ORIGINAL ARTICLE

Gender determination from pulpal tissue

Manisha M. Khorate,
Anita Dhupar[1],
Junaid Ahmed[2],
Ajit D. Dinkar
Department of Oral Medicine, Diagnosis, and Radiology, [1]Oral Maxillofacial Pathology, Goa Dental College and Hospital, Bambolim, Goa, [2]Department of Oral Medicine and Radiology, Manipal College of Dental Sciences, Mangalore, Karnataka, India

Address for correspondence:
Dr. Manisha M. Khorate, Department of Oral Medicine, Diagnosis, and Radiology, Goa Dental College and Hospital, Bambolim - 403 202, Goa, India. E-mail: khoratemanisha@rediffmail.com

Abstract

Objective: To evaluate the diagnostic performance of X (Barr body [BB]) and Y (F body [FB]) chromosomes observed in dental pulp tissue for gender determination of an individual. **Materials and Methods:** The study was carried out on 100 teeth (50 male and 50 female), which were indicated for extraction. The teeth were sectioned at various intervals (within 12 h to 49 days post-extraction), and the pulpal tissue was obtained. Two slides for each pulp tissue were prepared, one for 5% Quinacrine dihydrochloride stain (FB) and the other for Hemotoxylin and Eosin stain (BB). The slides were then observed under the fluorescent microscope for FB and under the light microscope for the BB respectively. **Results:** Gender determination from human pulp is possible up to 7 weeks. The percentage of FB and BB decrease gradually as the time interval increases. Further, an equation was derived from the data based on the canonical discriminant function coefficients. **Conclusion:** The determination of gender based on a joint search for the presence or absence of X (BB) and Y (FB) Chromosome is a reliable and cost-effective technique.

Key words: Barr bodies, F-bodies, forensic odontology, gender determination, pulpal tissue

Introduction

Owing to the scientific advancement and technology a drastic change is seen in the criminal scenario, road traffic/rail/aviation accidents, mass disasters, wars; and the bodies which are found are beyond recognition. If the whole skeleton is available, the accuracy of sex determination may be of the order of ninety-eight percent as the pelvic bones alone provide accuracy of around 95%.[1] However, the problem arises only when segments of the body or cranial cavity or isolated teeth are obtained from the site. In such scenario, sex identification becomes the most intriguing, complex, and sometimes controversial challenge.

Teeth, being the hardest substances in the human body, potentially can survive most of insults and consequences encountered at death and during decomposition. Tooth pulp remains protected in a hard tissue casing made up of dentin and enamel. The present study was conducted on pulp tissue derived from individuals belonging to age range of 16-55 years. The aim of this study was to evaluate the diagnostic significance of X (Barr body [BB]) and Y (F body [FB]) chromosomes observed in dental pulp tissue for gender determination of an individual, and to study the influence, if any, of age and the time interval on the demonstration of the sex chromatin (i.e., the percentage of BB or FB).

Access this article online	
Website: www.jfds.org	Quick Response Code
DOI: 10.4103/0975-1475.132540	

Materials and Methods

Sample collection

This study was conducted on a total of 100 (50 male and 50 female) teeth, which were extracted following orthodontic, pulpal or periodontal indications. Thus, along with the healthy teeth, teeth with exposed pulp, or with attrition/abrasion were also included. The sample was divided in ten groups with ten teeth in each group (5 male and 5 female cases). The teeth were kept at room temperature without any preservation and were processed at various intervals as per schedule of the study [Table 1].

Table 1: Groups as per time interval

Group	Time interval
1	0-12 h
2	12-48 h
3	2-5 days
4	5-7 days
5	7-14 days
6	14-21 days
7	21-28 days
8	28-35 days
9	35-42 days
10	42-49 days

Sectioning of teeth

A longitudinal groove was made with the help of an air-rotor handpiece using a cylindrical diamond bur, on the lingual surface of each tooth and was broken by striking with a chisel and mallet, at the junction of the crown and the root. The whole of the pulp tissue was separated out of the pulp cavity with the help of a probe and a #10 broach. The pulp tissue was washed in normal saline to remove any debris or calcified particles.

