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Barr Bodies Revelation from Dental Pulp – A Comparative Study on the Basis Of X- Chromatin Visibility and Nuclear Membrane Clarity

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ABSTRACT

Background: A convex or triangular shaped dynamic inactivated X chromosomal configuration is seen in female somatic cell nuclei. These morphologic variants are termed as Sex chromatin or barr bodies. Determination of gender is of valuable significance especially in cases where bodies are charred/ burnt and are beyond positive identification. Discovered by Murray Barr, these bodies are inactivated by a process known as Lyonization. Dental pulp, blood, hair follicles buccal smears etc proves to be impending sources from which barr bodies can be obtained.

Aims and objectives: The main aim and objective of the study is to assess barr bodies on the basis of X- chromatin visibility and nuclear membrane. Intraobserver variability of barr bodies were also evaluated and both histopathological and cytopathological methods were also evaluated

Material and methods: Total number of 60 subjects i.e. extracted teeth within the age group 10-40 years from 20 male & 40 females were taken. Dental pulp was obtained from the extracted teeth. Total number of two respective groups, Group 1 and Group II were categorized which included 10 males & 20 females each. Rapid tissue processing was done in group I and in Group II cytological method using centrifugation was inculcated.

Results: Nuclear membrane was extremely noticeable in rapid tissue processing histopathological method samples both in males and females. Conversely, in cytological method, the clarity of membrane was very indistinct. In Histopathological females of group 1, X-chromatin visibility was of paramount excellence. In case of males of both group I and II, X- chromatin was poorly visualized. A statistically significant difference was obtained

Conclusion: Nuclear membrane and X- chromatin visibility were superior in histopathological method especially in case of females. Histopathological method was found to be more feasible in revelation of barr bodies for medico- legal purposes

Keywords: *Dental Pulp, Inactivated X-chromosome, Sex Chromatin, tissue processing, cytological, nuclear membrane, X-chromatin, gender*

1. INTRODUCTION

Identification of deceased individuals is of utmost priority in forensic sciences. In criminal scenarios, road accidents, air plane crashes etc the establishment of identity is very difficult to encounter.[1] For such cases, gender can be determined by various methods such as craniofacial morphology, DNA analysis etc.[2] To serve this purpose by analysis of DNA, PCR can also be done. But this equipment is not cost effective and thus is not feasible to install at various institutions or forensic labs. Therefore, in the accountability to yield quick and effective results, presence and observation of barr bodies from human specimens such as blood (Davidson Bodies), saliva, buccal smears and dental pulp can act as efficient tool to determine

gender.[3] Dental pulp is surrounded by protective covering known as human enamel which acts a shield against harsh environmental circumstances such as high temperatures.[4] Also, in some disastrous episodes, the bodies are decomposed and charred and there is very minimal possibility to identify it.[5] In such exceptional cases, the pulp is often preserved due to enamel covering and thus can be an inexpensive method to retrieve barr bodies and to identify gender.[6,7] These sex chromatin bodies were discovered in 1949 by Murray Barr and he named the cells in which barr bodies were present as chromatin positive. In case of males, the cells lack this juxtanuclear chromatin and thus are considered as chromatin negative.[8] To determine the presence of barr bodies in dental pulp, tissue processing and cytological methods have been employed for gender determination from dental pulp in this study. So, in the department of Oral Pathology a comparative study was done by two different methods to evaluate barr bodies on the basis of two variable parameters i.e. nuclear membrane clarity and X chromatin visibility.

2. AIIMS AND OBJECTIVES

1. Barr Bodies estimation for determination of gender in human dental pulp using cytopathological and histopathological methods

2. To compare histopathological and cytological method for evaluation of barr bodies on the basis of nuclear membrane visibility

3. To compare histopathological and cytological methods for evaluation of barr bodies on the basis of degree of visibility of X- chromatin

