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Original Research

Efficacy of Sex Determination from Human Dental Pulp Tissue and its Reliability as a Tool in Forensic Dentistry

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Abstract:

Background: Sex determination is one of the primary steps in forensics. Barr body can be used as a histological method for identification of sex as it is found to be specific to female somatic cells and rare in male cells. To demarcate human dental pulp as an important identification tool of sex in forensic odontology (FO) and to evaluate the time period till which sex can be determined from pulp tissue using three stains H and E, Feulgen, and acridine - orange under fluorescence so as.

Materials and Methods: 90 pulp samples (45 males and 45 females) were subjected to Barr body analysis for determination of sex using light and fluorescent microscopy.

Results: Barr body was found to be positive for female samples and negative or rare in the male sample (<3%).

Conclusion: Barr body from human dental pulp tissue can be used as a successful determinant of sex identification in FO.

Key Words: Barr body, forensic odontology, sex identification, tooth pulp

Introduction

The term "forensic" is a Latin word, meaning forum or a place where legal matters are discussed. Forensic science is defined as a discipline associated with the application of science and technology for detection and investigation of crime and administration of justice, requiring the coordinated efforts of a multidisciplinary team.[1]

Forensic odontology (FO) also known as forensic dentistry is the science of dealing with evidence from dental and oral structures and is a specialty unto itself. The establishment of FO as a unique discipline has been attributed to Dr. Oscar Amoeda (Father of FO), who identified the victims of a fire accident in Paris, France in 1898.

This branch has applications in:

1. Identification of individuals in criminal investigations and/or mass disasters
2. Age estimation
3. Examination and evaluation of injuries to jaws, teeth, and oral soft tissues
4. Identification, examination, and evaluation of bite marks, which occur with some frequency in sexual assault and personal defense situation
5. As an expert witness in the legal case.

Construction of the biological profile is the first step to identify human skeletal remains; it includes the diagnosis of sex, age, nutritional state, height, and any other data that may allow an approximation to the characteristics of the subject alive.[2]

Natural disasters in areas of high population density, mass transportation accidents, development of conflicts, and terrorism usually require a large number of individuals to be identified which is easy if the bodies are well preserved or documentation enables forensic identification. The commonly used methods of forensic identification are DNA, dental records, and fingerprinting. However, when victims are carbonized, dismembered or have an advanced degree of tissue destruction, the process of establishing identity is difficult. In situations where the hands are charred or mutilated, fingerprints cannot be used for identification. Teeth are more durable, and identification using dental records may prove to be conclusive.[3]

Sex determination is considered to be the first and the most important step in forensic medicine for identification.[4] It holds importance in situations such as simple identification in the living, where the individual of one sex carries the features of the opposite sex; to decide an individual's power to exercise certain civil rights reserved for only one sex; for deciding questions relating to legitimacy, divorce, paternity, affiliation also to some criminal offences, or in identifying a dead individual in simple situations where primary sex organs are lost due to decomposition.[5] The various methods applied for identification of sex in FO are sexual dimorphism of canine teeth, rugoscopy in combination with molecular methods based on analysis of nuclear and mitochondrial DNA. Sex can also be determined by anthropological methods based on morphological characteristics of the bones.

However, in cases of murder or injury from assault where a single tooth is the sole material left behind at the scene of crime, even a single tooth might prove to be an important factor for identifying the individual by the histological and cellular techniques which provide an important contribution.[2]

The histological method for sex determination involves observation of the presence of chromatin body or Barr body in somatic cells.[6] The Barr bodies were initially studied by Barr and Bertram in the nuclei of the nerve cells of cats. Since then the study of Barr bodies has been an integral part of exploration by researchers in the field of FO. The sex chromatin/Barr body observation is possible in various cells and used for identification of biological sex.[7]

Barr bodies/sex chromatin is a small well defined, intranuclear body, which stains intensely with nuclear dyes.[2] 40% of female cells are found positive for Barr bodies which are known as chromatin positive while male cells are considered as chromatin negative. In 1952, Graham and Barr suggested that sex chromatin could be demonstrated in tissues other than neurons. The tissues examined included smooth, skeletal and cardiac muscle, cartilage, epidermis, endothelium, thyroid, liver, pancreas etc.[8] In 1991, Duffy *et al*. used experimentally dehydrated and cremated remains to isolate dental pulp cells for sex chromatin analysis.[9]