Staining pulpal cells

The pulpal tissue was then kept immersed in a fixative (3 Methanol: 1 Glacial acetic acid) for 24 h. It was then transferred to a mortar, few drops of 20% acetic acid were added to soften the tissue. Separation of the cells was carried out by crushing. The suspension thus obtained was centrifuged (REMI RM12C micro centrifuge) for 5 min at 6000 rpm. The supernatant was used to obtain a monolayer on chilled microscope slides, i.e., a single drop was dropped by a pipette, and was spread by a blunt ended glass rod to get a homogenous population of cells. Two smears were made from each suspension of the specimen. These slides were then air-dried and were fixed with a few drops of absolute methanol.

One slide was stained with Harris's (H and E) Hematoxylin and Eosin stain to study the BB. The slide was examined under oil immersion lens (×100) of light microscope for the BB. It appears as a dark spot usually lying against the nuclear membrane in the females.[2]

The second slide was stained with quinacrine dihydrochloride for the study of Y chromosomes (FB).[3] After natural evaporation of the methanol, the slide was stained with 0.5% quinacrine dihydrochloride for 20 min. The slide was then washed in distilled water thrice and was kept in McIlvaine's buffer (A 0.2 M solution of dihydrate salt of disodium hydrogen phosphate was prepared by dissolving 22.71 g of the solute in 800 mL of distilled water. A 0.1 M solution of monohydrate salt of citric acid was prepared by dissolving 10.50 g of the solute in 500 mL of distilled water. 0.2 M solute of dihydrate salt of disodium hydrogen phosphate was slowly added to 500 mL of 0.1 M citric acid solution, and the pH of the buffer was adjusted to 5.5) for 3 min. The slide was then washed with 0.4 g/L magnesium chloride for 10 min. Then a drop of glycerol was added and the slide was covered with a cover slip. The cover slip was then sealed with nail varnish. The slide was then observed with Olympus Fluorescent microscope BX 53 (SN OL399 T2 Tokyo, Japan) under oil immersion in dark field at an objective of ×40 and ×100, by Blue-violet (BV) exciting method (emitting a blue-violet color mainly at 4.047 and 4.038Å). Only those cells which contained the characteristic Y chromatin or FB, i.e., a brightly fluorescent spot attached to the nuclear membrane were counted as positive cells while those, which did not show any such fluorescent spot were labeled as negative.

Each slide was scanned for one hundred cells. An individual cell with fluorescent FB (quinacrine dihydrochloride stain) was counted. Similarly in H and E, stained slides an individual cell with visible BB was counted.

Results

In each slide hundred cells were observed. The presence of a cell with fluorescent FB was considered positive for male [Figures 1 and 2], and with visible BB was considered positive for female [Figures 3 and 4]. The mean, standard deviation and range of FB and BB were calculated for males and for females [Table 2].

In females, the mean percentage of BB was 34.6 ± 5.41 in the 0-12 h group. The percentage decreased with increasing post-extraction period and fell to 13.20 ± 2.39 over the period of 42-49 days, i.e., 7 weeks. The percentage of FB was in the range of 0-8 with mean to be 2.56 ± 2.31. In males, the mean percentage of FB was found to be 59.8 ± 6.06 in the freshly extracted teeth which decreased gradually with increasing time interval. The range of BB in male subjects was found to be 0-14 with a mean of 5.16 ± 3.05. These figures were found to be statistically significant with *P* value 0.000, [Table 3]. The range of FB in male teeth was found to be 7-69% with an overlap of 2%, and the range of the BB in female teeth was found to be 10-41% with an overlap of 5%. Canonical discriminant function coefficients were derived from these data:

$Y = (-0.927) + (\text{Number of FB}) \times (-0.05) + (\text{Number of BB}) \times 0.14$ (Male: $Y < 0$; Female: $Y > 0$)

Where Y provides us the discriminant score of any person, whose FB and BB scores are known. In case of males, the value of Y will be around −1.752, and in case of females it will be around +1.752. Thus, by substituting the number of FB and BB in the equation the sex of the individual to whom the tooth belonged could be determined. With the help of this model, 50 women were correctly classified as women and 50 men were correctly classified as men thus giving a total of 100% correct predictions [Table 4].