4. To assess the intraobserver variability in visualization of Barr Bodies.

3. MATERIAL AND METHODS

The study sample consisted of 60 teeth which are freshly extracted collected from patients ranging from 10-40 years. 40 samples collected were from male participants and rest 20 from females. Vital teeth were included while on the other hand, carious teeth were excluded. After fixation in formalin (10%) at normal circumstances, pulp was extracted by using a carborandum disc bur **(Figure 1)**. The teeth were categorized into Group I and Group II, consisting of 30 extracted teeth each having 20 females and 10 males. In group I, histopathological technique was employed while in Group II Cytopathological technique was done

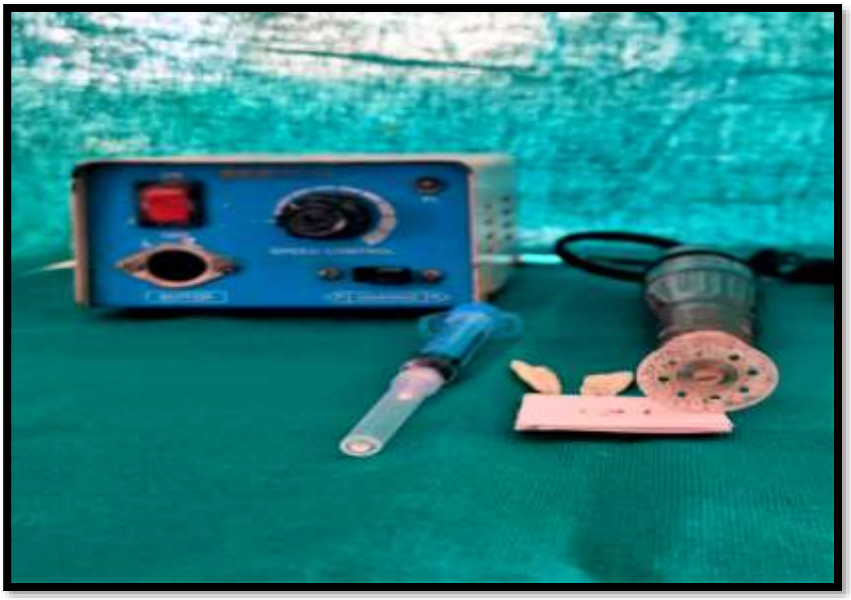


Figure 1: Vertical splitting of teeth for pulp retrieval by carborandum disc

4. METHOD EMPLOYED FOR HISTOPATHOLOGY[9]

The samples of tooth were immersed in Isopropyl Alcohol (10 minutes), Acetone (10 minutes) and Xylene (15 minutes). Tissues were then impregnated and embedded in wax. Sections were prepared **(Figure2, 3)** and stained with H&E

Figure 2: Semi – automatic microtome for preparation of paraffin sections

Figure 3: Thermostatic water bath

METHOD USED FOR CYTOPATHOLOGY[9]

Tissue samples were fixed (3METHANOL:1 GLACIAL ACETIC ACID) for 24 hours, crushed in mortar and suspensions obtained. Centrifugation was done and supernatant collected. **(Figure 4)** Pellets are obtained by repeating cycles of centrifugation (10min. at 1000 RPM). Smear preparation is done & stained with H& E. **(Figure 5)**

FIGURE 4: Centrifugal machine for cytological technique

Stained pulp tissue was visualized under oil immersion (100X) lens and barr bodies appeared as chromatin clumps in females. Now the nucleus showing Barr Bodies were counted from five represented fields from each section. Mean Barr body count value was calculated for female & male tissue samples by both techniques. Comparsion was done and calculated mean of barr bodies was obtained to conclude the competent technique amongst the two on the basis X-chromatin visibility and nuclear membrane clarity

Figure 5: Haematoxylin & eosin staining methods

5. OBSERVATION AND RESULTS

Stained pulp tissue was visualized under oil immersion (100X) lens and barr bodies appeared as chromatin clumps in females. Now the nucleus showing Barr Bodies were counted from five represented fields from each section. Mean Barr body count value was calculated for female & male tissue samples by both techniques. Comparsion was done and calculated mean of barr bodies was obtained to conclude the competent technique amongst the two on the basis X-chromatin visibility and nuclear membrane clarity.