Barr body can be observed with most of the nuclear stains such as hematoxylin and eosin (H and E), thionine, Papanicolaou, Feulgen, cresyl-violet, giemsa, aceto-orcein, and under fluorescence such as acridine orange (AO).[2]

Aims

Thus, the present study was undertaken to demarcate human dental pulp as an important identification tool of sex in FO, to evaluate the time period till which sex can be determined from pulp tissue using three stains H and E, Feulgen, and acridine – orange under fluorescence so as to estimate the best stain for visualization of Barr body and to assess the intraobserver variability for Barr body.

Materials and Methods

This study was conducted in the Department of Oral and Maxillofacial Pathology and Microbiology, I.T.S Centre for Dental Studies and Research, Muradnagar, Ghaziabad, Uttar Pradesh.

90 healthy pre-molars and molars in adult patients were divided into three groups; 45 males and 45 females and stored at normal room temperature for 15 days, 1 month, and 2 months. The pulp was obtained through normal access preparation on occlusion surface and using standardized k-files. The tissue was immediately stored in 5% formalin solution for 24 h after which the tissue was stained for H and E, Feulgen, and AO. These sections were systematically observed using a binocular microscope CX21 (OLYMPUS) and fluorescent microscope CX21 (MODIFIED) with ×100 magnification and analyzed to evaluate the mean number of cells positive for Barr body out of 100 random cells from five different fields.

Inclusion criteria

For teeth

Only healthy, normal teeth extracted due to periodontal disease or orthodontic purposes were considered.

For assessment of Barr bodies

Only those bodies which were situated at the periphery of the nucleus was considered to be a Barr body because as stated by Klinger *et al*. in 1962, human mucosal nuclei has high occurrence of other non-sex specific bodies, which lie freely in the nucleoplasm so can be confused with Barr body, also maximum studies show the occurrence of Barr body at periphery in human nuclei.

The usual number of Barr body usually present per cell is 0-1 in normal karyotypes,[8] thus, number of cells positive for Barr body was analyzed in the present study.

The normal distribution was confirmed from visualization of the collected data. Following this, descriptive statistics, including mean values, standard deviations, ranges (maximum and minimum), were calculated for each variable. The resulting data were analyzed using SPSS software. Data have been expressed as mean and standard deviation. The different variables were differentiated and analyzed using one-way ANOVA test followed by *post-hoc* analysis by Bonferroni method as all samples are independent in three variable time period.

The significance, i.e. $P < 0.05$ was considered to be significant. Beside this cut points/threshold values were calculated by applying area under the curve analysis (receiver operating characteristic [ROC]), the qualitative data were compared by linear coefficient depicting the trend in various stains and parameters under variable time period.

The agreement analysis was seen by applying interclass coefficient for quantitative and Kappa analysis for qualitative assessment as the observations were subjective.

Results

The female sample cells taken from dental pulp tissue were stained positive for Barr/chromatin bodies. Male sample cells would be stained negative or <3% for Barr/chromatin bodies (Figures 1-3). The three stains under variable time period showed a significant decrease in the mean number of cells positive for Barr body in the female sample with $P = 0.000$ in all the groups (Table 1).

ANOVA test and Bonferroni analysis were applied to test the level of significance which was found to be statistically not significant ($P < 0.05$) (Table 2).