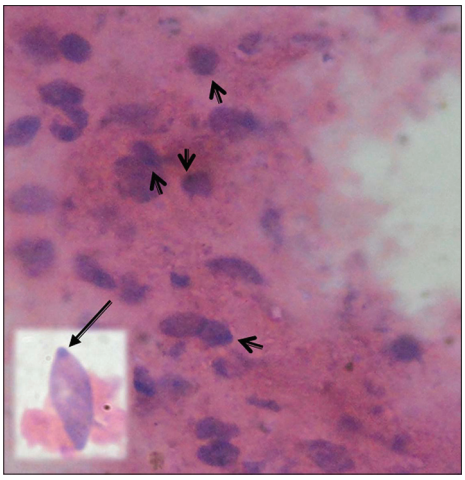


Figure 1: Photomicrograph of histologic section of female dental pulp stained with H and E, (original magnification, ×100). The arrows indicate the characteristic condensation of sexual chromatin (Barr body)

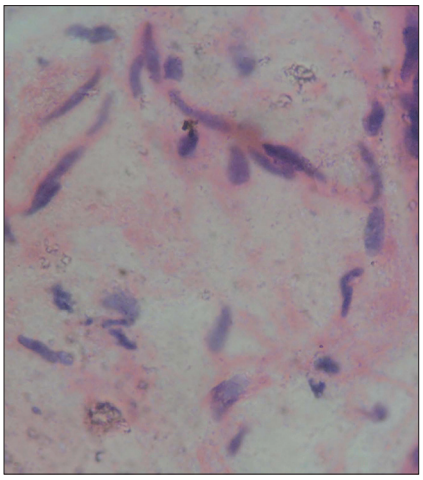


Figure 2: Photomicrograph of histologic section of male dental pulp stained with H and E, original magnification, ×100; showing absence of Barr bodies

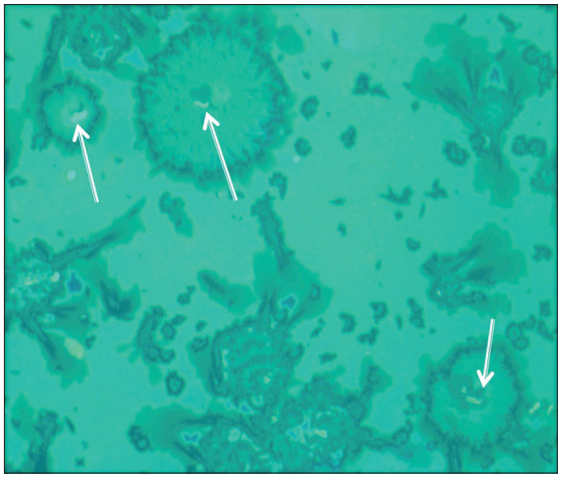


Figure 3: Photomicrograph of male dental pulp smear stained with Quinacrine dihydrochloride, (original magnification, ×100). The arrows indicate the nucleus with fluorescent F bodies

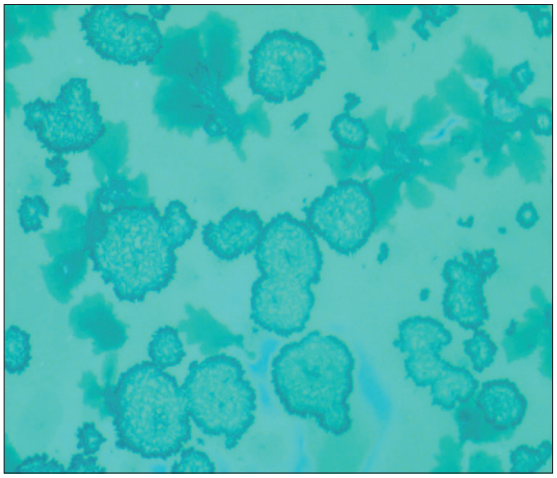


Figure 4: Photomicrograph of female dental pulp smear stained with Quinacrine dihydrochloride, (original magnification, ×100); showing absence of fluorescence

Table 2: Incidence of sex chromatin (F-body and Barr body) in male and female cases from the period 0-12 h to 42-49 days