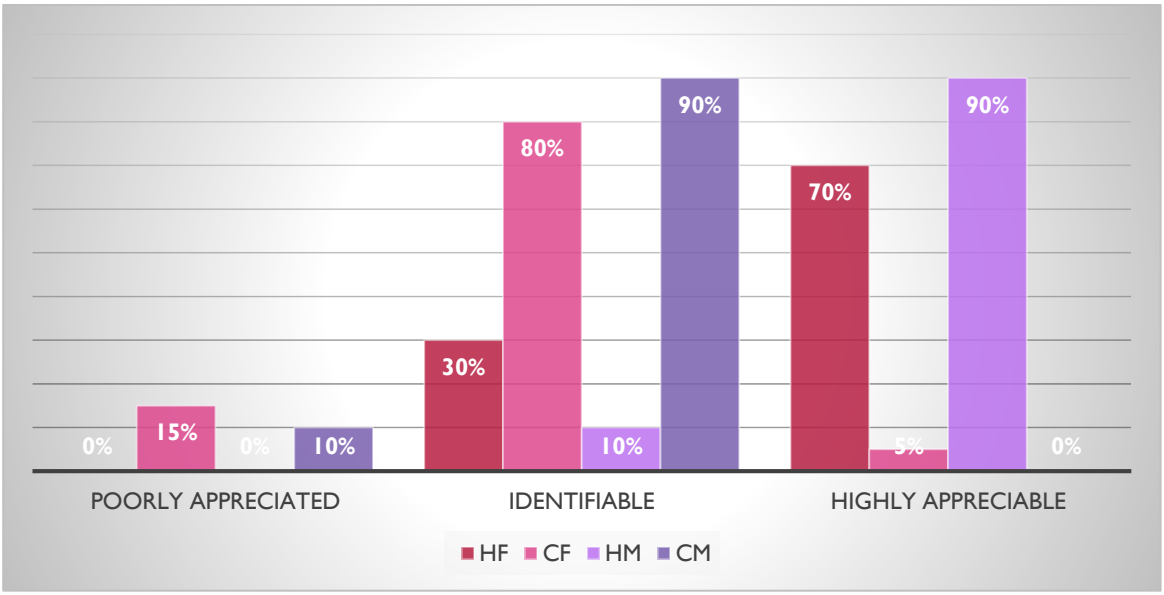
In the current study, the statistical analysis was done using SPSS (Statistical Package for Social Sciences, SPSS Inc. Version 16, and USA). The results showed that Nuclear membrane **(Table 1), {Graph1}** was highly appreciable in HM (90%) followed by HF (70%). Cytopathological method was not as successful in showing highly appreciable cells (CF=5%, CM=0%).The difference between histopathological and cytopathological methods was statistically significant ($p<0.001$).

X-chromatin **(Table 2), {Graph 2}** was best appreciable in HF (80%). HM showed identifiable (40%) and poorly appreciated (60%) X-chromatin. Cytopathological method was able to show 85% identifiable X-chromatin in CF. HF showed significantly better X-chromatin than all other groups ($p<0.001$).

			Group			
			HF	CF	HM	CM
Nuclear Membrane	Poorly appreciated	Count	0 (0%)	3(15%)	0(0%)	1(10%)
	Identifiable	Count	6 (30%)	16(80%)	1(10%)	9(90%)
	Highly Appreciated	Count	14(70%)	1(5%)	9(90%)	0(0%)
Total		Count	20	20	10	10

*Statistically significant (Chi-square=35.875, p-value<0.001)