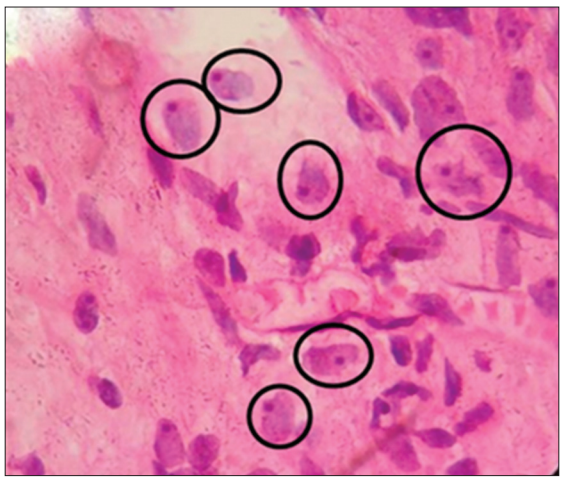

Figure 1: Cells stained positive in female samples for Barr body under hematoxylin and eosin stain.

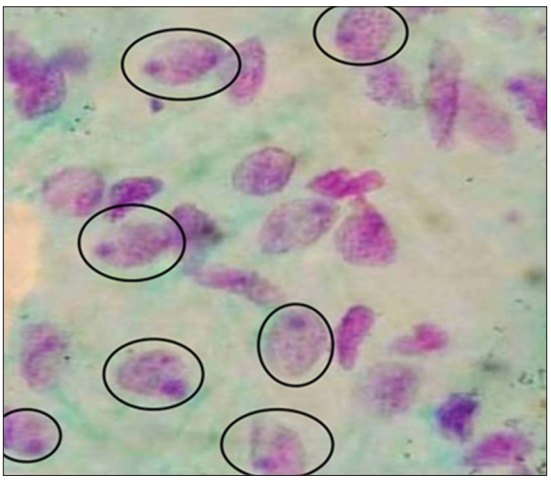

Figure 2: Cells stained positive in the female sample for Barr body under Feulgen stain.

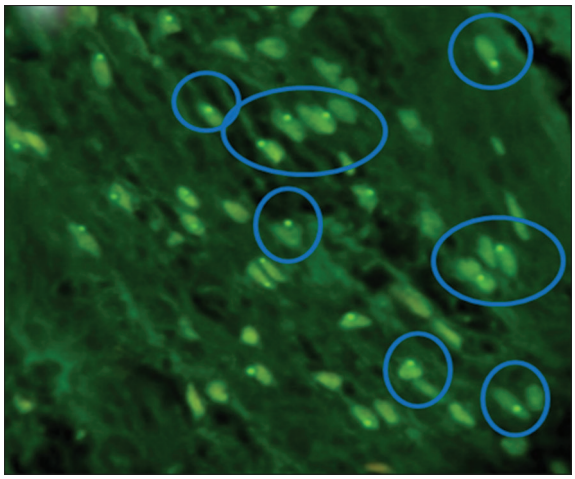

Figure 3: Cells stained positive in female samples for Barr body under acridine orange.

Different stains under variable time period showed not a significant decrease in the mean number of cells positive for Barr body in male sample with a $P = 0.599$ at 15 days, 0.650 at 1 month, and 0.739 at 2 months and under H and E, P value was found to 0.572, under Feulgen it was 0.454, and under AO, P value was 0.426 (according to Observer 2).

Cutoff points and their sensitivity and specificity under two stains for sex determination in different stains using Area under curve analysis (ROC analysis) were analyzed. The cutoff points of 7.5 under H and E, 7 under Feulgen, and 6.2 under AO, gave a sensitivity and specificity of 100% concluding that under any of the three stains samples showing more than 6 cells positive for Barr body could be considered as a female sample with 100% sensitivity and specificity (Table 3).

Discussion

Tooth being a source of pulpal tissue can be used for sex determination as pulp tissue is sequestered in hard tissue casing

Table 1: Comparative assessment showing mean number of cells positive for Barr body in female's samples (Observer 1) at variable time period in different stains.

Stains	At 15 days	At 1 month	At 2 month
H and E	51.4±4.01	31.6±3.44	16.8±2.42
Feulgen	37.33±3.77	21.33±4.89	13.86±2.89
Acridine orange	23.2±3.03	18.53±2.09	10.3±2.09
*F value	226.35	52.99	25.26
**P value (different stains at same time period)	<0.000 (H.S)	<0.000 (H.S)	<0.000 (H.S)
*F value	400.81	138.80	106.29
**P value (same stains at variable time period)	<0.000 (HS)	<0.000 (HS)	<0.000 (HS)

*ANOVA test, **P<0.05, ***HS: Highly significant, H and E: Hematoxylin and eosin

Table 2: Comparative assessment showing mean number of cells positive for Barr body in male's samples (Observer 1) at variable time period in different stains.

