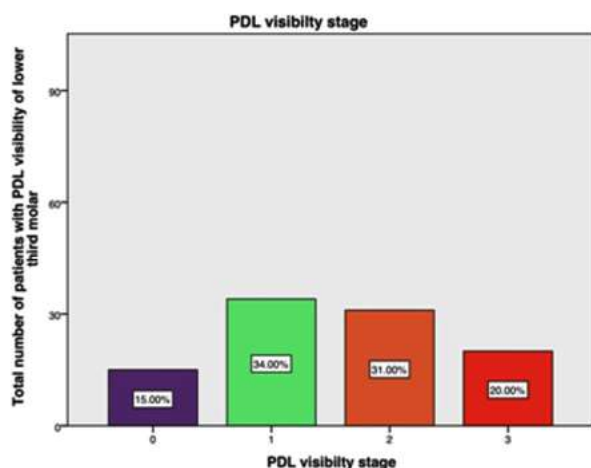


Fig. 3: The PDL visibility stage frequency distribution for each individual is displayed as a bar graph. Stages 0–3 are indicated by the X-axis. The percentage of participants in this study is indicated on the Y-axis. Highest prevalence was among stage 1 (light green) followed by stage 2 (orange) was 31%, stage 3 (red) was 20% and stage 0 (purple) was 15%.

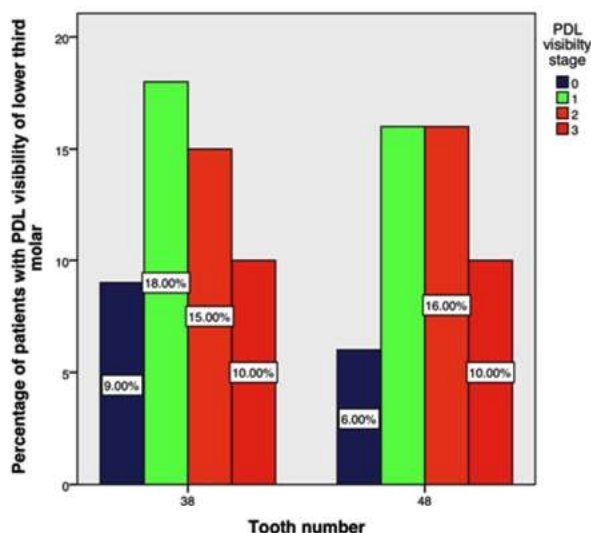


Fig. 5: The tooth number and PDL visibility stage are displayed in a bar graph. The tooth number is indicated on the X-axis, and the number of participants in our study is indicated on the Y-axis. The most common third molars in stage 1 (18%) were lower left (38) and most common in stages 1 and 2 (16%) were lower right (38) molars. The chi-square p-value of 0.01 indicated statistical significance.

statistically significant, with a p-value of 0.01. In stage 1, the lower right third molar (48) and lower left third molar (38), respectively, had the highest prevalence (18% and 16%). Statistics showed that the chi-squared p value=0.01 was significant.

In case of males, radiography visibility of 48 revealed the following results: The mean age was statistically significantly different for each stage category ($p < 0.01$), with the mean value being lowest in stage 0 and highest in stage 3. Stage 0 was

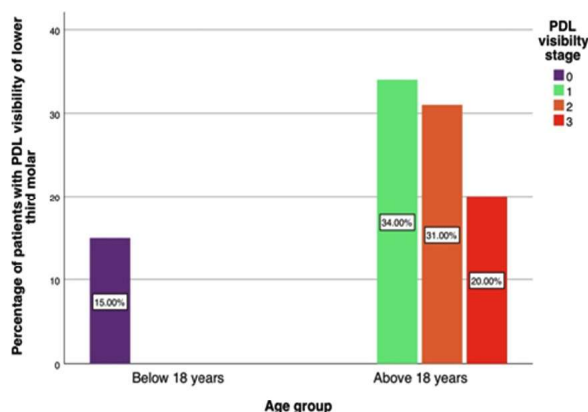


Fig. 4: Bar graph shows the age group and PDL visibility stage. The age group is shown on the X-axis, and the number of participants in our study is shown on the Y-axis. Stage 0 (purple) was seen only in individuals below 18 years (15%) and stage 1 (light green) was seen in the majority of the above 18 years old age group (34%) followed by stage 2 (orange) was 31% and stage 3 (vermillion) was 20%. Chi-square p value=0.00, was statistically significant.

initially identified at the age of 17, with a mean age of 18. At the age of 19, stage 1 was noted, with a mean age of 22. Stage 3's first indication appeared at age 25, with a mean age of 37.4 years, while stage 2's first indication appeared at age 24, with a mean age of 33.5 years. (Table 1).

Table 1: Mandibular right third molar age and stages of radiographic visibility of the periodontal ligament in males.

PDL Visibility stage	N	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
0	2	18.00	1.414	1.000	17	19
1	8	22.00	2.976	1.052	19	29
2	12	33.50	7.740	2.234	24	48
3	7	37.43	11.253	4.253	25	39

In female cases, radiographic visibility of 48 produced the following outcomes: There was a statistically significant difference ($p < 0.01$) in the mean age between the Stage categories, with the mean values being highest in Stage 3 and lowest in Stage 0. At 17.4, the first indications of stage 0 were observed, with a mean age of 17.4 years. At age 20, stage 1 was first observed, with a mean age of 21.7 years. Stage 2 was first noticed at age 21, with a mean age of 33, and stage 3 was noticed at age 32, with a mean age of 39.3 years. (Table 2).

Table 2: Age and stages of the female mandibular right third molar's periodontal ligament's radiographic visibility.

PDL Visibility stage	N	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
0	5	17.40	.548	.245	17	18
1	7	21.71	1.604	.606	20	25
2	4	33.00	8.042	4.021	21	37
3	3	39.30	14.422	8.327	32	40

In male cases, radiographic visibility of 38 showed the following results: A statistically significant ($p < 0.01$) difference in mean age was observed between the Stage categories. At the age of 17, with a mean of 17.67 years, stage 0 was first observed. For Stage 1 the first observed age was 20 years with mean age 23.8 years. Stage 2 was noted at 30 years old, with a 35.1-year-old mean. In stage 3, the mean age was 38.25 years, and the first observed age was 34 years. (Table 3).

Table 3: Mandibular left third molar age and stages of radiographic visibility of the periodontal ligament in males

PDL Visibility stage	Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum
0	17.67	3	.577	.333	17	18
1	23.80	10	2.348	.742	20	28
2	35.11	9	4.457	1.486	30	36
3	38.25	8	9.004	3.183	34	39

In female cases, radiographic visibility of 38 produced the following outcomes:

A statistically significant difference was observed ($p < 0.01$). With a mean age of 17.8, the first appearance of Stage 0 occurred at 17 years old. Stage 1 was first observed at 17 years old, with a mean age of 17.8 years. In Stage 2, the mean age was 31.5 years, and the first recorded age was 28 years. The mean age for Stage 3 was 39.7 years, with the first observed age being 30 years (Table 4).

Table 4: Age and stages of the female mandibular left third molar's periodontal ligament's radiographic visibility.

PDL Visibility stage	N	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
0	6	17.83	.408	.167	17	18
1	8	21.13	1.808	.639	19	25
2	6	31.50	4.278	1.746	28	37
3	2	39.70	16.971	12.000	30	39

DISCUSSION

Teeth are frequently used to estimate age because they are preserved for a very long time after all other tissues and bones have disintegrated. A quick, repeatable, non-invasive technique that can be applied to both living and unidentified deceased individuals is radiographic age assessment.¹⁷

Age estimation is one of the most crucial tasks for a forensic department when birth records are not well kept. It is an optical phenomenon that the periodontal ligament vanishes.¹⁸ This could have a biological basis in which the membrane becomes so thin that radiographs cannot show it.¹⁹ The development of third molar roots is typically taken into account in forensic dentistry when estimating age, particularly when establishing the 18-year-old cut-off to distinguish between a person's status as a child or adult.²⁰

It was discovered that individuals under the age of eighteen, regardless of gender, exhibited stage 0. Males' right and left third molars showed signs of stage 1 onward when they surpassed the age of 18. It follows that if a person is discovered in any of the stages starting with Stage 1, they have achieved major status and are older than 18 years old. Our findings support the observations made by Sequeira *et al.*¹⁵ and Olze *et al.*¹²

The age range in our study was 17–40 years, whereas the age range in Demirjian *et al.*'s study was only 3–17 years, despite the fact that they presented a very good method for age estimation from the radiological appearance of the mandibular third molar.²¹ Timme *et al.*¹³ found that the minimum age for stages 2 and 3 was at least 21 years old in their investigation. Our study's results are consistent with this finding.

Another observation from our investigation was that, starting from stage 2, the participants were older than.²¹ Consequently, stage 2 could be applied to establish the 21-year-old cut-off point. Another significant age in forensics is 21. This is consistent with the findings of Guo *et al.*¹⁴ regarding the Chinese populace. But Sequeira *et al.* pointed out that in the Portuguese population, Stage 3 would fulfill this function, emphasizing the variation seen in various populations. Therefore, in order to increase prediction efficacy, it is recommended that this methodology be evaluated and used in particular populations.¹⁵

In both the left and right mandibular molars, there was a significant positive correlation ($p = 0.000$) between the periodontal ligament visibility

and chronological age. This was consistent with other research.²¹ Since the entire study depends on OPG observation a crucial step in standard forensic age estimation the same X-ray can be utilized to assess the third molars' periodontal ligament visibility.

In our study, strong positive association was observed between chronological age and the periodontal ligament visibility in right as well as left mandibular molars ($p = 0.000$). This was in accordance with other studies.²² The technique's benefit is that it will reduce costs and time spent without increasing radiation exposure.²³ Additionally, exhibits strong intra- and inter-observer agreement and is a straightforward methodology that is applicable on a regular basis.²⁴ There are certain restrictions associated with this technique. Specifically, third molars must have fully developed roots, be free of caries or other dental disease, and be fully erupted without being

impacted. Future research could make use of this and assess it.

CONCLUSION

In our study, both males and females had to be younger than 18 years old to be eligible for Stage 0. For both males and females, the minimum age at which Stages 1, 2, and 3 initially manifested was greater than 18. We can therefore conclude that a person should be older than eighteen if they exhibit Stages 1, 2, or 3. Third molar growth and calcification are unquestionably not the preferred development marker. Still, it makes sense because there aren't any other promising signs in the late teens and early twenties.

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Assessment of Attitude and Knowledge of Law Students Towards Medico-Legal Autopsies

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ABSTRACT

BACKGROUND: Autopsies play an important role in aiding the judicial system. Forensic Medicine is the bridge that connects medical knowledge and the law together and Autopsies are the vital part in this link. In order to create better efficiency in the judiciary, strengthening and sharing knowledge is important on both the Forensic Expert's and the Lawyer's view. The attitude or perception of autopsies is equally important as much as the knowledge about the same.

AIMS: The purpose of this study is to assess the knowledge and attitude of law students about and towards medico legal autopsy and to identify the reasons if any and suggest some corrective measures.

MATERIAL AND METHODS: A cross sectional prospective study done in a Law College in Mangalore, with a sample size of 140 undergraduate law students using a semi-structured questionnaire distributed online.

RESULTS: Response rate was 98.57% with a female to male ratio of 1.8:1; mean age of the participants came to 24.52 years. 40% of participants knew what is an autopsy and why autopsies are done. 12% knew about exhumation while 66.7% were aware about the need for a complete autopsy. 44.2% said autopsies are beneficial and 31.9% participants said it does not harm socio-religious norms and were in favor of conducting an autopsy of their kin, if it was necessary.

CONCLUSION: Most participants have a positive outlook towards medico-legal autopsies although further enhancement on knowledge on the same is needed.

KEY MESSAGE: Even a minor knowledge gap is of prime significance as their knowledge and

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positive perception about medico legal autopsies is crucial towards delivering justice. Thus, a clear understanding of medico-legal autopsies should be inculcated in law students. It can be achieved through workshops, seminars, exhibitions, guest lectures etc.

Keywords: Autopsies; Law Students; Expert witness; Exhumation; Judiciary.

INTRODUCTION

The term namely autopsy or necropsy deals with post mortem examination to identify the deceased and the cause of death.¹ While Forensic or Medico-legal autopsy is solely recognized as a part of inquest that is mainly recognized as a blender of legal and scientific investigative approach.² An autopsy can be marked as complete when all the body cavities are opened and examined thoroughly to come to a conclusive cause of death.³ A partial autopsy is worse than no autopsy at all, as it is more likely to lead to a miscarriage of justice.⁴

Autopsy is one of the sensitive medical procedures that are bound by legal jurisdictions. Individuals with different notions and lack of awareness about the procedure are more likely to question and object to it. This rejective attitude may lead to conflicts related to the entire process of autopsy.⁵ Without autopsy, identifying the cause or any unnatural deaths would become nearly obsolete. The number and types of deaths involving autopsy differs significantly from place to place.^{6,7} Requests for an autopsy are primarily done by police officers under the section of 174 of Criminal Procedural Code (Cr.P.C). Also, a Magistrate can call for an autopsy under the section of 176 of Cr.P.C.⁸

The salient aim of this research is to analyze the views of law students according to the term autopsies as they have a large impact on the judgments of a case that indirectly impacts on the perception of other people. This particular research can be considered as an attempt to develop a clear notion about the perception and knowledge of law students, about autopsies.

METHODOLOGY

Studies were done prior on attitude and aptitude of the general public and medical undergraduate students towards autopsies. However at the time of conducting this study, no prior studies on the topic were done, involving law students

- **Design of study and participant characteristics:** A Cross sectional prospective study using an online questionnaire based survey was conducted among final year undergraduates in SDM College of Law, Mangalore.
- **Site of study:** SDM College of Law, Mangalore.
- **Process:** After obtaining clearance from

the Institutional Ethics Committee, a well-structured online questionnaire in English, designed by medical professionals and validated by the peer colleagues was given to the mentioned participants, to conduct the survey. Participants were able to access the questionnaire after consent was given.

- Data analysis was drawn using descriptive statistics and using SPSS version 25.0

RESULTS

- Out of 140 questionnaires distributed, 138 students responded, giving a response rate of 98.57% (Women 64.5%; Men 35.5%).
- Female: male ratio of 1.8:1.
- Mean age of the respondents = 24.52 years.
- 76 (55.07%) out of 138 respondents knew the term medico legal autopsy (Fig. 1). Among the 76 respondent's answers for reasons to perform medico legal autopsy, the most common one was to know cause of death 56 (73.68%).

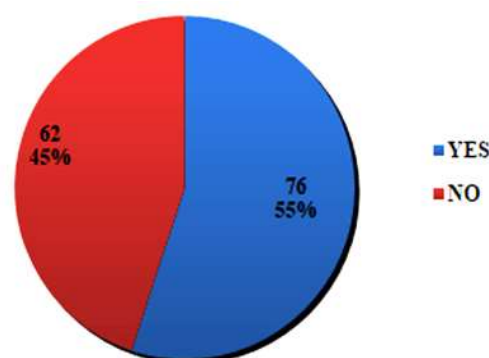


Fig. 1: Do you know the term Medico Legal Autopsy?