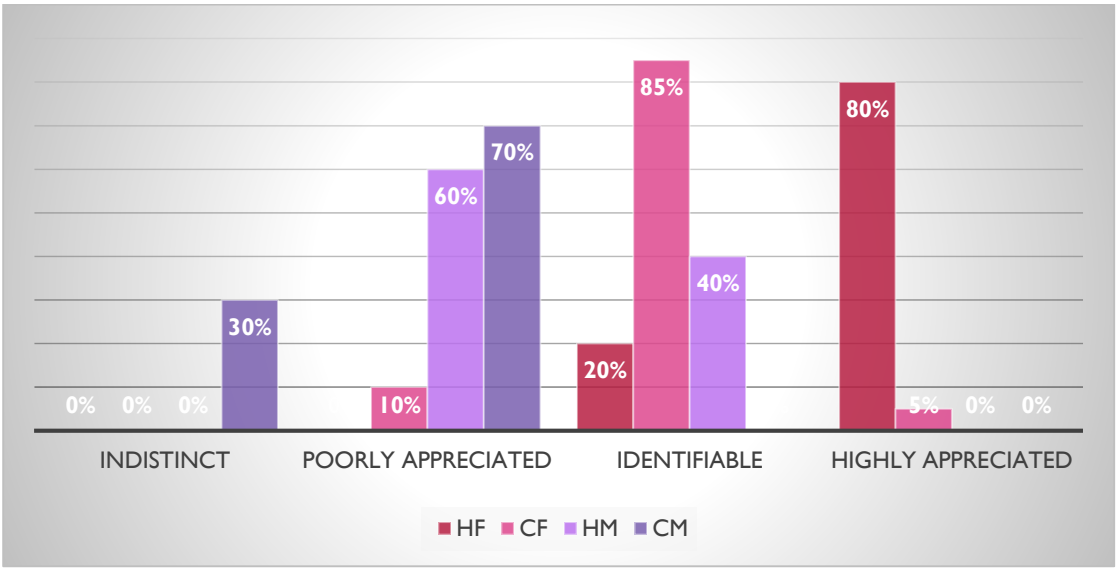
Table 1: Comparison of nuclear membrane in histopathological males, histopathological females, cytopathological males and cytopathological females



Graph 1: Nuclear membrane in various groups

			Group			
			HF	CF	HM	CM
X-Chromatin	Indistinct	Count	0 (0%)	0(0%)	0(0%)	3(30%)
	Poorly appreciated	Count	0(0%)	2(10%)	6(60%)	7(70%)
	Identifiable	Count	4(20%)	17(85%)	4(40%)	0(0%)
	Highly Appreciated	Count	16(80%)	1(5%)	0(0%)	0(0%)
Total		Count	20	20	10	10

Table 2: Comparison of X-chromatin in different groups



GRAPH 2: X chromatin in various groups

6. DISCUSSION

In Forensic medicine, different ways for identification of deceased individuals are provided and special protocols for complete profiling are assigned particularly for decomposed and dead bodies .[10] The preliminary and most significant stride for establishing individuality is gender/sex determination. Various methods are available for gender determination in forensic odontology. Gender determination can be done with hard tissues and soft tissues. Bones and teeth are the sources of hard tissue. In case of various natural and manmade disasters, teeth and the soft tissue within it i.e. dental pulp containing genetic information are often preserved due to hard encasing of calcified tissue like enamel and dentin.[8] Barr bodies or sex chromatin body was first identified in the neurons of female cats for the purpose of indirectly assessing the chromosomal status of the cells, by an anatomist from London named Murray barr[11]. This exceptional nuclear sex chromatin prototype evident only in females plays a considerable role in forensic identification. Barr bodies appears to be basophilic and Plano or biconvex in morphology. This inactivated X chromosome can be reactivated and the process of lyonization can be reversed if the body is under stress. Also, the frequency of barr bodies can also be decreased due to premalignant and malignant lesions, pregnancy and females under oral contraceptives.[12] We in this study compared two methods namely histopatholgical and cytological for the detection of barr bodies from dental pulp using X-chromatin and nuclear membrane as two different parameters.

In our study, on the basis of clarity & visualization of nuclear membrane, we scored nuclear membrane from 1- poorly appreciated, 2-Identifable & 3- highly appreciated. Nuclear membrane was highly appreciated (Score 3) in 14 samples of HF (70%) followed by 6 samples of HF (30%) showing identifiable (Score 2) nuclear membrane visualization. No HF sample showed poorly appreciated nuclear membrane (Score 1) **(Figure 6, 7).** In case of HM, nuclear membrane was highly appreciable (Score 3) in 9 samples (90%) & the rest 1 sample (10%) showed identifiable nuclear membrane (Score 2). No