Stains	At 15 days	At 1 month	At 2 month
H and E	2.0±1.56	2.06±1.75	2.2±1.82
Feulgen	2.26±1.90	1.93±1.48	2.0±1.89
AO	1.66±1.23	2.2±1.14	1.06±1.33
*F value	0.519	0.436	0.305
**P value (different stains at same time period)	0.599 (NS)	0.650 (NS)	0.739 (NS)
*F value	0.567	0.804	0.872
**P value (same stains at variable time period)	0.572 (NS)	0.454 (NS)	0.426 (NS)

*ANOVA test, **P<0.05, ***NS: Not significant, H and E: Hematoxylin and eosin, AO: Acridine orange

Table 3: Cutoff points and their sensitivity and specificity under two stains for sex determination in different stains using AUC analysis (ROC analysis).

Stains	Cut point	AUC	Sensitivity (%)	Specificity (%)
H and E	7.500	1.00<0.000	100	100
Feulgen	7.000	1.00<0.000	100	100
Acridine orange	6.250	1.00<0.000	100	100

AUC: Area under the curve, H and E: Hematoxylin and eosin, ROC: Receiver operating characteristic

where it is buffered from the detrimental effects of impact trauma and heat. In situations where desiccation of pulp tissue is rapid, its cells become firmly embedded in the dried fibrous pulp matrix, and thus can be used for identification of sex.[10]

Human dental pulp been an excellent source of DNA can be a source of biological evidence for identification of an individual and which means the comparison of genetic material such as DNA.[7]

Pulp tissue has been used for sex determination from polymerase chain reaction (PCR) analysis. Another advanced technique that has used the dental pulp for sex determination is the amelogenin protein or AMEL identification.

An intermediate and less costly procedure is the histological and cellular techniques. One such histological technique is demonstration of nuclear sex. The various methods for the demonstration of nuclear sex are karyotyping, fluorescent body (Y-chromatin), Davidson body in the polymorphonuclear leukocytes, and Barr bodies (X-chromatin). However, the study of Barr bodies analyses has an advantage over other techniques due to simple staining techniques and its less time consumption.[8] Barr chromatin or Barr bodies and sexual identification methods have been described in various tissues,[9] including the dental pulp.[7] It is presented as a rapid technique and is easily implemented due to requirement of simpler equipment in contrast to techniques such as PCR and loop-mediated isothermal amplification method.[5]

In situation where tooth is a sole evidence at a crime scene, sex determination from tooth by Barr body analysis is considered to be a reliable method as dental pulp,[5] obtained from teeth contains fibroblasts which is an excellent source of DNA in the form of Barr body.[8] The present study showed the number of cells positive for Barr body in female samples under three different time periods and three different stains by two different observers.

Barr bodies were best identified under H and E stain at a time period of 15 days (51.4% cells positive) followed by Feulgen (37.33%/cells) and least under AO (23.2% cells). The overall values under different stains and time periods and the agreement between the two observers was found to be significant ($P < 0.05$) (Table 1).

The number of cells positive for Barr body in male samples were best identified under Feulgen stain at a time period of 15 days (2.53% cells positive) followed by H and E (2%/cells) and least under AO (1.87% cells). The overall values under different stains and time periods and the agreement between the two observers was found to be significant ($P > 0.05$) (Table 3).

The mean and standard deviation for each stain and time period was statistically calculated and was found to be noteworthy for determination of sex from Barr body count from female pulp tissue sample.