- Most of the 138 respondents i.e. 98 (72%) thought all autopsies involved police (Fig. 2) and the commonest reason for responding

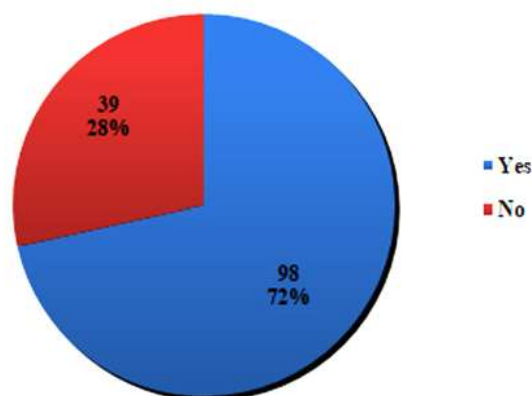


Fig. 2: Do all autopsies involve police?

“yes” to the question was involving unnatural deaths 77(77.7%).

- The study shows majority of respondents i.e. 89 (64.5%) replied “no” when asked if the doctor can start the autopsy immediately after receiving the body (Fig. 3). 112 (81.2%) respondents thought that it is the autopsy surgeon who hands over the body to the family of the deceased and not the police.

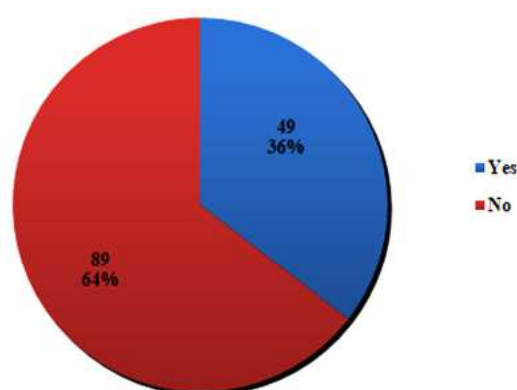


Fig. 3: Can the doctor proceed with the autopsy immediately after receiving the body?

- It was observed that 27.5% of the respondents were of the opinion that autopsies can be conducted outside mortuaries out of which the majority (60.5%) said that it can be conducted outside mortuaries in case of re-

examination / during exhumation.

- Almost two third of the responses i.e. 92 (66.7%) were implying that an autopsy must always be complete and should never be prejudiced only to the site of visible external injuries only.
- Among the unwilling fraction, fear of blood was the major limiting to witness an autopsy; others included not being able to comprehend the procedure, felt like wasting the time of the forensic surgeon, fear of the mortuary itself, etc. (Fig. 4)

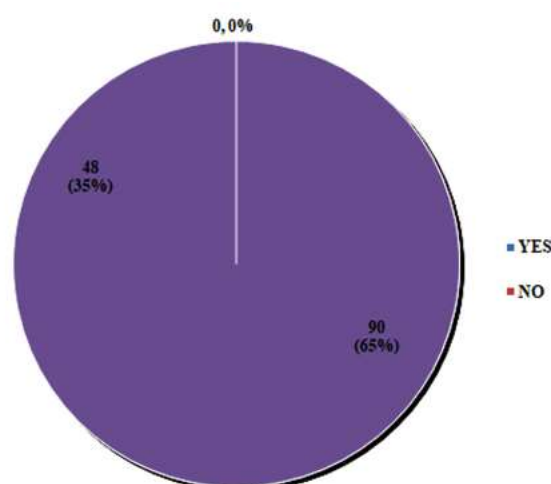


Fig. 4: Given a chance, I would witness an autopsy

Table 1: Aptitude and Perception towards autopsies (N=138)

Statements	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1. Autopsy will cause disfigurement of the body and will only add further misery to the relatives of the deceased	16	27	61	29	5
2. Post mortem examination is of no use to the deceased	61	24	22	20	11
3. By using technical aids like Computerized Tomography and Magnetic Resonance Imaging scans, the efficiency of an autopsy would be better over dissecting the body.	25	19	48	32	14
4. I would not be in favor of conducting an autopsy, if the body is of my own relative	44	37	35	13	9
5. Autopsy would disturb the peace of the family of the deceased	24	25	42	27	20
6. I feel that autopsies are violating the cultural and religious views of the family of the deceased.	43	44	29	12	10

- Bulk of the respondents i.e. 61, were neutral (44.2%) while 16 strongly disagreed (11.6%), 27 disagreed (19.6%), 29 agreed (21%) and only 5 (3.6%) strongly agreeing to the mentioned statement ‘Autopsy will cause disfigurement of the body and will only add further misery to the relatives of the deceased’. (Table 1)
- The response trend to ‘post mortem examination being of no use to the deceased’ were having a majority of 61 responses (44.2%) strongly disagreeing, 24(17.4%) disagreeing, 22(15.9%) being neutral, 20(14.5%) agreeing and the rest 11(8%) strongly agreeing. (Table 1)

- To the implementation of virtual autopsies over conventional autopsy, majority of the responses were neutral, 48(34.8%), while 25(18.1%) strongly disagreed, 19(13.8%) disagreeing, 32(23.2%) agreeing and 14(10.1%) strongly agreeing. (Table 1)
- About 44 (31.9%) respondents had a favorable attitude in conducting autopsy on a deceased kin, if needed. (Table 1)
- Most participants remained neutral to the comment that autopsies will have a negative impact in the mental peace of the family of the deceased. (Table 1)
- It was also observed that 44(31.9%) participants were of the opinion that autopsies will not hamper any socio-religious or cultural beliefs. (Table 1)

DISCUSSION

Previous literature^{9,10} citing the knowledge and attitude to autopsies, were done among students of arts, medicine and commerce along with the general population. The present study has accounted the same on law students, and have shown a generally favourable attitude in the context of autopsy, but needs more improvement in terms of basic technical aspects of an autopsy. The present study has shown similarities with that of one conducted in Malasiya, citing that autopsies are solely used to identify the cause of death.¹¹ The notion about this is found to be more clearer only in participants from a medical background.⁹ Although the majority were aware of the fact that the forensic surgeon had to wait to obtain the inquest report before the commencement of the autopsy, only a minor fraction of participants knew that the body of the deceased was handed over to the family by the police and not by the forensic surgeon.

Majority of the study population were willing to witness an autopsy, if given a chance which shows a positive outlook to the concept of necropsy and willingness to inculcate more knowledge about it. Fear of blood was the main reason for the latter fraction to disagree. In this study, there were more positive responses on consenting to conduct an autopsy of a relative if needed as compared to the study done in Manchester, on medical students.¹² This is also in conjunction with a study conducted in Delhi¹³ that also highlighted where most participants showing willingness to conduct autopsies of their deceased kin, if needed.

Post mortem imaging or virtual autopsies are an adjuvant but not a replacement to conventional autopsy practices.¹⁴ This particular study reveals that most of the participants are not clear about the term 'Virtual autopsies'. Compared to a study done in Shanghai, China¹⁵ where the major fraction of general population strongly agreed about having fear of disfigurement of the body during autopsy, the response obtained in our study were less apprehensive.

Conflicting responses were also seen among a study in Malaysia¹⁶ where the study population who had good knowledge on autopsy but did not show interest in it leading to autopsy refusal; main factors for refusal included body disfigurement after the autopsy, considered "no use" in conducting it, etc. In our study, most participants felt that medico legal autopsy were beneficial to the family of the deceased; similar to that of study done among the general public in Mangalore, India.¹⁰ This indicates that their outlook towards medico legal autopsy is slowly progressing to a promising positive scale.

People who have sufficient knowledge regarding the process and need for autopsies are not likely to show keen interest in the context of using it. Although there can be no absolute alternative for postmortem examination to date, trying to understand the apprehension, empathize can be a trust building exercise.¹⁰

This particular study also revealed a positive attitude from law students in the context of cultural aspects. Cultural aspects mainly depend on several religious views of a locality. Cultural and values related to a specific country are also found to be involved in the concept of autopsy as revealed by the present research.¹⁶ It is important to note that autopsies can also provide peace of mind or closure and more importantly, justice for the bereaved family in certain situations. Therefore, a comprehensive education on the necessity of autopsies should be encouraged in all communities regardless of religion upon all unnatural death for the check of justice.³ The study is the first to date that assess the attitude and aptitude of law students towards medico-legal autopsies. It highlights that most participants have a positive outlook towards medico-legal autopsies; but there still is a requirement to strengthen the awareness and aptitude among the law students, related to medico legal autopsy and forensic science. The limitation of the study is that only one setup could be involved in the conduction of data collection, as multiple study setups would have given a comprehensive input for assessment.

A run through of the medico legal procedure should be restructured and delivered to the law students in a simplest way possible through workshops, seminars, exhibitions, guest lectures and so on. It may be helpful to involve the support of religious organizations in these educating programs, so that resistance to conducting autopsies is reduced. An aggressive attempt to educate not only law students but also the general public, on the importance of post mortem examination and its value, is necessary.

To conclude, enhancing the aptitude and attitude of students of law, towards medico legal autopsy is a major necessity. Even a minor knowledge gap is of prime significance as their knowledge and positive

perception about medico legal autopsies is crucial towards delivering justice.

CONCLUSION

To conclude, enhancing the aptitude and attitude of students of law, towards medico legal autopsy is a major necessity. Even a minor knowledge gap is of prime significance as their knowledge and positive perception about medico legal autopsies is crucial towards delivering justice.

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A Study of p53 Immunostaining in Breast Carcinomas: Correlation with Histopathological Prognostic Factors

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ABSTRACT

BACKGROUND: Breast cancer is the most common cancer in women worldwide and 2nd common cancer after cervix in India. Presence of p53 gene alteration like loss of function p53 mutants correlate with poor prognosis and tend to be more frequent in high grade, large size, node positive, and in estrogen-progesterone receptor negative (ER-, PR-) tumors, suggesting that mutant p53 may become increasingly critical for breast cancer progression.

AIMS: To determine the frequency of expression of p53 immunostaining (IHC) in breast carcinomas and to correlate its relationship with tumor size, histological grade, lymph node status and with hormonal receptors status wherever possible.

MATERIALS AND METHODS: Sixty-five breast carcinoma cases were studied for various clinicopathological parameters like tumor size, histological type, grade, axillary lymph node status, lympho vascular invasion, in situ component, Nipple areola complex, deep resected margin involvement, Nottingham Prognostic Index (NPIG) and subjected to p53 immunostaining. 37 out of 65 cases were studied for ER, PR, Human Epidermal Growth Factor Receptor 2 (HER2/neu) antibodies. p53 expression was correlated with known prognostic factors. Furthermore, autopsy studies can be used as supplement to know the disease reservoir.

RESULTS: p53 positivity was found in 72.3% of breast carcinomas with significant association between p53 expression with histological grade (p-value = 0.013) and Nottingham Prognostic Index Groups (NPIG) (p-value = 0.011). No significant correlation was seen between p53 expression and tumor size, lymph node status, hormonal receptor (ER, PR) status and HER2/neu expression.

CONCLUSION: Our study revealed that the higher the tumor grade, the higher the p53 expression and its association with NPIG. Thus, p53 can be considered as a prognostic marker and useful for management.

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KEYWORDS: Breast carcinoma; Immunohistochemistry; p53; Nottingham Prognostic Index Groups.

KEY MESSAGE: Timely mammography and self-breast examination may prevent morbidity by early diagnosis and biopsy should be done if physical findings suggest breast cancer, even if mammogram results are negative.

INTRODUCTION

Breast cancer is the most common cancer in women and accounts for 29% of all cancers diagnosed each year worldwide accounting for 19% of the total cancer burden. The development of distant metastasis is the primary cause of death in breast cancer patients. The involvement of axillary lymph nodes, larger size of the tumor, hormone receptor negativity, presence of the mutation of p53 and vascular invasion determine poor prognosis and treatment options upon initial diagnosis.¹

Predisposition to develop carcinoma breast depends upon several etiological factors like age at menarche, parity, age at menopause, family history, diet, alcohol consumption, socioeconomic status, history of radiation exposure and use of oral contraceptive pills. Recently cigarette smoking also has been identified as a risk factor.²

Like other tumors, it is a disease with a complex, heterogeneous genetic and biochemical background. No single genomic or metabolic condition can be regarded as decisive for its formation and progression. However, a few key players can be pointed out and among them is the TP53 tumour suppressor gene, commonly mutated in breast cancer.¹ It leads to an accumulation of non-functioning p53 protein in the cell nuclei, which can be detected by immunohistochemical techniques.³

Numerous studies show that in breast cancer, the presence of p53 gene alteration like loss-of-function p53 mutants correlate with poor prognosis and tend to be more frequent in high-grade, large size, node positive, and in estrogen-progesterone receptor negative (ER-, PR-) tumors, suggesting that mutant p53 may become increasingly critical for breast cancer progression.⁴

MATERIALS AND METHODS

It was a cross-sectional study of 2 years (September 2016 to August 2018) undertaken in the Department of Pathology, JSS Medical College and Hospital, JSSAHER, Mysore. All breast carcinomas diagnosed on lumpectomies and mastectomies were included whereas non-epithelial malignant tumors and post chemotherapy cases were excluded from the study. Furthermore, autopsy studies can be used as supplement to know the disease reservoir.

Method of collection of data

All mastectomy specimens of invasive breast

carcinoma - NST (IBC - NST) were studied noting the clinical details. After formalin fixation, paraffin embedding and staining with haematoxylin and eosin (H&E), histopathological features were studied. Histopathological grade was assessed using Bloom and Richardson method, modified by Elston and Ellis. Tumor, Node, Metastasis staging (TNM staging) according to AJCC classification 8th edition was used for pathological staging. Immunohistochemistry (IHC) for p53 was performed on all 65 cases on paraffin embedded wax sections.

Procedure for p53 IHC Staining

Paraffin blocks best representing the tumor in each case were selected after reviewing the H&E slides. Three to four μ m thick sections were taken on Poly-L-Lysine coated slides and air dried. The slides were baked at 60°C for one hour in hot air oven. Slides were deparaffinised and rehydrated. Retrieval solution (Tris buffer for antigen retrieval) was brought to boil in the pressure cooker. Slides were placed in metal staining racks and lowered into pressure cooker ensuring that the slides were completely immersed in the retrieval solution. When the pressure cooker reached operating temperature and pressure, it was timed for up to two to three whistles. The pressure cooker was removed from the heat source and the slides were allowed to cool for 30 minutes in the same solution. The slides were washed with wash buffer for one minute. Peroxide block was applied for ten minutes and washed with wash buffer for one minute. The sections were incubated with primary antibody (DAKO, FLEX Monoclonal Mouse Anti-Human p53 Protein Clone DO-7 Ready to Use, Code IS616) for 20 minutes and washed twice with wash buffer. The sections were then incubated with LABELLED POLYMER - HRP [Horse radish peroxidase] (DakoEn Vision + Dual Link System - HRP, DAB+ [3,3' Diaminobenzidine], Code K4065) for 20 minutes and washed thrice with wash buffer. The bound antibody was visualized using a DAB-chromogen substrate which was prepared by adding 50 μ l of DAB Chromogen (DakoEn Vision + Dual Link System - HRP, DAB+, Code K4065) to one ml of DAB buffer (DakoEn Vision + Dual Link System - HRP, DAB+, Code K4065). The sections were washed with wash buffer and counterstained with haematoxylin and again rinsed in water for five minutes. Sections from colorectal carcinoma were taken as positive control.