HM sample showed poorly appreciated nuclear membrane (Score 1)**(Figure 8).**16 samples (80%) in CF showed identifiable nuclear membrane (Score 2) ,3 samples (15%) showed poorly appreciated (Score 1) nuclear membrane & 1 sample (5%) showed highly appreciated nuclear membrane (Score 3)**(Figure 9)**. 9 samples of CM (90%) showed identifiable nuclear membrane (Score 2) & the rest 1 sample (10%) showed poorly appreciated (Score 1) nuclear membrane. No sample in CM showed highly appreciated (Score 3) nuclear membrane **(Figure10)**. Therefore, we found that nuclear membrane was highly appreciable in HM followed by HF. Cytopathological method was not as successful in showing highly appreciable cells. The difference between histopathological and cytopathological methods was statistically significant ($p<0.001$).

On the basis of degree of visibility of a densely stained mass (X chromatin) lying next to nuclear membrane we scored X chromatin from 0- indistinct, 1- poorly appreciated, 2-Identifable & 3- highly appreciated. X chromatin was highly appreciated (Score 3) in 16 samples of HF (80%) followed by 4 samples of HF (20%) showing identifiable X-chromatin. No HF sample showed indistinct (score 0) & poorly appreciated X chromatin (Score 1) **(Figure 6, 7)**. In case of HM, X-chromatin was poorly appreciated (Score1) in 6 samples (60%) & the rest 4 samples (40%) showed identifiable X chromatin (Score 2). No HM sample showed indistinct (Score 0) & highly appreciated X chromatin (Score 3) **(Figure 8**). 17 samples (85%) in CF showed identifiable X chromatin (Score 2), 2 samples (10 %) showed poorly appreciated (Score 1) X chromatin &1 sample (5%) showed indistinct X chromatin (Score 0). No sample in CF showed highly appreciated X chromatin (Score 3) **(Figure 9).** 7 samples of CM (70%) showed poorly appreciated (Score 1) X chromatin & the rest 3 samples (30%) showed indistinct (Score 0) X chromatin .No sample in CM showed highly appreciated (Score 3) & identifiable (Score 2) X chromatin. So, we found that X-chromatin was best appreciable in HF (80%). HM showed identifiable (40%) and poorly appreciated (60%) X-chromatin **(Figure 10)**. Cytopathological method was able to show 85% identifiable X-chromatin in CF. HF showed significantly better X-chromatin than all other groups ($p<0.001$).

A similar study was done by Johnson J[13] et al in which they took four different parameters including nuclear staining, nuclear membrane integrity, cytoplasmic staining & cytoplasmic transparency to conclude the best stain for Barr body determination in buccal smears. They found that nuclear Staining (Light - Deep stained), Nuclear Membrane Integrity (Smooth-Rough), Cytoplasmic Transparency (High-Low) and Cytoplasmic Staining (Light Stained-Dark Stained) in Aceto-Orcein and PAP Staining. The data shows that between both stains the PAP Stain excelled in both efficiency and accuracy from the Aceto-Orcein stain.

We visualized barr bodies under oil emersion under oil immersion (100 X) lens of light microscope & barr bodies appeared as clumps of chromatin on the inner nuclear membrane. Along with this, we assessed the intra observer variability of barr bodies. In our study, we found that the reliability analysis of all the three observers observer1 (O1), observer 2(O2), observer 3(O3) showed statistically significant mean difference using Intraclass correlation coefficient. Among the three observers, Observer 1 & observer 2 showed Intraclass correlation coefficient of 0.998 ($p<0.001$), Observer 1 and Observer 3 showed an intra-class correlation coefficient of 0.999 ($p<0.001$) & Observer 2 and 3 showed an intra-class correlation coefficient of 0.996 ($p<0.001$). **(Table 3)**

	Intra-class Correlation Coefficient	**p-value**
O1 and O2	0.998	<0.001*
O1 and O3	0.999	<0.001*
O2 and O3	0.996	<0.001*