The Barr body was analyzed in a human oral cavity in 1956 when Hermann and Davis analyzed oral smears of 100 persons for Barr body. The results showed that 84% of the males had an incidence of the sex chromatin of 0-2%, while the females showed an uniform distribution between 10% and 32%.[4]

Galdames *et al.*, in 2010 conducted a study on histological sections of human dental pulp using H and E stain and found out that the mean of Barr body - positive cells was 20.4 (SD 0.44) in female samples. There was no Barr body - positive cells in preparations of male subjects.[6]

In 2004, Das *et al.*, examined pulp tissue smears for determination of the sex of an individual. They reported that 24.92% of women pulp cells were positive for Barr body observation and that with an increase in post-mortem time number of Barr bodies decreased, and sex identification was possible till 4 weeks. They also found that 36.5° of temperature is suitable for Barr body study and with further increase in temperature, the number of Barr bodies decreased.[4,8]

Reddy *et al.* in 2012 examined mucosal samples stained with AO to assess confocal microscopy for the detection of Barr body positive cells. The authors found out that female sample showed 18-72% cells showing Barr body positive cells whereas male samples showed 1-3%.[11] Our results were similar to Duz *et al.* studies in females and Reddy *et al.* studies for males.[6,11]

The time period up to which the determination of sex from Barr body analysis in human dental pulp varies by different authors. Duffy *et al.* in 1991 examined human dehydrated pulps from extracted teeth to assess sex chromatin from fibroblasts in artificially mummified and heated pulp tissue. The authors found that at room temperature there is prolonged stability of sex chromatin, i.e. up to 1 year with a frequency of 37-75% in females and 9-28% in males and in human molars heated at 100°C for 1 h, sex could be determined up to 1 year with a frequency of 40.2% Barr bodies in females and 13.6% in males. The authors concluded that sex chromatin is shown to preserve in dehydrated pulps and mummified pulp for up to 1 year and that the human pulp tissue retains sex diagnostic characteristics when heated to 100° for up to 1 h and these findings can be implicated in actual forensic situations.[10]

The second phase of present study estimates the reliability of time period up to which the sex determination can be made which revealed that sex can be determined from Barr body analysis up to 2 months under all three different stains by both the observers with average number of cells positive for Barr body been 17.13% under H and E followed by 13.93% under Feulgen and 10.3% under AO at 2 months of time period with

overall significant values in all three groups ($P < 0.005$ as these values under 2 months were in accordance with the study of Davis and Herman (1956) who stated that sex chromatin frequency varies from 10% to 32% in female mucosal smears.[4] Although Barr bodies were found in dental pulp samples, details regarding the post-mortem time necessary for a proper identification are varied.

Seno and Ishiz indicated that the corpuscle can be identified up to 5 months after death, whereas Whittaker *et al*. and Das *et al*. indicated it to be 5 and 4 weeks post-mortem, respectively. In another study by Ionesy (1980), who stored teeth at room temperature for 1 year and identified a reduction in the ability of sex chromatin staining compared with freshly extracted teeth.

Seno *et al*. in 1977 found that in 25 cm teeth buried in mud and sand in the open, sex can be determined in 100% of cases after 1 month; however, followed by a reduction of 20% after 3 months due to cellular decomposition. On the contrary, there was deterioration of cellular structure seen in teeth at 3 days when immersed in running water. Ionesy (1980)[12] extracted and analyzed the dental pulp, refrigerated to −4°C to −8°C for 15, 30, and 40 days, concluding that it is possible to determine the sex till 30 days.[4]

Our studies were found to be in accordance with Seno *et al*. who stated that there is 20% decrease in number of cells positive for Barr body after 3 months.

This study was first of its kind where the cutoff points were calculated to calculate the specificity and sensitivity of the three stains used. The cutoff points of 7.5 under H and E, 7 under Feulgen, and 6.2 under AO gave a sensitivity and specificity of 100% concluding that if any of the three stains samples showed more than six cells positive for Barr body that sample could be considered as a female sample with 100% sensitivity and specificity.

The present study shows the extent of interobserver agreement between the two observers for Barr body count under various stains and different time periods which was found to be best under H and E with a 98.62% agreement followed by Feulgen stain with an agreement of 97.61% and least in AO with an agreement of 93.29% in females samples. The agreement between the observers was found to be significant in all three stains. In male samples, the agreement was found to be best under Feulgen with an agreement of 71.34% followed by AO with an agreement of 58.06% and least under H and E with an agreement of 44.59%. The agreement between the observers was found to be non-significant in all the three groups.