Method of reporting IHC

Brown granular nuclear reactivity was taken as positive. Staining characteristics of the tumors were agreed upon by two people involved in the study. Results of the immunohistochemical and histological analysis were recorded. The entire section was screened to determine the region with the maximum proportion of stained nuclei. p53 over expression was recorded as intensity of staining and score based on the percentage of the cells staining positively. First, percentages of the total number of tumor cells are assigned to one of six categories:

Scores

0 for < 5%, 1 for 5-30%, 2 for 30-50%, 3 for 50-70%, 4 for 70-90%, 5 for > 90%.

Second, the intensity of p53 immunostaining is scored as follows:

Staining Intensity

1+ for weak, 2+ for moderate, 3+ for intense.

Then the scores of percentages and staining intensity are multiplied to produce a weighted score for each tumor, as a score of < 8 designated as low expression and >8 as high expression.

The immunohistochemical staining technique using a modification of the avidin-biotin method was used for estrogen receptors, progesterone receptors and HER-2/neu and staining was performed with primary antibodies against ER, PR and HER-2/neu (A0485, Dako Corp., diluted 1:1,600). Scoring for ER, PR and HER-2/neu was done by All Red Method.

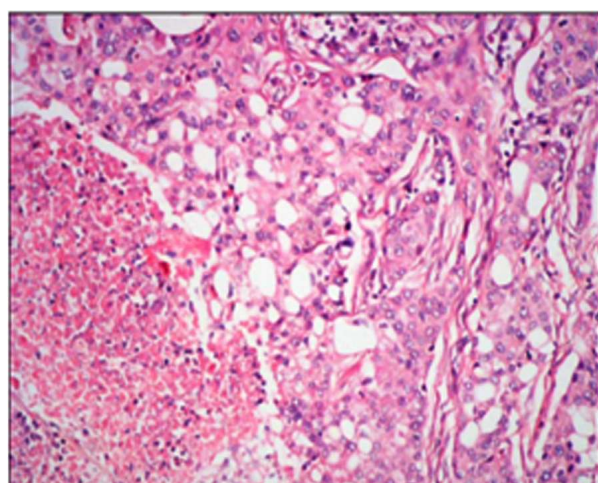


Fig. 1c: Invasive breast carcinoma of No Special Type, Grade 3: Areas of necrosis with tumour cells showing marked pleomorphism and atypical mitosis (H&E, x200)

STATISTICAL ANALYSIS

The statistical analysis was performed using statistical package for social sciences (SPSS) version 22. Percentages was used for categorical data and charts were generated using Microsoft excel 2007. The Association between p53 immunostaining and clinicopathological features including tumor size, nodal status, tumor grade, Estrogen/Progesterone receptors and HER-2/neu status were evaluated using the chi-square test. p-value <0.05 was considered statistically significant.

RESULTS

The age group of patients ranged from thirty years to seventy-five years, with mean age of fifty years. Maximum number of patients (24/65) were in sixth decade of life accounting for 36.9% of total cases. Right side breast was most affected with thirty-seven cases (56.92%) of tumor in the right breast and in twenty cases (43.07%) in the left breast [Fig./Table 1a & 1b].



Fig. 1a: Gross picture of modified radical mastectomy specimen showing nipple retraction



Fig. 1b: Cut section of modified radical mastectomy specimen showing a grey white infiltrating tumour

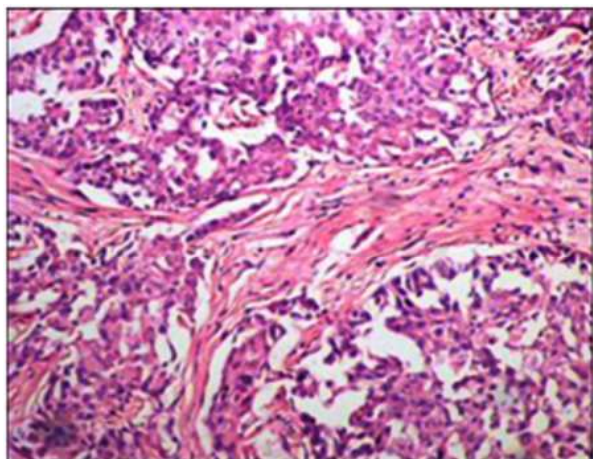


Fig. 1d: Invasive breast carcinoma of No Special Type, Grade 2: Tumour cells showing mild to moderate pleomorphism with areas of desmoplasia (H&E, x200)

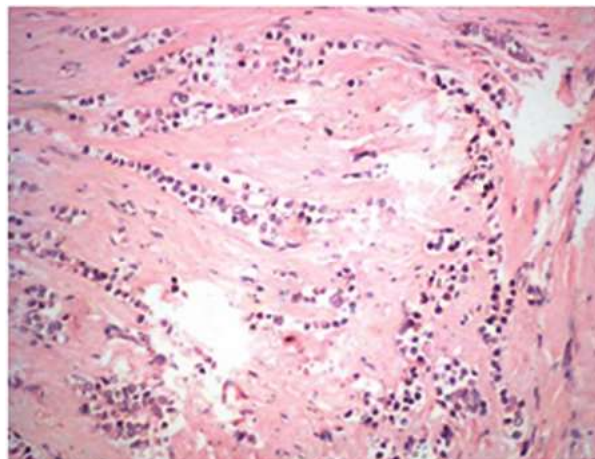


Fig. 1e: Invasive lobular carcinoma with typical Indian file arrangement of tumour cells (H&E, x100)

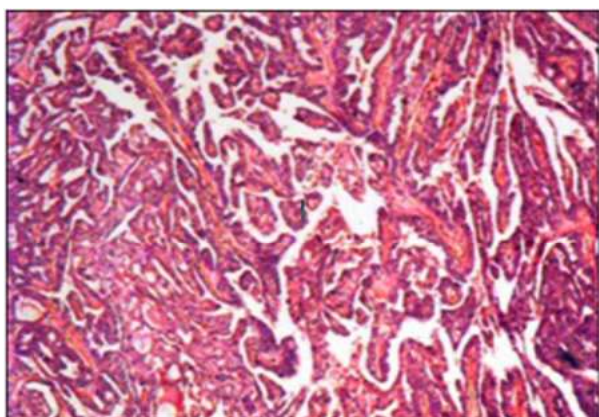


Fig. 1f: Invasive papillary carcinoma: Tumour cells arranged in papillary pattern with central fibrovascular core (H&E, x100)

Histopathological diagnoses included fifty-seven cases (87.69%) of invasive breast carcinoma No Special Type (IBC – NST) [Fig./ Table 1c & 1d], three cases of invasive lobular carcinoma [Fig./ Table 1e], two cases of invasive papillary carcinoma [Fig./ Table 1f] and one case each of mixed ductal and lobular carcinoma.

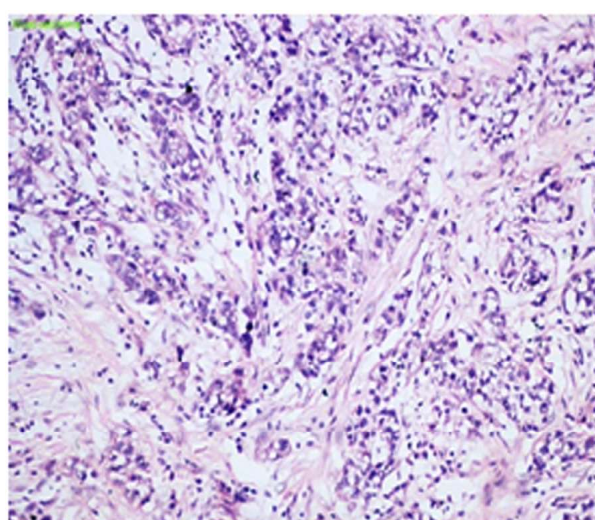


Fig. 2a: Mixed ductal and lobular carcinoma (H&E, x100)

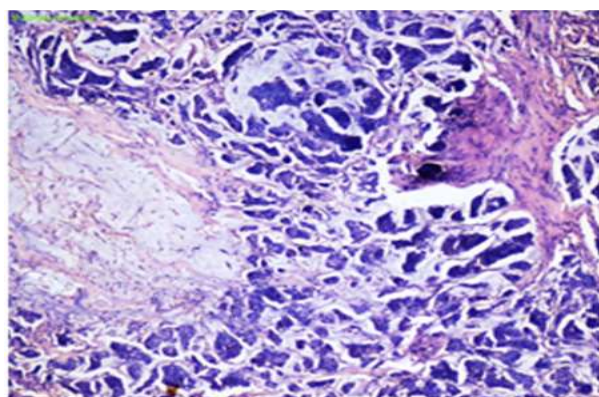


Fig. 2c: Mucinous carcinoma: Tumour cells arranged in clusters in a pool of abundant mucin (H&E, x200)

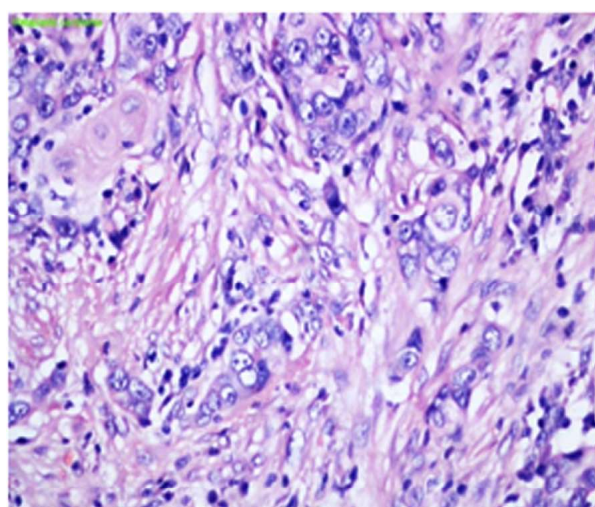


Fig. 2b: Metaplastic carcinoma: Tumour cells arranged in nests with focal area of squamous differentiation (H&E, x400)

[Fig./Table 2a], metaplastic carcinoma [Fig./Table 2b], and mucinous carcinoma [Fig./Table 2c].

Histopathological prognostic markers

Histologic grade was assessed in all cases of IBC – NST and metaplastic carcinoma using Elston and Ellis modification of the Bloom-Richardson grading system. Maximum number of cases were found to be of grade 3 accounting for 55.2% cases followed by grade 2 (44.8%). There was no case of grade 1 tumor.

The size of tumors ranged from two cm to twelve cm in greatest dimension with a mean size of 4.2 cm. The tumors were divided into four categories based on tumor size using TNM system of staging. Maximum number of cases were of pT2 category accounting for 67.7%.

Out of all, in four cases axillary lymph nodes could not be assessed. From rest, axillary lymph node involvement was seen in forty cases. Twenty cases showed metastasis in 1-3 lymph nodes (pN1),

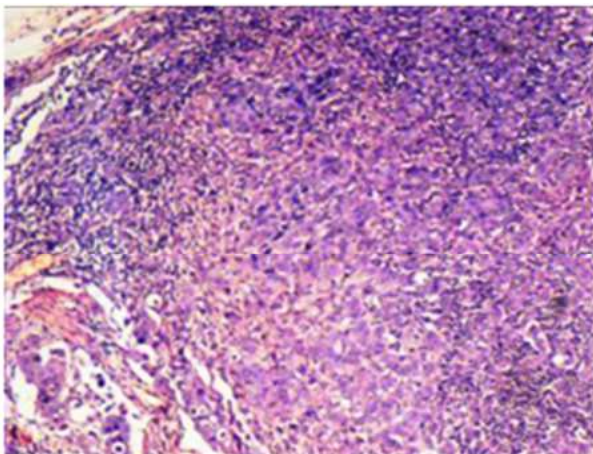


Fig. 2d: Lymph node: Tumour cells showing metastatic deposit (H&E, x100)

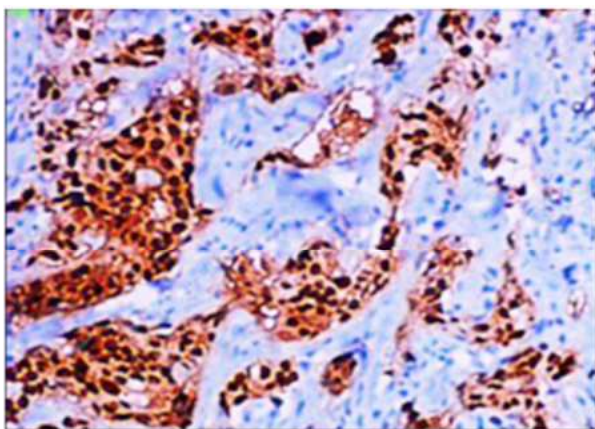


Fig. 2e: ER Positive: Tumour cells showing nuclear positivity with estrogen receptor immunostain (IHC, x200)

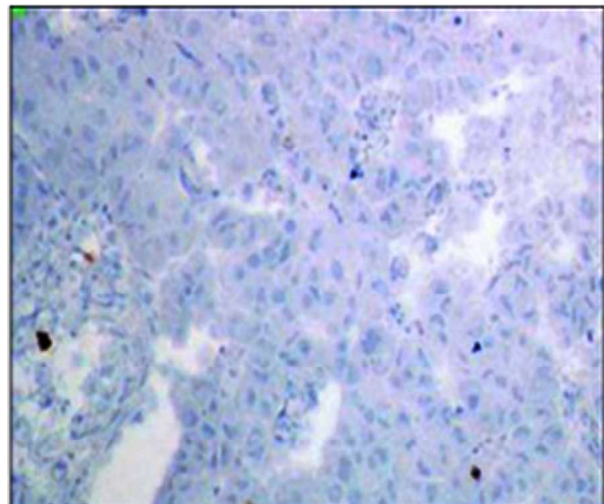


Fig. 2f: ER Negative: Tumour cells showing no staining with estrogen receptor immunostain (IHC, x200)

7 cases showed metastases in 4-9 lymph nodes (pN2) and 13 cases showed metastases in >10 lymph nodes (pN3) [Fig./Table 2d].

Twenty-nine cases showed in situ component, 2 cases showed involvement of nipple and areola and 7 cases showed involvement of deep resected margin. ER and PR status were analysed only in 37 cases. 73% of cases (27 cases) were positive for ER [Fig./Table 2e & 2f] and 70% of cases (26 cases) were positive for PR [Fig./Table 3a & 3b].