***Statistically significant ($p<0.05$)**

TABLE 3: The reliability analysis was done using Intra-class correlation. There were a total of 3 observers. Observer 1 and observer 2 showed an intraclass correlation coefficient of 0.998 ($p<0.001$), observer 1 and observer 3 showed an intra-class correlation coefficient of 0.999 ($p<0.001$). Observer 2 and 3 showed an intra-class correlation coefficient of 0.996 ($p<0.001$)

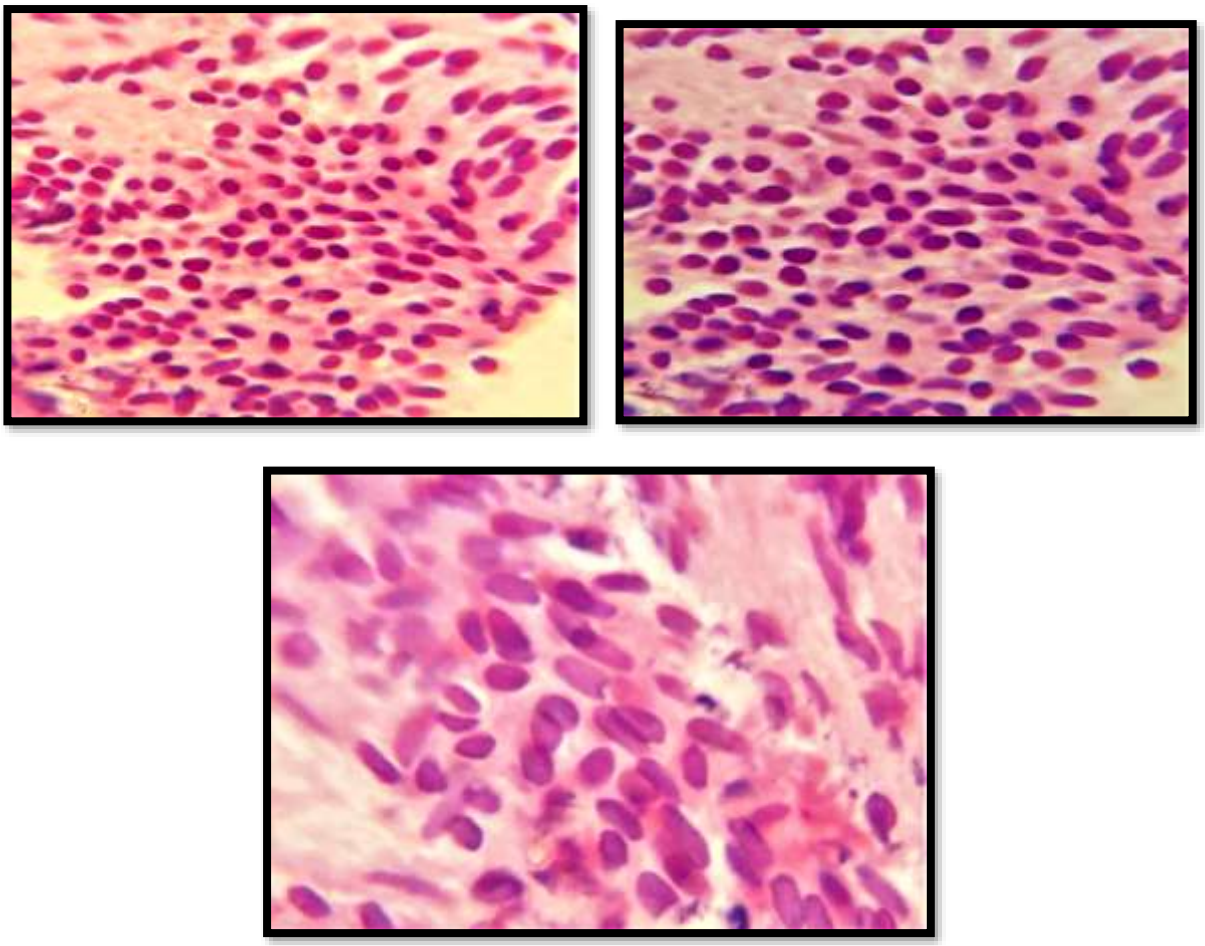


Figure 6& 7: Pulpal tissue in histopathological section in females showing highly appreciated X-chromatin visibility & nuclear membrane clarity *(*H&E stain; 100X)