On assessing the interobserver variability of two observers in each of the three stains under three different time period in the present study, an overall significance was found in all female samples ($P < 0.05$) and overall non-significant results were observed in male samples.

Although the interobserver agreement between the two observers under AO stain was lowest ever, the interobserver agreement was significant with an agreement ranging from 88.02% of Observer 1 to an agreement of 96.38% of Observer 2. The least variability in range between the two observers was seen under H and/E hence validating that the interobserver variability is least under H and E stain. Interobserver agreement has been studied by Datar *et al*. (2013) in cytological smears for Barr body between two observers. The authors found out an agreement of 95% and 98% in all female samples.[13]

In another study by Basavaraj *et al*. 2011, interobserver variability was between two observers for analysis of Davidson body from mucosal smears. The authors found out Davidson body in all the females samples with mean values of 3.6% and 3.7% concluding it to be a reliable method for sex determination.[10]

The third phase of the study was carried out to assess the varying methods of visualizing Barr bodies in human dental pulp, i.e., using H and E, Feulgen, and AO stains (Figures 1-3). H and E stain was used due to its ease in use and availability. Feulgen stain is considered to be specific for Barr body and a better stain for sex chromatin as it is DNA specific and also is negative for nucleus and Nissl bodies which can be confused with Barr body/sex chromatin. AO was used because of its high degree of specifity and accuracy.

The results showed that quantitative assessment of Barr body was best under H and E with a mean and standard deviation of 51.4 ± 4.01 at 15 days, 31.6 ± 3.44 at 1 month, and with a mean and standard deviation of 16.8 ± 2.42 at a time period of 2 months for Barr body, followed by Feulgen with a mean and standard deviation 37.33 ± 3.77 at 15 days, 21.33 ± 4.89 at 1 month, and 13.86 ± 2.89 at 2 months and least under AO with a mean and standard deviation of 23.2 ± 3.03 at 15 days followed by value of of 18.53 ± 2.09 at at time period of 1 month and 10.3 ± 2.09 at a time period of 2 months. The overall values under all three stains were significant ($P < 0.05$).

The qualitative assessment of the three stains was done by scoring four different parameters for each stain at each time period which was the first study of its kind. The Kappa test was applied on the scoring of two observers over the parameters of staining intensity, nuclear outline, Barr body outline, and identification of nuclear disintegration. The maximum agreement between the two observers was found to be in Barr body outline clarity under AO stain which was 0.963, followed by identification of Barr bodies which was seen best under H and E with a Kappa value of 0.891, staining intensity was seen

best under AO with kappa value of 0.829, and nuclear outline was seen best under Feulgen with a kappa value of 0.857. There was almost perfect to very good agreement observed between the two observers for different parameters observed under different stains.

The parameter of Barr body outline was best observed at a time period of 15 days under Feulgen stain, followed by AO and least observed under H and E. However, at a time period of 1 month this parameter was best analyzed by both Feulgen and AO showing good scoring followed by H and E stain. Even at the time period of 2 months, Barr body outline was best observed under Feulgen and AO.

The parameter of nuclear outline clarity was best observed with a good scoring at a time period of 15 days under Feulgen stain, followed by AO and least under H and E. However, at a time period of 1 month, this parameter was best analyzed under AO showing good scoring followed by Feulgen and H and E stain with good scoring. At a time period of 2 months, Barr body outline was best observed under Feulgen, followed by H and E and AO.

The parameter of overall staining intensity was best observed with a good scoring at a time period of 15 days under Feulgen stain, followed by H and E, and least under AO. However, at a time period of 1 month, this parameter was best analyzed under Feulgen showing good scoring followed by AO and H and E stain showing good scoring.

At a time period of 2 months, Barr body outline was best observed both under Feulgen and AO followed by H and E.