HER2/neu status was analysed only in 37 cases. 32.4% of cases (12 cases) were positive for HER2/neu with a score of 3. Twenty-five cases were

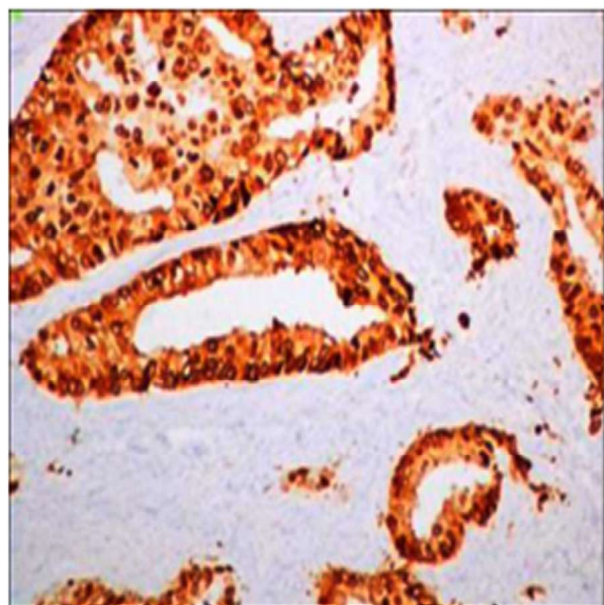


Fig. 3a: PR positive: Tumour cells showing nuclear positivity with progesterone receptor immunostain (IHC, x200)

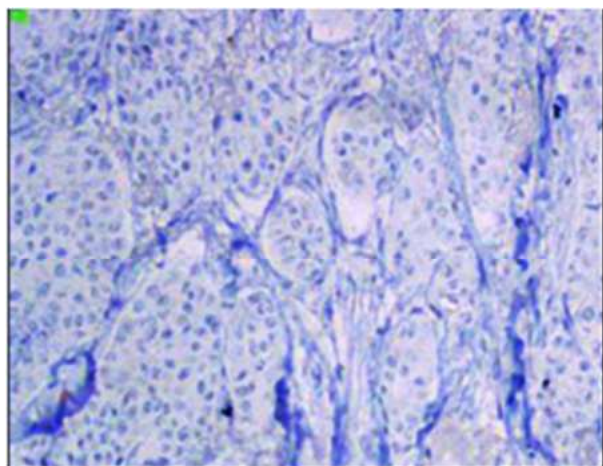


Fig. 3b: PR negative: Tumour cells showing no staining with progesterone receptor immunostain (IHC, x200)

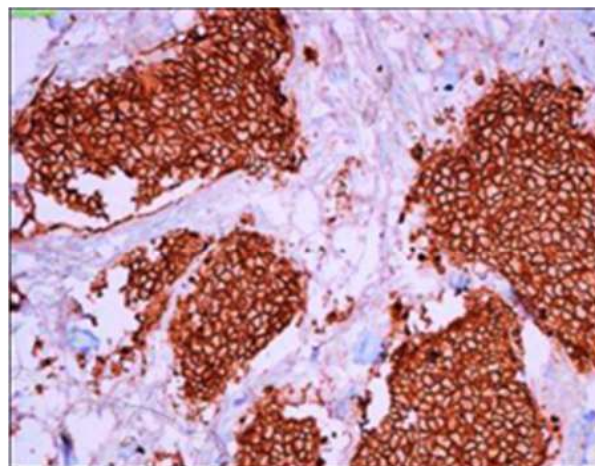


Fig. 3c: HER2/neu Score 3: Tumour cells showing strong membrane positivity with HER2/neu receptor immunostain (IHC, x200)

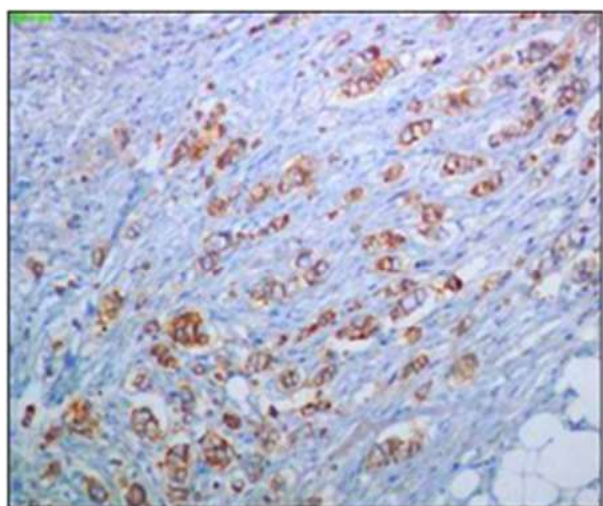


Fig. 3d: HER2/neu Score 3: Tumour cells showing moderate staining of >10% of cells with HER2/neu receptor immunostain (IHC, x200)

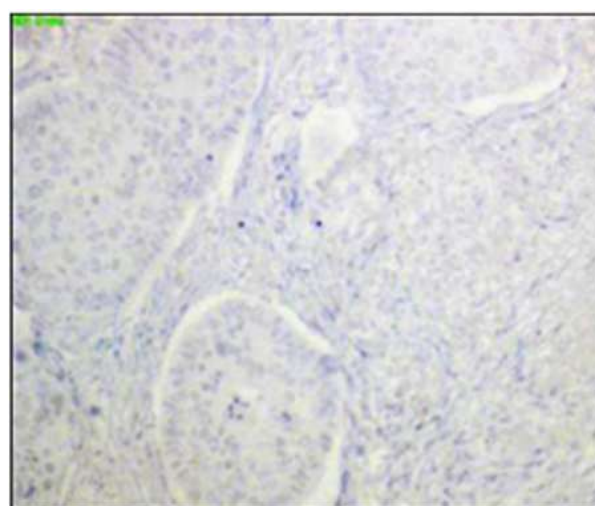


Fig. 3e: HER/neu Score 0: Tumour cells showing no staining with HER2/neu receptor immunostain (IHC, x200)

negative (12 had a score of 0 and 4 had a score of 1) and 9 cases were inconclusive (score 2) [Fig./ Table 3c, 3d & 3e]

NPI was calculated by {size (cm) x 0.2} + {lymph node stage (1 – 3)} + {grade (1 – 3)}. Patients were categorised into 3 groups. Maximum number of cases were in moderate group (50%)

p53 Expression in breast carcinomas

p53 expression was found in nuclei of tumor cells and the percentage of tumor cells showing p53 positivity and intensity of p53 positivity varied between tumors. The expression was assessed and evaluated by applying scoring system used by Jin-Woo *et al.*⁵ All the cases were evaluated by applying

this scoring system. 72.3% of cases (47 cases) showed p53 positivity and 27.7% of cases (18 cases) were negative for p53. Out of 47 positive cases, maximum number of cases (15 cases) were with score 1 (5-30% tumor cells showing positivity) and minimum (5 cases) were with score 5 (>90% tumor cells showing positivity). Intense staining (intensity score of 3+) was noted in 26 cases (55.3% cases) and weak staining (intensity score of 1+) was noted in 10 cases (21.3%) whereas 25 cases (53.2% of cases) showed high expression (weighted score >8) and 22 cases (46.8% of cases) showed low expression (weighted score <8) of p53. So, 38.5% of cases showed p53 high expression while 33.8% of cases were with low expression. p53 was not expressed in

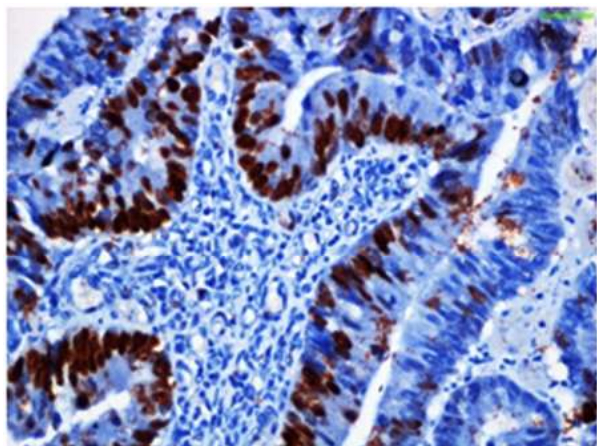


Fig. 4a: p53 nuclear positivity with high expression in carcinoma colon (control) (IHC, x200)

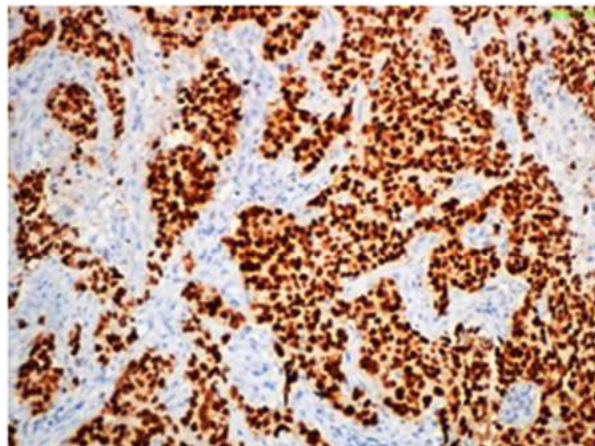


Fig. 4b: p53 nuclear positivity with high expression (weighted score $5 \times 3 = 15$) (IHC, x100)

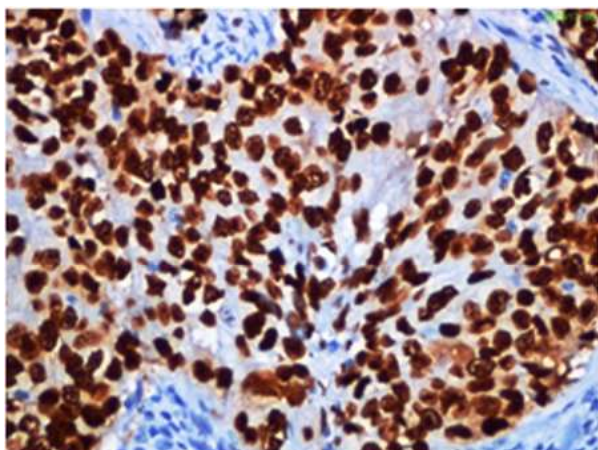


Fig. 4c: p53 positivity with high expression (weighted score $5 \times 3 = 15$) (IHC, x200)

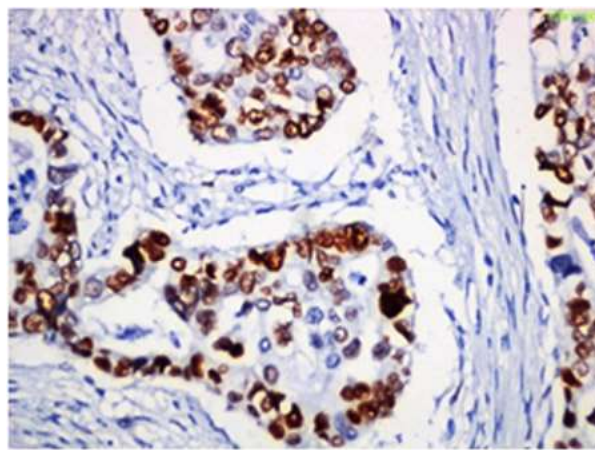


Fig. 4d: p53 positivity with high expression (weighted score $4 \times 3 = 12$) (IHC, x200)

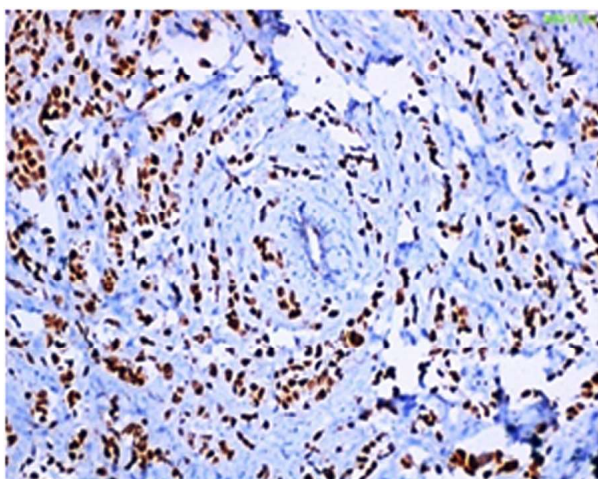


Fig. 4e: p53 positivity with high expression (weighted score $3 \times 3 = 9$) (IHC, x40)

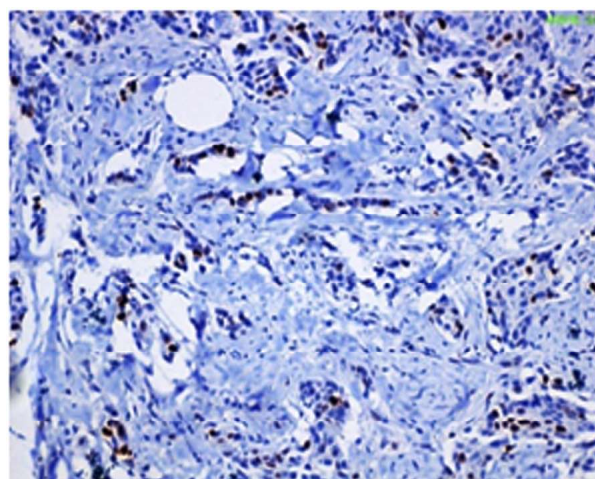


Fig. 4f: p53 positivity with low expression (weighted score $2 \times 3 = 6$) (IHC, x40)

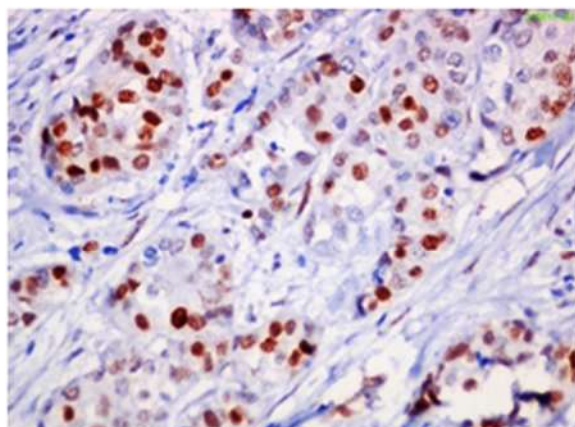


Fig. 5a: p53 positivity with low expression (weighted score $2 \times 2 = 4$) (IHC, x200)

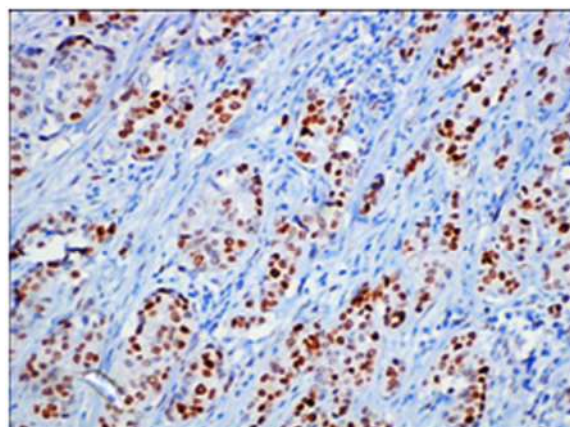


Fig. 5b: p53 positivity with low expression (weighted score $3 \times 1 = 3$) (IHC, x40)