Figure 8: Pulpal tissue in histopathological section in males showing no X chromatin visibility & appreciable nuclear membrane clarity *(*H&E stain; 100X)

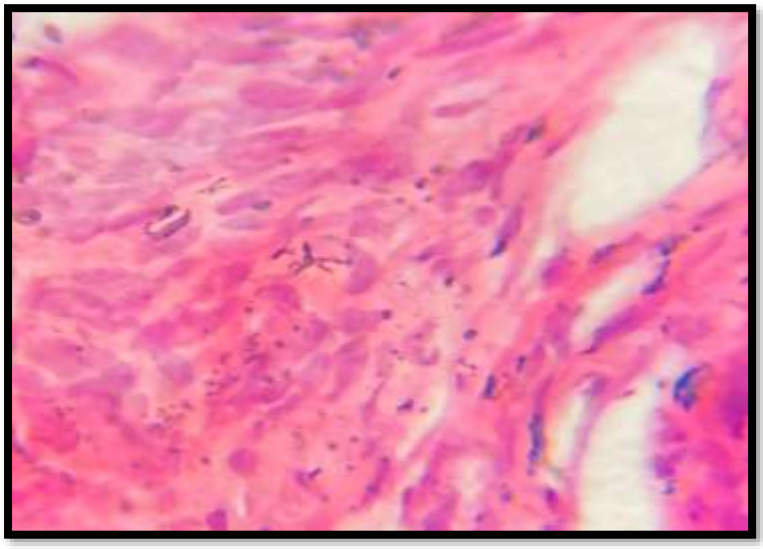


FIGURE 9: Cytopathological preparation of pulp showing poorly appreciated X chromatin visibility & poor clarity of nuclear membrane in females *(H*&E stain; 100X)

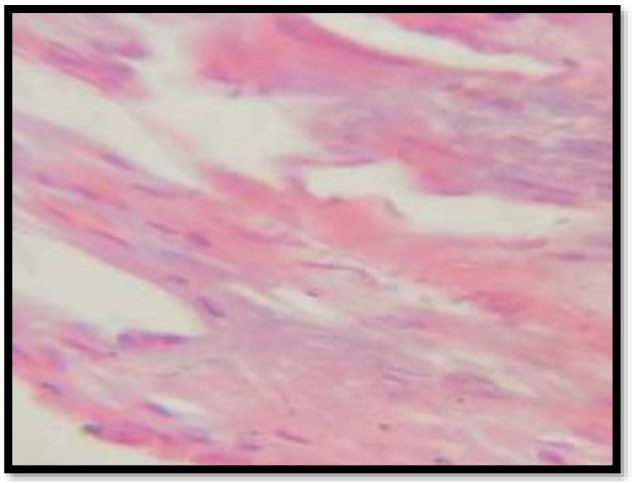


Figure 10: Cytopathological preparation of pulp showing no X chromatin visibility & very diminished nuclear membrane in males (H&E stain; 100X)

Therefore, in the present study a drastically high percentage of barr bodies was depicted and established in females. The method which gives tremendous results is manual and rapid histopathological tissue processing technique. Cytological method showed obscure and unclear proportion of barr bodies. Significantly negligible number of barr bodies was observed in males. Sometimes males also show positivity for barr bodies because they carry and inherit primary sex organs of both the sexes.[12] Thus the number and frequency of barr bodies in males and females were highly distinguishable. Histopathological females (HF) showed diagnostically superior precision in both X chromatin and nuclear membrane parameters as compared to Cytological females and males (CF & CM).

7. CONCLUSION

Gender determination from dental pulp using barr bodies proves to be simple, feasible and reliable criteria in the field of forensic odontology. However, parameters such as X chromatin visibility and nuclear membrane clarity were appreciated better in histopathological method with an accuracy and precision of 95%. Cytopathological method proves to be less unswerving in context of visualization of barr bodies. The mean number of barr bodies in females was more as compared to males. Thus, barr body determination for gender identification is economical, rapid and gives good results in field of cytogenetics and forensic medicine.

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