The parameter of identification of nuclear disintegration was best observed with a good scoring at a time period of 15 days under AO stain, followed by Feulgen, and least under H and E. However, at a time period of 1 month, this parameter was best analyzed under both Feulgen and AO, showing good scoring followed by H and E showing good scoring. At a time period of 2 months, Barr body outline was best observed under AO in 5 out of 15 cases followed by Feulgen and least in H and E.

This analysis concluded that different parameters can be best visualized under different stains and at different time period. Our results showed that Feulgen is a better stain then H and E in relation to the qualitative assessment of Barr body which is in accordance with study by Moore and Barr (1955) who carried out a study of sex chromatin in benign tumors in man and used H and E and Feulgen stain for their study. They found out that Feulgen was a better stain for sex chromatin as it is DNA specific and also is negative for nucleolus and Nissl bodies which can be confused with Barr body/sex chromatin.[10] In one of the other studies, Moore and Barr in 1957, in malignant tumors in man where again they found out that Feulgen stain was better than H and E as malignant tissues have multiple nucleoli, which can be confused with Barr body under H and E stain, however, Feulgen stain had been negative for nucleoli, thus proves to be more specific for Barr body/sex chromatin.[10]

AO on the other hand, being a fluroscent stain is said to have high degree of specificity and accuracy.

Conclusion

Thus, on the basis of the finding from our studies sex determination can be used by analysis of Barr body from human dental pulp tissue and can be implicated in FO with reliability only till a fixed time period of 2 months and at room temperature of 37°. After that there is a fall in average number of cells positive for Barr body as a result of post-mortem decomposition and degenerative changes. Thus, this technique can only be implied reliably and easy to perform the technique in that forensic situation where an individual tooth has been traced as evidence or where DNA amplification is not possible. However, in situations where long buried remains have been traced or under pressure or even in edentulous patients, this technique cannot be implicated.

References

1. Saxena S, Sharma P, Gupta N. Experimental studies of forensic odontology to aid in the identification process. J Forensic Dent Sci 2010;2(2):69-76.
2. Mittwoch U. Sex chromatin. J Med Genet 1964;1(1):50-76.
3. Barr ML, Bertram LF, Lindsay HA. The morphology of the nerve cell nucleus, according to sex. Anat Rec 1950;107(3):283-97.
4. Davis AM, Herrmann W. The determination of chromosomal sex by oral smears. Yale J Biol Med 1956;29(1):69-74.
5. Galdames IS, Roa I, Cantin M. Sex chromatin in dental pulp performance of diagnosis test and gold standard generation. Int J Morphol 2010;28(4):1093-6.
6. Galdames IS, Flores A, Roa I, Cantin M, Zavando D. Sex determination by observation of Barr body in teeth subjected to high temperature. Int J Morphol 2011;29(1):199-203.
7. Das N, Gorea RK, Gargi J, Singh JR. Sex determination from pulpal tissue. J Indian Acad Forensic Acad 2004;26(2):5.
8. Moore KL, Barr ML. The sex chromatin in human malignant tissues. Br J Cancer 1957;11(3):384-90.
9. Duffy JB, Waterfield JD, Skinner MF. Isolation of tooth pulp cells for sex chromatin studies in experimental dehydrated and cremated remains. Forensic Sci Int 1991;49(2):127-41.
10. Basavaraj BP, Chandrashekar M, Shashikala P, Vijaykumar BJ. Reliability of Davidson body to determine the nuclear sex of the individual: Interobserver variability between pathologists. Indian J Forensic Med Toxicol 2011;5(2):11-3.

11. Reddy DS, Sherlin HJ, Ramani P, Prakash PA. Determination of sex by exfoliative cytology using acridine orange confocal microscopy: A short study. J Forensic Dent Sci 2012;4(2):66-9.
12. Ionesiy AG. On the possibility of teeth sexing by cytological proceeding. Sudebno-Meditsinkaia Expertizia, 1980;23:27-8.
13. Datar U, Angadi PV, Hallikerimath S, Kale AD. Cytological assessment of Barr bodies using aceto-orcein and papanicolaou stains in buccal mucosal smears and their sex estimation efficacy in an Indian sample. Acta Cytol 2013;57(5):516-21.