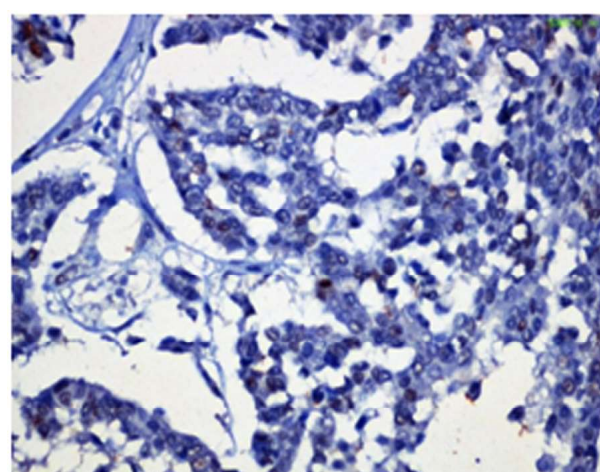


Fig. 5c: p53 positivity with low expression (weighted score $1 \times 1 = 1$) (IHC, x100)

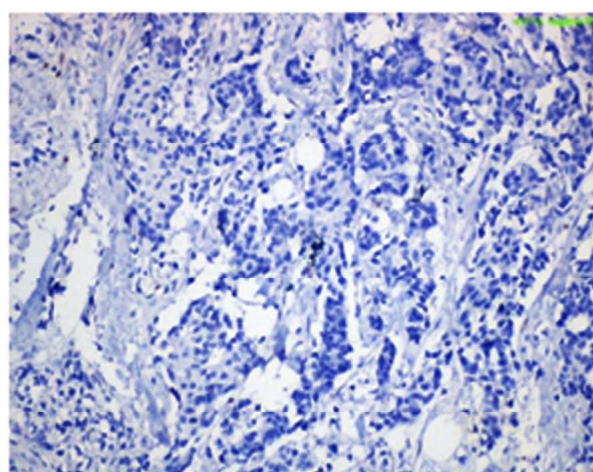


Fig. 5d: p53 immunostaining negative (IHC, x40)

Table 1: Correlation of p53 expression with known histopathological prognostic factors

Histopathological Parameters		No. of Patients (%)	p53			P – value	Significance
			High Expression No. (%)	Low Expression No. (%)	Negative Expression No. (%)		
Tumour size	T1	7 (10.8)	1 (14.3)	4 (57.1)	2 (28.6)	0.601	Not significant
	T2	44 (67.7)	19 (43.2)	13 (29.5)	12 (27.3)		
	T3	11 (16.9)	3 (27.3)	4 (36.4)	4 (36.4)		
	T4	3 (4.6)	2 (66.7)	1 (33.3)	0		
Tumour grade	2	26 (44.8)	5 (19.2)	11 (42.3)	10 (38.5)	0.013	Significant
	3	32 (55.2)	18 (56.3)	9 (28.1)	5 (15.6)		
Lymph node status	N0	21 (34.43)	4 (19.05)	10 (47.62)	7 (33.33)	0.175	Not significant
	N1	20 (32.79)	10 (50)	3 (15)	7 (35)		
	N2	7 (11.47)	3 (42.86)	3 (42.86)	1 (14.28)		
	N3	13 (21.31)	5 (38.46)	6 (46.15)	2 (15.38)		

Table to cont...

NPIG	Good	10 (17.9)	0 (0)	3 (30)	7 (70)	0.011	Significant
	Moderate	28 (50)	13 (46.4)	10 (35.7)	5 (17.9)		
	Poor	18 (32.1)	8 (44.44)	7 (38.9)	3 (16.7)		
ER Status	Negative	10 (27)	5 (50)	3 (30)	2 (20)	0.809	Not significant
	Positive	27 (73)	10 (37)	9 (33.3)	8 (29.6)		
PR Status	Negative	11 (29.73)	5 (45.5)	4 (36.4)	2 (18.2)	0.812	Not significant
	Positive	26 (70.3)	10 (38.5)	8 (30.8)	8 (30.8)		
HER2/Neu Status	0	12 (32.4)	6 (50)	2 (16.7)	4 (33.3)	0.435	Not significant
	1	4 (10.8)	0	3 (75)	1 (25)		
	2	9 (24.3)	3 (33.3)	3 (33.3)	3 (33.3)		
	3	12 (32.4)	6 (50)	4 (33.3)	2 (16.7)		

the remaining 27.7% of cases. [Fig./ Table 4a, 4b, 4c, 4d, 4e, 4f, 5a, 5b, 5c, 5d]

Correlation of p53 expression with prognostic factors

Significant correlation of p53 expression was found with tumor grade and Nottingham Prognostic Index Groups with p-value of 0.013 and 0.011 respectively.

DISCUSSION

Breast carcinoma is one of the most common malignancies and the leading cause of cancer death in women. Early and correct diagnosis is the most important factor for treatment outcome of the patient. There are various known prognostic factors for breast carcinoma like tumor size, histopathological grade and lymph node status.⁶ Now-a-days these known prognostic factors are supplemented with measurements of steroid hormone receptors, proliferation index, tumor suppressor genes, oncogenes and oncoproteins for predicting outcome of the disease and decision making for optimal treatment. Recent attention has been directed at molecular classification and molecular and genetic testing are found to have greater prognostic and predictive value but still they are expensive and not yet widely available.⁵

The p53 mutation remains the most common genetic change identified in human neoplasia. The p53 gene encodes for 53 kDa nuclear phosphoprotein, which has been implicated in controlling cell cycle regulation, differentiation, DNA repair and apoptosis. Unlike normal p53, non-functional mutated p53 accumulates in the nucleus of tumor cells and can be detected by

IHC. According to many studies, p53 mutation is associated with greater degree of progression of tumor, high proliferation rate, more aggressive disease, greater probability of micro metastases and worse overall survival in breast carcinoma.⁷⁻¹⁰ Careful studies with micro dissected tumor material have shown that p53 mutation may occur in ductal carcinoma in situ (DCIS) before the development of invasive cancer and the frequency increases from around zero in low grade DCIS to 30 – 40% in high grade DCIS.¹¹⁻¹³ These results emphasize the important role of p53 alterations early in the carcinogenesis of breast.¹⁴

In this study total 65 cases of breast carcinomas were studied for expression of p53 and out of 65, 37 cases were studied also for expression of hormonal receptors like ER and PR and for HER2/neu. p53 expression was correlated with known histopathological prognostic factors. There was significant correlation between p53 expression with histological grade and NPIG but not with tumor size, lymph node status, hormone receptors status or HER2/neu status.

p53 Expression

p53 IHC positivity was identified by distinct brown colour nuclear staining due to oxidised DAB. The result of IHC was quantified as scores.¹⁵ As there is no specific scoring system for evaluation of p53 expression, different scoring systems were used by different studies. The present study followed the scoring system used by Ryu *et al.*¹⁶ Which showed p53 positivity in 72.3% of cases. This is like studies done by Patnayak *et al.*¹⁷ (69.2%), Sekar *et al.*¹⁸ (71.67%) and Gupta *et al.*¹⁹ (88.9%) but according to many other researchers the frequency of positivity of p53 is comparatively less. These variations can be explained by the effect of genetic

and environmental factors in the study population, the type of tissue used for the study (formalin fixed, frozen, stored etc.), the type of antibody used and interpretation of results.²⁰

Correlation of p53 expression with histological prognostic factors

Tumor size is an independent prognostic factor in breast carcinoma and survival rate decreases with increasing size of the tumor.^{21,22} Hence the p53 expression was correlated with the size of tumor in the present study. The tumor size ranged from 2 cm to 12 cm with maximum number of tumors between (T2) 2 – 5 cm in size. This can be explained as most of the breast carcinomas are relatively painless, they usually get ignored by the patients till they reach a significant palpable size.¹⁸ There was no significant correlation between tumor size and p53 expression in this study (p-value = 0.601) which was comparable to the findings of Gupta *et al.*¹⁹ AI – Joudi *et al.*²⁰ and Sheipour *et al.*²³ and Lebe *et al.*²⁴

Histologic grade of breast carcinoma is validated to be a poor prognostic factor by multiple independent studies. In the present study grade 3 tumors were more in number in comparison to grade 2 which was similar to findings of Cass *et al.*²⁵ It was found that there was a significant correlation between histological grade and p53 expression (p-value – 0.013) in the study. The studies done by Gupta *et al.*¹⁹ Cass *et al.*,²⁶ Sirvent *et al.*²⁷ AI-Jaudi *et al.*²⁰ Prabal *et al.*¹⁵ and Singh *et al.*²⁸ also yielded similar result.

Axillary lymph node status is an important prognostic factor in breast carcinoma. The risk of distant recurrence is directly related to number of positive lymphnodes.²⁶ Furthermore, the mortality rate in node positive cases is four to eight times higher than node negative cases with lymphnode involvement being a valuable indicator of long term survival.^{27, 29} In the present study there was no correlation between p53 expression and lymphnode status which was similar to findings of Han *et al.*,³⁰ AI-Jaudi *et al.*²⁰ Sirvent *et al.*²⁷ and Sheikhpour *et al.*²³ However, the studies conducted by Gupta *et al.*¹⁹ Singh *et al.*²⁸ and Ivkovic – Kapicl *et al.*³¹ contradicted this. The variation in result can be explained as in the present study, lumpectomy specimens were also considered and in 4 cases lymph nodes could not be assessed. Hence large number of cases are required to find out the association between p53 expression and axillary lymph node status.

Nottingham prognostic index is a numerical index used for prognostication of breast cancer. It depends on tumor size, grade of the tumor and nodal status. In the study, NPI has significant association with p53 status which is contradicting the study done by Kurshumilu *et al.*³² and Esin *et al.*³³

Correlation of p53 expression with ER/PR status and Her2/neu status

Hormonal receptors have been known to be very important for therapy and prognosis in breast cancer. The receptor positive patients are considered to have better prognosis than negative patients who present lower survival rate and more tumor relapse.^{34,35,36} In the present study there was no correlation between p53 expression and hormonal receptor status which was comparable with studies done by AI – Jaudi *et al.*²⁰ and Sheikhpour *et al.*²³ However, studies done by Sirvent *et al.*²⁷ Han *et al.*,³⁰ and Alireza *et al.*³⁷ showed significant relationship between these two. The difference can be due to small study population.

The breast cancers positive for HER2/neu are very aggressive in nature and have higher chances of tumor progression and metastasis.³⁸ There was no significant correlation between p53 expression and HER2/neu status in the present study. The similar result was found by Han *et al.*,³⁰ Sirvent *et al.*²⁷ and Shokouh *et al.*³⁹ but contradicted by Rasheed *et al.*⁴⁰ and Patnayak *et al.*¹⁷ The variation in result may be due to a smaller number of cases taken in the study.

Use of Autopsy Series to know the Disease Reservoir-How much more Breast Carcinoma Cases can be Detected?

Autopsy studies may be used to estimate the prevalence pool of incidental cancer (and pre-cancerous lesions) among people not known to have specific cancers during their life. A substantial reservoir of insitu carcinomas is undetected during life. How hard we the pathologists look for the disease and, perhaps, our threshold for making the diagnosis are potentially important factors in determining how many cases of insitu lesions will be diagnosed. The latter has important implications for what it means to have the disease.

CONCLUSION

Carcinoma breast is considered a major concern these days since it is contributing to a large part of mortality and morbidity worldwide. Many

researches from the last decade have correlated a significant association between the development of carcinoma breast and polymorphism of p53, which is the well-established and most studied tumor suppressor gene.

The percentage of tumor cells stained with p53, and the intensity of staining varied among cases in the study which can be explained on the basis of genetic variation in the population. The level of p53 expression increased with increase in grade of the tumor that is most of the grade 3 tumors showed positivity with high expression. However, association was not that evident with tumor size and axillary lymph node status, hormone receptors expression or HER2/neu status.

In conclusion, studying the p53 expression with more molecular markers, larger number of cases

and with longer duration will provide a better idea about its prognostic significance and usefulness in treatment. And autopsy studies may be used as an adjunct to estimate the prevalence pool of incidental carcinoma cases.

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Rank Order Impediments to First Responder Care on Road Traffic Accident Victims among the Auto Rickshaw Drivers of a Selected District of Karnataka

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ABSTRACT

BACKGROUND: The mortality rate of road traffic accidents (RTAs) is high in India. RTAs lead to disability and mortality in young adults in the country. It is a global problem as approximately 1.24 million deaths occur annually worldwide because of RTAs. India lacks an organized system of trauma care to attend to trauma cases immediately at the accident site.

OBJECTIVE: The present study attempted to identify the impediments of auto rickshaw drivers to provide first responder care to RTA victims.

MATERIALS AND METHODS: The study used a quantitative approach with a cross-sectional survey design after institutional ethics clearance. The sample size was 1040, with a confidence level of 95% on the literature review knowledge score. A convenient sampling method was used for sample selection.

RESULTS: A majority (n = 335; 32.2%) of the participants belonged to the age group between 41 and 50 years and were educated up to secondary school (n = 551; 53.0%). A majority of the participants (n = 345; 33.2%) exhibited 16–30 years of driving experience. Among the participants, 602 (57.9%) exhibited experience in administering first aid. Of the total participants, 898 (86.3%) reported that legal issues and inadequate community support and resources were the major barriers to administering first aid, whereas 888 (85.4%) participants reported psychological problems as the major barrier. Additionally, attitude toward first aid was the

barrier for 814 (78.3%) of the participants, whereas poor knowledge was the barrier for 589 (56.6%) for providing first aid care.

CONCLUSION: The present study identified the various barriers faced by the participants in providing first responder care to RTA victims and exhibited the significance of providing training to common people which will be contributed to SDG 3 and 4.

KEYWORDS: First care; First injury care; First responder care; Road traffic accident care; Road traffic injuries.

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INTRODUCTION

According to the report by the World Health Organization (WHO) on road safety, 1.35 million people die annually worldwide due to road traffic accidents (RTAs). Road traffic injuries are a leading cause of death in the age group of 5–29 years. Approximately 50 million individuals underwent nonfatal injuries following RTA, with most of them exhibiting disabilities from these injuries. The majority of victims of RTAs in both developed and developing countries are foot travelers, cyclists, and motorbike riders. Immediate attention is required to set interventions to prevent RTAs and save lives. The WHO addressed the RTA mortalities as an “epidemic” because they would become the world’s fifth biggest killer by 2030. Although developed countries have been able to reduce their RTA mortality rates, they are on the rise in the developing and underdeveloped nations.¹

The Decade of Action for Road Safety 2021–2030 was launched by the WHO in October 2021 with the primary goal of preventing at least 50% of road traffic injuries and deaths by 2030. The strategies underline the importance of a 360 degree tactical plan for road safety. In the strategic plan, the provision of timely life-saving emergency care for the injured is emphasized as a global plan.^{1,2}

India is a rapidly developing nation in the South East Asian region.² Approximately 6 million new motor vehicles are launched annually on Indian roads.³ India has registered the maximum number of road accidents in the world. According to the National Transportation Planning and Research Centre experts, the number of road accidents reported in India is thrice that reported in developed countries.⁴ Approximately 13 people lose their lives every hour in RTAs. According to a global report, India has the second highest mortality rate from road traffic injuries globally at 29.2 per 100,000 people.^{5,6}

Approximately 1.27 million people in India sustain serious injuries due to RTAs. India accounts for 6% of the total global RTA mortality even though it has only 1% of the world’s motor vehicles.⁷ A majority of major accident survivors are either bed or wheelchair bound for the rest of their lives due to brain or spinal cord injury.⁸ The chance of survival from a serious RTA is maximum if the victims are brought into the casualty department within the first hour of trauma, referred to as the “golden hour”.⁹

Most RTA deaths are reported during the pre-

arrival time to the hospital due to a lack of travel facilities or long travel distances, especially in rural and remote areas. Efficient first response care can save the lives of RTA victims.^{10,11}

Autorickshaws are a three wheeled public transport vehicle operated by low horsepower engines and is observed in all major Indian cities. Their center of gravity allows them to swivel in impossible twists around the heavy traffic roads. Thus, they are the perfect vehicle for people who do not have personal transport and do not wish to take buses. A city has more than 1,00,000 autorickshaws compared with 100 ambulances, thus making auto rickshaws the most accessible vehicle during a medical emergency.¹²

Although the emergency ambulance service is provided by the government, the efficiency of the service is questionable due to various factors.^{13,14} Thus, the present study attempted to assess the barriers faced by the auto rickshaw drivers, who are easily accessible in a community, in providing first responder care to RTA victims in the urban and rural areas of the Udupi district of Karnataka.

Research gaps Identified:

An observational study was conducted in 220 licensed drivers to assess their skill in immediate care to save RTA victim lives in Thailand. An observational checklist was used to collect the data from the sample. The majority (71%) of drivers exhibited poor skills in managing head injuries, whereas 67% exhibited poor skills in the care of fracture extremities and spinal cord injuries.¹⁵

A few large scale studies have been conducted in India. The study by XYZ *et al.* reported the readiness and accurate care techniques to save the life of accident victims. Approximately 7% of roadside fatalities could be prevented if members of the public are equipped to save lives.¹⁶ Thus, the present study attempted to assess the impediments faced by the common people in administering first responder care to RTA victims. Auto rickshaw drivers were deemed ideal candidates for assessment as they were observed in all community areas with their vehicle.

Objectives:

The present study attempted to identify the impediments of autorickshaw drivers in providing first responder care to RTA victims which will be contributed to SDG 3 and 4.

MATERIALS AND METHODS

A quantitative research approach with a cross-sectional survey design was used.

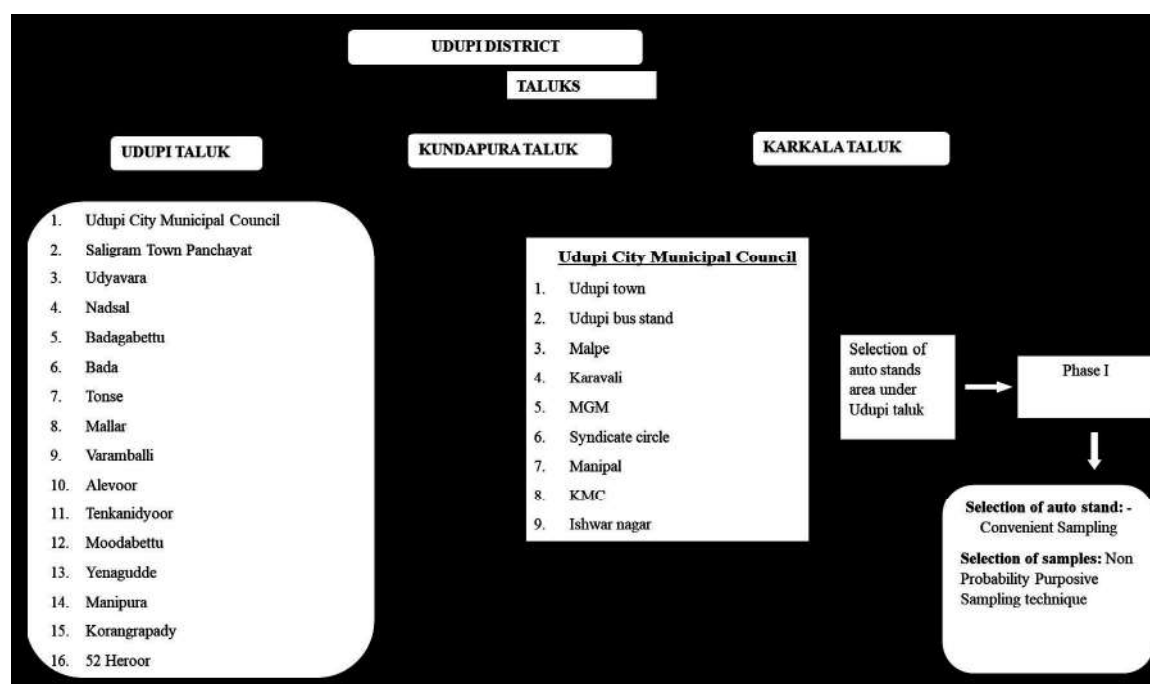


Fig. 1: Sample selection process

Fig. 1 illustrates the sample selection. The Udupi taluk was selected from various taluks of Udupi district and has 16 major autorickshaw stands, of which the Udupi municipal stand was selected. These selections were performed using the simple random selection method. The samples from each sub-autorickshaw stand were selected using the convenient sampling technique from the various autorickshaw stands in the Udupi district. The sample size was calculated using the following formula:

Sample size for estimation of proportion

$$n = \frac{(z_{1-\alpha/2})^2 pq}{d^2}$$

The confidence level was set at 95% and based on previous study scores. The margin of error (d) was set as 0.03. The final sample size was 1040.

$$N = \frac{(1.96)^2 \times 0.42 \times 0.55}{(0.03)^2}$$

Inclusion criteria:

Authorickshaw drivers aged less than 65 years

who were willing to participate in the study were included.

Exclusion Criteria:

Authorickshaw drivers with any serious health issues, those who were known for homophobia, and those who were unable to read English or Kannada language were excluded from the study.

Operational Definition:

In the present study, first responder care refers to the care provided to victims within the first hour of RTA to reduce the accident complications by providing recovery position; primary management for bleeding, extremity fractures, and head and neck injuries, and shifting of the victim to a hospital. First responder refers to the person who initiates immediate care for RTI victims before shifting the victim to a hospital or arrival of the medical care team at the accident scene.

Data collection Procedure:

Authorickshaw drivers were met at their workplaces after obtaining permission from the

authorities. Consent was obtained after explaining the procedure and objectives of the project. The impediment survey tool was used to collect responses on the impediments to the first responder care.

Data Collection Instrument:

An impediment survey tool comprising 35 statements that were validated by experts in various fields was used to collect data. It was initially implemented on 20 autorickshaw drivers to check its reliability and was modified based on the responses obtained from them, before being used for the final data collection. The final tool was pretested with 10 subjects to check its accuracy. These 25 subjects were not included in the final data analysis. The finalized tool was used to collect the data.

Ethical Consideration:

Permission was obtained from the Institutional Research Committee (IRC 92/2017) and the Institutional Ethical Committee (IEC 473/2017). The study was registered in CTRI, and the CTRI registration id is CTRI/2017/09/009715. Official permission was obtained from the autorickshaw driver's association. Participant information sheets were distributed to participants, and written consent was obtained from each participant. Special care was taken to maintain confidentiality and reassure participants that the findings of the study will not affect their careers.

DATA ANALYSIS

Data analysis was performed using SPSS Software. SPSS's latest version was used for computing the statistics. Frequency distribution and percentage were computed for all the variables.

RESULTS

Table 1: Frequency and percentage distribution of samples based on demographic variables
N=1040

Age in yrs	Frequency	Percentage
18 - 30	96	9.2
31 - 40	286	27.5
41 - 50	335	32.2
Above 51	323	31.1

Gender

Male	1040	100
Female	00	00

Educational status

Primary	391	37.6
Secondary	551	53.0
P.U.C.	86	8.3
Graduate	12	1.2

Years of driving experience

less than 5 years	145	13.9
between 6 - 15 years	263	25.3
between 16 - 30 years	345	33.2
above 30 years	287	27.6

The majority of the participants (n = 335; 32.2%) were in the age group of 41–50 years, 323 (31.1%) participants were in the age group of 51–55 years, and 96 (9.2%) participants were in the age group of 18–30 years (Table 1). All participants were men, with no female drivers in the study. The majority of the participants (n = 551; 53%) had completed secondary school education, whereas 391 (37.6%) had only primary school education. Only 12 (1.2%) participants had education up to graduation. Most of the participants (n = 345; 33.2%) exhibited 16–30 years of driving experience, 287 (27.6%) exhibited more than 30 years of driving experience, and 145 (13.9%) participants exhibited less than 5 years of driving experience.

Table 2: Frequency and percentage distribution of samples based on experience in providing first responders care, accident time and type of vehicle
n=1040

Previous experience in providing first responders care	Frequency	Percentage
Yes	602	57.9
No	438	42.1
Time of attending the victims*		
Morning (6 am - 12N)	218	36.22
After Noon (12N - 5 pm)	86	14.28
Evening (5 pm - 10 pm)	95	15.78
Night (10 pm - 6 am)	203	33.72
Type of Vehicle met with the accident*		
Motor cycle with or without gear	407	67.61
Auto rickshaw	29	4.82

Table to contt...

Car	68	11.29
Bus	40	6.65
Lorry/Truck	58	9.63

*n=602

Only 602 (57.9%) participants had experience in providing first aid care, whereas 438 (42.1%) did not have any such experience (Table 2). Of the 602 accidents, most accidents (n = 218; 36.22%) occurred in the morning time (between 6 am and 12 pm), whereas 203 (33.72%) accidents occurred at night (between 10 pm and 6 am). The least number of accidents (n = 86; 14.28%) occurred in the afternoon (between 12 pm and 5 pm). Motorcycles (n = 407; 67.61%) were most commonly involved in RTAs, whereas auto rickshaws (n = 29; 4.82%) were the least involved in RTAs.

Table 3: Frequency and percentage distribution of samples based on Barrier assessment score
n=1040

Barrier	Frequency	Percentage
Legal issue	898	86.3
Community support & Resources	898	86.3
Psychological	888	85.4
Attitude	814	78.3
Knowledge	589	56.6

Data in table 3 presents the barriers faced by autorickshaw drivers in providing first responder care to RTA victims in the rank order. Of the total participants, 898 (86.3%) reported that legal issues faced by them after first responder care, inadequate community support in providing immediate life-saving care, and lack of resources to provide first responder care were the major barriers in providing first aid to RTA victims. Psychological problems of the providers was reported by 888 (85.4%) participants as another barrier. The attitude of the participants toward first responder care to RTA victims was the barrier for 814 (78.3%) participants, whereas some participants (n = 589; 56.6%) reported the lack of knowledge about first aid care as a barrier.

Table 4: Rank order of the impediments received from the first aid care responder's
n=1040

Barrier	f	%
Legal problems		
• Arrest by police	966	92.88
• Legal burden in future	837	80.48

• Pay the treatment cost	798	76.73
• Samaritan law	586	56.35
• Providing personal details	420	40.39
• Civil or criminal case	222	21.34

Community Support & Resources

• Adequate training in first aid	924	88.85
• Other's help	772	74.23
• Reaction of hospital people	747	71.83
• Availability of ambulance	646	62.12
• Access to any equipment	628	60.38

Personal Psychological issues

• Worried about the steps	819	78.75
• Giddy or irritable mood	793	76.25
• Consciousness about other's reaction	748	71.93
• Self-confidence	537	51.64

Attitude towards caring for the victims

• Don't like to give care	853	82.02
• Accidents happen due to the victim's mistake	762	73.27
• Giving care to the accident victims is not my responsibility	474	45.58
• Readiness to use own vehicle	456	43.85

Knowledge on first responders' care

• Concept of RTI	738	70.97
• Meaning of first responders' care	575	55.29
• Wound care	479	46.06
• Head injury care	468	45
• Spinal cord injury care	447	42.99
• Airway management	443	42.6

The researcher has identified the barriers faced by the first aid care responders in initiating the care and is ranked in table 4. As discussed, the impediment is broadly categorized into legal issues, support from the community and resources, psychological problems faced by the first aid care responders, and attitude and knowledge of the responders. From the above categories, the researcher has ranked the barriers according to the response received from the first aid care responders.

The result highlights that a majority of the responders are concerned about the arrest (92.88%) by the police, which ranked as top in the category of legal formalities. Any possibility of legal liability in future related to the incident has resulted in 80.48 % of the respondents from providing care. Financial

liabilities for the treatment are also a major concern as reported by 76.3%. 56.5% identifies the lack of knowledge about the good samaritan law as a barrier to providing care. Around 40.39% and 21.34% are worried to provide personal details and the remaining identifies the possibility of any legal cases such as criminal or civil common barrier. Looking at the support received by the first aid care responders from the community and the availability of resources have highlighted the dominance of lack of adequate training among the barriers, which is ranked the highest by 88.85% of the responders. This is followed by lack of other's help (74.23%), reaction of hospital people (71.83%), lack of ambulance for immediate transport of the victim (62.12%) and access to equipment (60.38%) for first aid provision as other barriers.

The attitude of the responders was assessed using a few indicators such as willingness to provide care, responsibility for the care and transportation of victims to the healthcare organization. The survey revealed that 56.3% of the responders always like to provide care and 42% reported that the responsibility of providing care doesn't belong to them. However, another 51.3% of the responders agreed to immediate transportation of the accident victims in their vehicles. Similarly, the keen cause of accidents is identified as the poor driving skills of victims. 73.27% ranked that the cause of accidents is due to the victim's mistake. Giving care to the victims is not the responsibility of auto rickshaw drivers have been ranked as third by 45.58% of the respondents.

Likewise, readiness to use own vehicle is ranked last in the category of attitude of the respondents by 43.85%. The knowledge of the respondents have highlighted that lack of knowledge on RTI as the top barrier in providing care by 70.97%. This is followed by lack of knowledge of first responder's care (55.29%), knowledge on wound care (46.06%), head injury (45%), spinal cord injury (42.99%) and airway management (42%).

DISCUSSION

The present study attempted to identify the impediments faced by autorickshaw drivers in providing first responder care to RTA victims. To the best of our knowledge, this study is the first of this type conducted in Karnataka. Thus, the results of the study can be used by policy makers to solve problems faced by the people in providing first responder care to RTA victims.

The majority of the participants (n = 335; 32.2%)

were in the age group of 41–50 years. This finding is contradictory to those of some studies conducted among drivers.^{17,18} Most studies revealed that the average age of drivers was 30 years. However, in the present study, only 96 (9.2%) participants were in the age group of 18–30 years. Additionally, the majority of participants were of late middle age. Age increased their responsibility for their work and fear to involve in risky incidences. Additionally, their physiological illness or other personal problems prevented them from providing quick immediate first responder care to the RTA victims.

All participants were men, and there were no female autorickshaw drivers. This finding is concurrent with those of other studies conducted among drivers.^{17,18} These findings may be attributed to biological reasons or the cultural background of the geographical area. Driving is a job requiring more physical strength and is probably the reason for the lack of female drivers. Additionally, some societies are not ready to accept women as drivers for commercial or transport vehicles.

The majority (n = 551; 53%) of participants had completed secondary school education. This finding is contradictory to that of another study reporting that most drivers had completed primary education.^{19,14} Higher educational status ensures increased awareness about first responder care.

Most of the participants (n = 345; 33.2%) exhibited 16–30 years of driving experience. These findings are concurrent with those of another study.¹⁷ A rich driving experience is crucial for minimizing the chance of accidents and providing superior service and care to victims.

Only 602 (57.9%) participants had experience in providing first responder care to RTA victims. This finding is concurrent with those of other studies.¹⁹ Most participants exhibited some experience in providing care to RTA victims, exhibiting their intention to help others during times of crisis. In the present study, most accidents (n = 218; 36.22%) occurred during the morning time between 6 am and 12 pm. This finding is contradictory to that of another study.⁷ This may be due to heavy traffic, congested roads, and careless and busy pedestrians during school and office hours. Effects of alcohol, careless driving, and the use of mobile phones during driving may also contribute to accidents.

The present study revealed that motorcycles (two-wheelers) (n = 407; 67.61%) were most commonly involved in accidents when compared with other vehicles. This finding is concurrent with that of other studies.^{20,7,21} Accidents mainly occur due to overspeeding, risky driving, or unsteadiness of the

vehicle. Poor road conditions and bad weather also contribute to accidents to some extent.

A majority (n = 898; 86.3%) of the participants reported that legal issues they were forced to face after first responder care, inadequate community support in providing immediate life-saving care, and lack of resources to provide first responder care were the leading barriers in providing emergency life-saving care to RTA victims. This finding is concurrent with that of a study conducted in Karnataka.¹³ The major complaint raised by the participants was that they were called and interrogated by police officers in connection with the accident. Additionally, they were taken to court several times in connection to the RTA cases, exhibiting the significance of generating awareness among the general population about the legal protections and responsibility of the first responders to RTA care. The participants also reported the lack of support from other people when they initiated care for RTA victims. No emergency equipment or resources were available for first responder care. As per traffic rules, first aid boxes are compulsory in vehicles. However, the majority of transport vehicles do not maintain the first aid box properly. Additionally, ambulances often do not reach the accident spot on time. This is a serious problem faced by the first responder, which can be solved only with the formulation of a comprehensive algorithm for first responder care by policymakers of the country. Additionally, legal protection should be provided to the people administering first responder care to RTA victims.

Psychological problems were reported to be another barrier in providing first responder care by 888 (85.4%) participants. The major problems faced by the participants under this barrier were the fear of the accident scene, blood, and failure. This finding is concurrent with that of another study, which stated that the mental health of drivers was also crucial for proper driving and care.²² The fear of failure scared the participants in providing care to RTA victims this is a serious problem and can be solved only with proper guidance and counseling.

Most participants (n=814; 78.3%) in the present study exhibited a negative attitude toward providing first responder care to RTA victims. This finding is concurrent with that of another study conducted on drivers.²² Most participants felt that immediate care for RTA victims was to be provided by the emergency department or police and that it was not the drivers' job to look after them. They also expressed that by providing emergency care by investing their time, energy, and sometimes money,

they were encountering trouble later may be from the hospital administrators, police, and relatives of the victims, while not benefiting in any form. This problem can be solved by providing proper recognition and support to caregivers with all the necessary compensation and protection.

Another major finding of the present study is the lack of knowledge about first aid care being the barrier for some participants (n = 589; 56.6%) in providing first responder care to RTA victims. This result is supported by that of another major study conducted in Iran²³ stating that emergency care providers require knowledge and training in first responder care. This can be assured by providing compulsory training programs in first responder care for drivers during the renewal process of driving licenses. In India, although motor vehicle rules mandate a first aid class for obtaining a driving license, the efficiency of the participants in providing first responder care is not measured. Knowledge deficit in providing first responder care can be solved by tightening the rules regarding attending the first aid class and also by arranging refresher training courses on first responder care at regular intervals.

The present study is one of the first studies conducted in Karnataka to identify the barriers in providing first responder care to RTA victims. The single-center design of the study is a limitation of the study. However, the result can be generalized for all the drivers as the participants in this study were from various backgrounds. Further multicenter studies can be conducted in a wider range to identify additional barriers faced by people in providing first responder care.

CONCLUSION

The present descriptive study is relevant in the present time to understand the barriers faced by autorickshaw drivers in providing first responder care to RTA victims. These problems must be addressed and solved permanently in a timely fashion. If policymakers implement programs to create awareness among the general public about first responder care, numerous lives that are lost due to RTAs can be saved.

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Chelioscopy Study on Lip Prints of Male/Female/Transgender/Illness: A Review

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ABSTRACT

Like fingerprints, lip prints are completely one-of-a-kind. Lip wrinkles come in a wide variety of shapes and sizes, but this review will focus on the differences between the sexes. There are forensic and non-forensic uses for lip prints. Chelioscopy is a powerful method for solving crimes by examining physical evidence left at the site. Greek chelioswich, meaning “lip” is where the term “cheiloscopy” originates. Both destructive and nondestructive techniques are used to categorize samples. There are numerous classifications that may be applied to lip prints due to their distinctive wrinkling and shape patterns; for instance, the clauco martin santos, Suzuki, and tsuchihashi classifications. Reviewing a large population, we find that both sexes share a preponderance of type IV groove patterns, that male and female lip prints exhibit no discernible variation over time, and that lip prints from the third gender display strikingly different characteristics.

KEYWORDS: Personal identification; Suzuki and Tsuchihashi; Lip prints; Destructive and non-destructive methods; Trimester; Age; Gender; Distinctive folds; Rooves.

INTRODUCTION

The skin, muscles, glands, and mucous membrane that make up the lip folds form an

anatomical barrier to the oral cavity at its entrance. In the same manner as the upper half of the lip extends laterally toward the cheeks to generate the nasolabial sulcus, the bottom part of the lip is bounded inferiorly to form the labio sulcus. The buccal gap, or space between the two lips, is distinct. Skin and a mucous membrane cover the lip area. Lip wrinkles or grooves emerge when the labial cord and Klein's zone contract. Neither inflammation, herpes, diabetes, or any other medical condition can alter the unique pattern of these lips' fingerprints.^{1,2}

Other few Methods of Classification:

1. Martin santos classification

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2. Suzuki and tsuchihashi classification
3. Renaud classification
4. Afchar-bayat classification
5. Josemaria Dominguez classification^{4,9}

METHOD AND MATERIAL

Lipstick, a brush, cellophane tape, bond paper, and a magnifying glass are all necessities for the sample collection. Put some pressure on the paper where the lipstick is and see what happens. The paper was taped to ensure its survival for future reference. Under magnification, the prints were identified as belonging to the Suzuki and tsuchihashi schools. There were a total of 2112 persons analyzed in the study conducted by Tim Peter Thermadam, Laxmikanth Chatra, and Auswaf Ahsan of the Department of Oral Medicine and Radiology at the KMCT Dental College in Calicut, Kerala. After getting institutional ethical permission and the subject's informed agreement, the researchers employed lipstick to record the lip groove patterns, which were later examined under a microscope. The statistical analysis was performed using SPSS 22.0.^{4, 6, 13}

1. Apply lipstick in the lips using earbuds.
2. Ask the person to cling the lips against bond paper.
3. And the impression is obtained clear with the fissure marks.
4. Fix a cellophane tape over the sample collected.
5. Using the help of magnifying glass make the view of sample more clear.
6. Study the variation of each sexuality/gender.
7. Classify the lip prints and note it.⁶

Chelioscopy:

Chelioscopy was initially described by R. Fischer in 1902. In 1932, the eminent French criminologist Edmond Locard advocated for the use of lip prints as a means of identification. The purpose of this research is to determine the individuality of lip prints and their usefulness in personal identification. The human lip has a transition zone between the inner labial mucosa and the outer skin, and this zone is where fingerprints can be taken in the form of creases and grooves. Chemo-microscopy is a common term for this type of research.⁹

Advantage of Chelioscopy:

Because of its ease of use and low cost, cheiloscopy is increasingly being incorporated into forensic investigations. Our research found no association between ABO blood groups and the prevalence of any given lip print pattern among populations.¹²

LITERATURE REVIEW

Various Methodology of Analysis:

Before each application, the lipstick (REVLON, No. 02 created by Aero pharma Pvt Ltd, Mumbai, India; it was black in color and non-glossy to ensure the optimum print visibility) was wiped off with a tissue paper, as stated by researchers Muniapillai Siva Kumar and Ravi Aravindha. When the subject opened their mouth, lipstick was applied quickly and evenly to their upper and lower lips.^{4,6,7,8,29} "After applying the lip stick, the volunteers were told to rub their lips together. In order to get a good lip print, the person had to keep their mouth open the whole time. To make a lip impression on a glass slide, we first dabbed its center and then pressed our lips uniformly on its upper and lower corners. The lip print can be created with different powders or cyanoacrylate and then photographed if it is preserved on the proper surface. Lip prints taken in latent form must be completely dry before they can be developed, and the same powder is used for both."⁹

BASIC DUSTING OF LATENT PRINTS

Many crime scene investigators employ conventional, non magnetic powder, which accounts for more than half of the powder they use. This rule applies to any domestic crime scene where windows, counters, televisions, and other goods are moved or handled. At commercial break-in scenarios, it can be applied to metal filing cabinets, painted doors, broken glass, and metal window frames. White, dichromatic, silver/gray, and black are only some of the colors available in regular powders. The proper hue is chosen to provide sufficient contrast.⁹

Technique

Muniapillai Siva Kumar and Ravi Aravindan are two practitioners of these methods. Lip designs in order Slides were inspected thoroughly at 10X magnification and then categorized according to suzuki and tsuchihashi's (1970) quadrant wise classification. Only the central 10 mm of the lips

were considered to avoid errors caused by lip prints from the right and left sides of the lips overlapping. Using Suzuki and Tsuchihashi's criteria, the lip prints were categorized, and then the kind and number of grooves were input into the subject's pre-existing proforma, one quadrant at a time. After compiling the information, a comprehensive subject chart was made.^{8,9}

COMPARISON STUDIES OF LIP PRINT

Reddy KS claims that extensive identification is crucial to any criminal inquiry. At the turn of the last century, fingerprints were the only reliable technique of human identification, and their development was largely due to the efforts of Sir William Herschel, Sir Francis Galton, and Sir Edward Henry. In India in 1858, Sir William Herschel used fingerprinting for the first time.^{1,2} Fingerprints and lip grooves, both of which are unique to each individual, can be used for identifying purposes. According to his research, finger printing is much simpler because the details are more distinct.

Kasprzak j, utsuno H, Kanoh T, Tadokoro O, Inoue K as a, state that criminals are familiar with the processes police use while looking into crimes. Lip prints, which are reddish brown to pink in hue and extend from the nasolabial folds to the lower edge of the face, are the subject of chelioscopy. Which is more distinctive than fingerprints due to patterns, and which forms in the womb between the ages of four and six months and cannot be altered unless in the case of certain diseases, such as inflammation and herpes. Lip prints found on napkins, drink glasses, mirrors, or articles of clothing at the scene of a crime might be submitted as evidence in court by the forensic dentistry division.^{1,2,3,6,24}

Cases involving murder, rape, and other serious crimes rely heavily on scientific and investigative controls such as fingerprints, lip prints, DNA, and dental records. As we've already established, there are a number of different categorization methods for chelioscopy, but the one developed by Suzuki and Tsuchihashi in 1970 is by far the most often used. These grooves are broken down into five distinct categories and detailed in Table 1.

Table 1: Types of Lip print patterns according to Suzuki and tsuchihashi.⁸

Classification	Patterns
TYPE - I	Complete Vertical Grooves
TYPE - I	Partial Vertical Grooves
TYPE - II	Branching Grooves

TYPE - III	Intersected Grooves
TYPE - V	Reticular Grooves
TYPE - VI	Other Undifferentiated Grooves

The study makes use of brand new computer programs. Statistics are performed using SPSS 2.0. The four sections of a lip print are as follows: The square test was utilized as the significance examination of choice. The letters A, B, C, and D From 0% to 34.70% to 0% to 12.10% in month zero to 28.80% to 35.70% to 0% to 0% in month six. Agreement testing for gender identification using latent variable analysis Gender identification is an important part of forensic science. Significant quadrant based intra and inter-observer agreements were measured using kappa values at 0 and 6 months by both observers. There was statistically significant agreement between the two observers on their assessments of the morphologic pattern of the lip in each of the four quadrants. The calculated kappa values show that the two observers had "excellent" to "very good" agreement when identifying gender. Kappa (K) values were calculated to examine levels of agreement between and among groups of observers. Gender identification is an important part of forensic science. It is based on research in dental rehabilitation. Graph 4 displays the results of an assessment of strong intra and inter-observer agreements using kappa values in each quadrant by both observers at 0 months and 6 months. After six months, Observer measured a gender determination agreement of approximately 67.5% and Observer II measured a gender determination agreement of approximately 57.5%, indicating statistically significant inter and inter-observer agreement when evaluating the morphologic pattern of then accordance. After six months, Observer I's measurement of stability was 0%, whereas Observer II's measurement was 27.8%. Gender identification is an important part of forensic science. It is based on research in dental rehabilitation.

Inter-observer and inter-observer ag that are statistically significant were measured using kappa values in each quadrant by both observers at 0 months and 6 months, respectively. Observer I found an agreement of 95.40%, 95.90%, 100%, and 97.50% between the lipstick and photo methods for determining gender in quadrants A, B, C, and D during the first month, and 82.40%, 85.50%, 87.30%, 92.20% after six months. Gender identification by digital image is discussed as a potential gold standard in Graph 3. The accuracy

of the latent technique to identifying gender in photographs is evaluated.⁷

Using a cross-sectional design, Muhammad hammad 1, Haroon habib 2, Yasir Ali bhatti 3, sadia zia 4, Fouzia javeds 5, mudaser Hussain abbasi 6, rana Muhammad akhtar 7, hassan barkat 8, and salahuddin analyzed data from 1,000 MBBS students. Chi square was computed in the lip print using SSPS software. Tables 2 and 3 both provide chi-square results in the final column.

Table 2: Number of people involved in the research and number of people with types of patterns

-	Male	Female	Total
Type I-Long vertical grooves	15	45	60
Type II-Short vertical grooves	2	6	8
Type III-Branching grooves	6	14	20
Type IV-Diamond grooves	1	4	5
Type V-Reticular grooves	2	3	5
Type VI-Undifferentiated grooves	1	1	2

Table 3: The count of each lip print is a calculated in Pearson chi square, likelihood ratio, linear by linear association N of valid cases and they a cell of (58.3%) have excepted count less than⁵

-	Value	Df	Asymp. sig (2 sided)
Pearson chi square	1.319 ^a	5	.933
Likelihood ratio	1.233	5	.942
Linear by linear association	.680	1	.409
No of valid cases	100	-	-

Type V was the least common, and there was no significant correlation between male and female lip prints.²³ Cheiloscopy Vahan Wala sonal 1, chintan shah 2, sandeep pagare 3, kapil gavand 4, claimed that, the first proposed in 1950, and in the 1960s and early 1970s, experimentation was undertaken on lip prints because, as of right now, unless other than health difficulty at birth, lip prints will not alter.¹⁰⁻²⁰ A recognizable fingerprint may have been acquired. Forensic cheiloscopy specialists say that studying the third gender categorization would provide a good place to begin, and that courts should support such research. The authorities can utilize this information to better identify third gender victims of crime". At all, Pattern types I, I', and II are more common in females, as reported by vahanwala S.P *et al.*²³ The signature of Type III is Male has been identified. Typically males. Because

of the complexity of patterns, errors are possible. Cheiloscopic gender determination may still leave room for doubt. That's why we got result.¹⁰ Eunuchship, as defined by Peeran and Ramalingam, is a peculiar behavioral pattern seen in men who are physiologically born much later in life. It was shown that the majority of the sample population had lip groove types I, I', 4, and 5. Men were more likely to have a type 1' or type 1 lip groove pattern, whereas women were more likely to have a type 4 or type 5 pattern. Comparable findings were found when comparing male and female upper and lower lips independently.

Researchers Aparna 1, Ahmed Mujib. B 2, Rashmi Naik 3, Shruti K Patil 4, and Arun Kumar 5 found that the vast majority of persons with hypertension and diabetes mellitus were classified as having type IV (14) diabetes. The purpose of the research is to compare children with and without²⁹ down syndrome. This research employed an inductive strategy, a cross-sectional design, and extensive direct observation. Among the 68 participants in this cheiloscopy investigation, 34 have Down syndrome and the remaining 34 do not. Labial grooves (I-complete vertical, I'-Incomplete vertical, II-bifurcated, III-cirfor the research purpose cross, IV-reticular, or V-undefined) and lip thickness (thin, thick, medium, and mixed) were evaluated in eight labial regions called sub-quadrants in the city of Joo Pessoa, PB, Brazil. Analysis of the data was performed.¹⁵ There are more women than men in the type II category. Type 2 lip print patterns were more common in Bihar and West Bengal than Type 1 patterns were in Odisha.^{7,13,16}

The study's findings suggest that establishing identification is crucial to any criminal inquiry. If available, lip prints should be analyzed with other methods to establish the offender's link to the crimes they committed. Forensic anthropologists rely heavily on cheiloscopy, the study of lip prints, because they are just as unique as fingerprints. Estimating sexuality with lip prints. The study included 434 students, 143 male and 291 female. Dark lipstick and cellophane tape were used to record²⁸ each person's lip print, which were then transferred to white paper. The designs of the upper and lower lips were each divided into three sections, for a total of six sections.^{22,28} Lip print fig. 1 shows the many lip print designs.

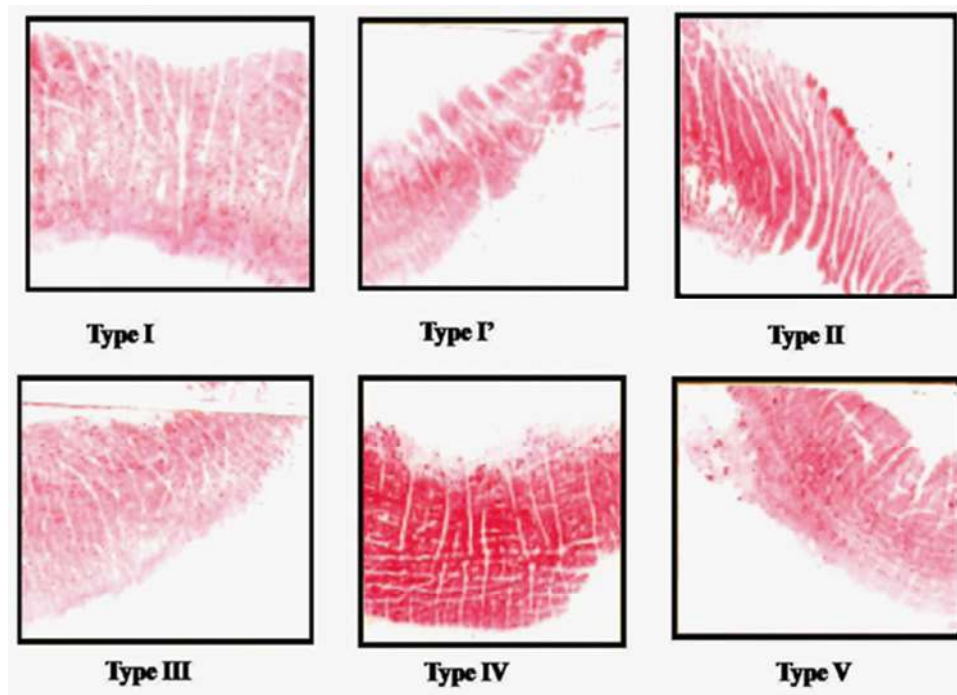


Fig. 1: Different patterns of lip print type I-Full vertical grooves, typeI'-Partial vertical, typeII-Branching grooves, typeIII-Intersecting grooves, typeIV-reticular, typeV-other grooves.

Researchers Vikash Ranjan 1, Mysore K Sunil 2, and Raghav Kumar 3 came to the conclusion that the creases and grooves in each participant's labial skin resembled those produced by the conventional way of applying lip stick. Lip prints were unique to each individual, making them a potential form of personal identification. Types I and II predominated in the first quadrant.⁸ Each individual also has their own unique pattern combinations.

Therefore, there is no scientific basis for genetic features like rugae patterns in finger prints or lip prints, nor for castrated men or nice guys behaving in a feminine manner (gay), as both are physically male.²⁷ The present According to studies, more extensive research with a larger sample size is still required to fully understand the transgender community.¹⁰ The amount of rugae on the mid palatine raphe on the right and left sides According to the length of the rugae: The rugae were classified into the following three groups, based on their length:

First >5 mm

3 to 5 mm secondary

3 mm of fragmentation.

A number of researchers, including Eshani saxena 1, B. Rchandraskar 2, Sudheer hongal 3, Nilesh torwane 4, Pankaj goel 5, and priyesh mishra 6, have

come to this conclusion: Shorter than 2 millimeter long rugae were disregarded. The length of a rugae was determined by using a divider to measure its biggest dimension, regardless of its shape.²⁶

The rugae were divided into the following classes according to their outward appearance:

They were shaped like a crescent and had a small curvature. Curved rugae were those in which the or end showed any sign of curvature at all. A wavy rugae was one that was curved all the way along its length but just slightly curved at its start or finish. They don't pause between their launch and their destination. Circular rugae are those that form a clear, continuous circle. Two clusters of rugae, one in each direction, were formed. Rugae that pointed ahead were associated with positive angles. Rugae that faced backward were associated with negative angles. Joining of rugae together. When two rugae met at their beginning or end, they were said to be joined. Two distinct groups of rugae emerged as a result of the consolidation. When two rebels begin in the same midline location but shortly split off in opposite directions, we say that they have diverged. Rugae that branched off the midline but met at the sides are said to converge. The palatal rugae pattern was assessed by a single trained and certified analyst. To determine intraexaminer reliability, we re-examined 10 randomly selected casts after a 3

hour interval.¹¹ Type III was the most common male lip print type, whereas Type I was the most common female lip print type. The outer four sections of the lip showed statistically significant differences between sexes. Lip print patterns showed that the central third of the lip played no role in identifying gender. Lip thickness was significantly different between sexes, and this difference could be used in a logistic regression model to identify gender. Not only do the lip prints of men and women differ greatly in pattern, but also in size. A person's sex can be determined using these features.¹⁸ Sudan III dye and aluminum powder generated statistically significant results ($p < 0.05$) when used to make visible lip prints, but had no effect when used to make latent impressions. The use of indigo dye resulted in statistically significant results for the appearance of both latent and obvious lip prints ($p < 0.05$).¹⁹ In one quadrant, there was a statistically significant correlation between parental and offspring lip print patterns, while in the other three quadrants, there was no correlation at all. The good resemblance between family members can be

attributed to genetics and heredity. Lip prints are an extra method of identification.²⁰

According to vahanwala sonal 1, chintah shah 2, Sandeep pangree 3, kapil gavand 4, Hemant bhuntani 5, Naveen shetty 6, mandavi waghare 7, they collected 50 samples of eunuchs, Mumbai roads, and 50 samples of men using the traditional lipstick method; these were analyzed using the Suzuki and Tsuchihashi method to determine whether or not there was a significant difference between the two sexes. Even while they undergo physiological changes characteristic of women, the results indicated that type I and I' that are predominate in nature.¹¹

Researchers K Randhawa 1, R S Narang, and P C Arora say they used lip prints to determine the ages of 600 people (289 men and 311 women) and then split the larger group into three age brackets based on the results: group 1 (individuals aged 1 to 20 years old), group 2 (individuals aged 21 to 40 years old), and group 3 (individuals aged 41 years or older).



Fig. 2: Lip prints of female with type I-complete vertical grooves



Fig. 3: Type II' in lip print of female



Fig. 4: Type IV in male

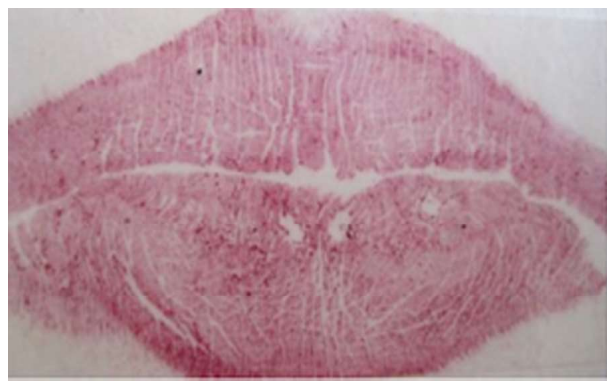


Fig. 5: Type V in male

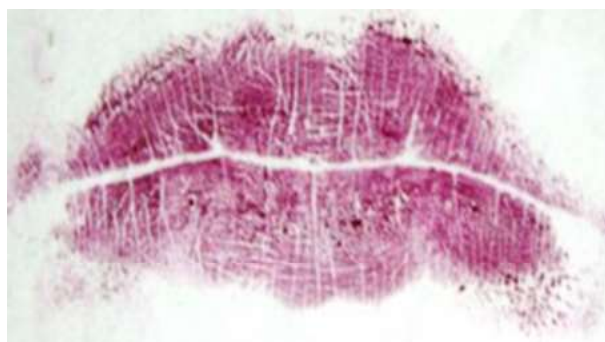


Fig. 6: 9 years old child lip print 1/3 of the lower lip has reticular patterns

According to Rashmi venkatesh 1, mariapriscilla David 2 they organized a research with 200 subjects as a sample for the research purposes and they made them under the group of 4 and the samples of father, mother child A and child B is taken traditional lip sick method and cellophane tape is stuck against it the glued part into sample bond paper and the analyses is conducted and as a part of result the lip prints between identical twins are different considering the family lip print the child is either is having mother or father same lip print pattern with their owninduvial miniatures and group 3 the lip prints where taken after an interval od 3 months and the prints are similar to the first set of lip prints hence the lip prints permeance is proved.²⁹

DISCUSSION

The lip prints of people with diabetes and hypertension are distinct from the lip prints of those without these diseases, and the lip prints of brothers and sisters are also different. Because there is a distinct difference in the lip prints of every indivual, it is possible to easily recognize a person's gender. This is because the lip prints of the third

gender are also different, and these lip patterns differ based on the hormonal changes.

CONCLUSION

According to these researchers, the research population shows a separate change between male and female in which male has type I or I' in the maximum study population and female has type II very few researchers who have examined the lip print of third genders until the person either with diabetes, hypertension, herpes, inflammation and trauma only that can change the lip print during the period of this illness other than this nothing can change the lip print. And even this lip prints of patients with diabetes and herpes have predominance of type III in common without considering genders.²⁵

Forensic Signifances of Chelioscopy:

Most people of both sexes share the same type 4 lip print pattern, which is significant according to the research of 1. Avanindra Kumar, 2. Swami Nand Prasad, 3. Vaibhav Kamal, 4. Swati Priya, 5. Mukesh Kumar, 6. Avanish Kumar. The research suggests that lip prints can be used as an additional method of determining a person's race. Cheiloscopy is a newer technique among the various identifying methods available to forensic experts. Several important conclusions have already been drawn from this line of inquiry, including the fact that an individual's lip print can be used to verify their identification, that it does not change over time, and that it can be used to distinguish between the sexes. With greater study, cheiloscopy has the potential to become a reliable evidence method.^{13,21}

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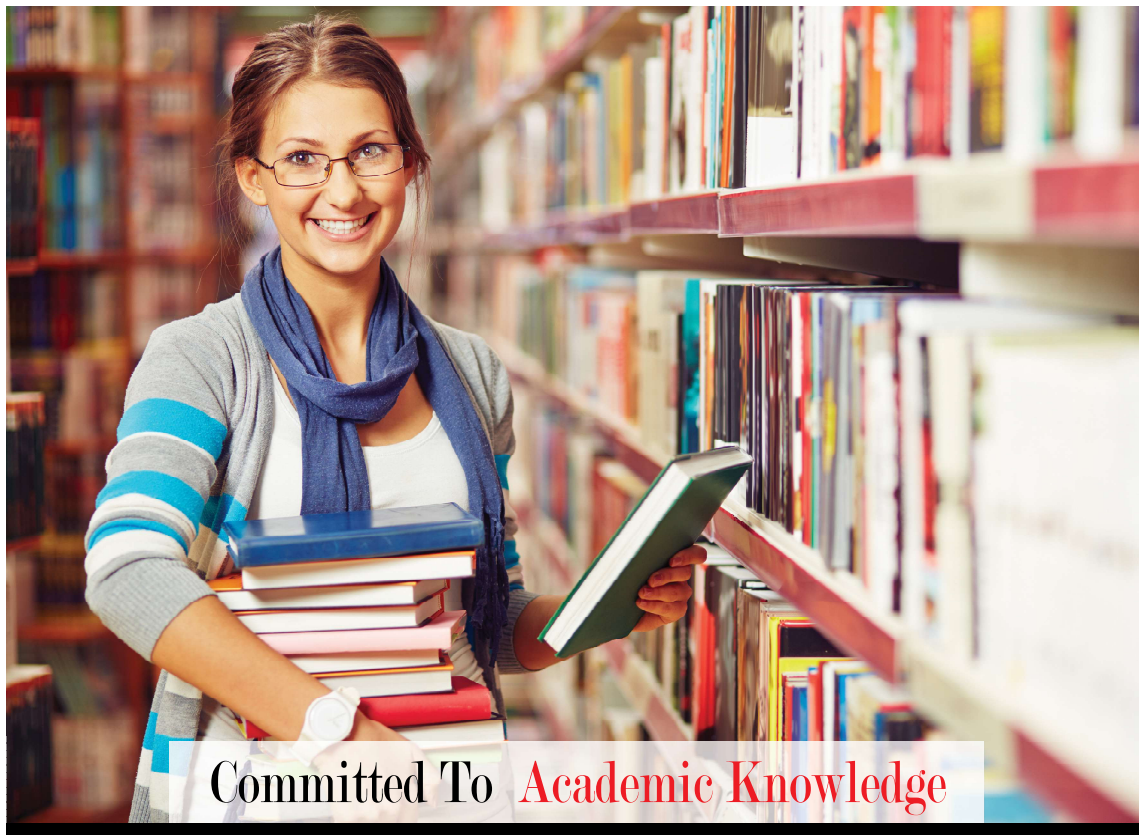
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