Time interval	Males				Females			
	Number of F-bodies		Number of Barr-bodies		Number of Barr-bodies		Number of F-bodies	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
0-12 h	59.8±6.06	54-67	4.00±1.23	2-5	34.6±5.41	27-41	5.00±1.58	3-7
12-48 h	60.0±8.74	46-69	3.40±1.1	2-5	27.80±3.11	25-32	2.60±2.79	0-6
2-5 days	40.4±6.88	31-48	5.60±3.05	2-7	24.20±3.2	20-28	6.00±1.58	4-8
5-7 days	37.60±8.4	27-46	5.8±2.17	4-9	20.00±1.88	18-23	4.20±1.64	2-6
7-14 days	27.40±5.07	24-36	7.20±5.63	0-14	22.40±4.62	18-30	1.40±1.34	0-3
14-21 days	20.20±8.13	13-30	6.2±2.17	3-8	15.40±2.51	13-18	1.20±1.30	0-3
21-28 days	22.4±2.6	20-26	8.6±2.61	6-12	14.20±2.17	11-16	1.00±0.71	0-2
28-35 days	16.00±2.73	12-19	5.00±1.88	3-8	15.20±1.64	14-18	2.00±1.58	0-4
35-42 days	15.00±2.34	12-17	2.20±0.84	1-3	14.20±2.95	11-19	1.80±1.79	0-4
42-49 days	8.00±1.23	7-10	3.60±2.88	0-7	13.20±2.39	10-16	0.40±0.55	0-1
Mean±SD	30.68±18.32	7-69	5.16±3.05	0-14	20.12±7.4	10-41	2.56±2.31	0-8

Discussion

Sex determination in forensic odontology can be done using tooth alone. Various features of teeth, such as morphology, crown size, and root length; Deoxyribonucleic acid (DNA) amplification, X/Y chromosomes from the pulp tissue etc.,

can assist a forensic odontologist in gender determination of the remains. Due to continuous wearing of the teeth along occlusal and proximal surfaces, the accuracy and reliability of the sex determination based on the morphology of the tooth is questionable, especially, after 22-25 years of age. Sex identification by DNA amplification shows high accuracy; however, requires more complex work and time to complete the analysis and needs special equipment.[4] Study of X and Y chromosomes in the pulpal cells, which are not undergoing active division, is considered to be an easily accessible, less expensive and reliable method.[5]

The first demonstration of the sex chromatin body was by Barr and Bertram.[6] A normal human female has only one BB per somatic cell while a normal human male has none. Moore, Graham and Barr described a simple method of chromatin testing in human skin biopsies and demonstrated the practical value of the test as a guide to chromosomal sex in ambisexual subjects.[7] It is seen as an intensely stained body lying against the nuclear membrane and can be observed with most of the nuclear stains, such as H and E, Papanicolaou, Feulgen, cresyl violet, aceto-orcein, carbol-fuchsin, and fluorescence.[8] BB and sexual identification method have been described in various tissues as bone cells,[9] cells of retina,[10] oral mucosal cells[11] including dental pulp.[12,13]

Caspersson *et al*., while using the fluorescent dyes to give banding of chromosomes, noticed particularly a bright fluorescence of the long arm of the Y chromosome.[14] Zech described a simple technique to demonstrate the Y chromosome using the quinacrine mustard.[15] The alkylating agent such as quinacrine, accumulate in DNA regions rich in guanine and is responsible for the bright fluorescence of the Y chromosome. Bobrow and Vosa used quinacrine dihydrochloride (Atebrin) to demonstrate the Y chromosome in the interphase nuclei as a characteristic fluorescent body; thus, providing the ideal counterpart of the BB or X chromosome.[3]

Determination of FB and BB

In the present study, the mean percentage of male pulp cells showing FB was found to be 30.68 ± 18.32% and in females 2.56 ± 2.31%, which was found to be almost similar to the figures obtained by the other studies. As far as BB are concerned the mean percentage obtained in the present study was 20.12 ± 7.4% in females and 5.16 ± 3.05% in males, which was similar to that reported by Das *et al*.[12] [Table 5].

Effect of time interval

In our study, we were able to differentiate sex with certainty up to 7 weeks, though the percentage of FB and BB gradually decrease as the time interval increases [Graphs 1 and 2]. Highest level of FB in our study was 69 and of BB was 41, which was seen in the time period of 0-48 h. There was a remarkable fall in the BB after 48 h, and a drop was seen in the count of BB after 14 days of the time period [Graph 1].

Table 3: Independent *T* test

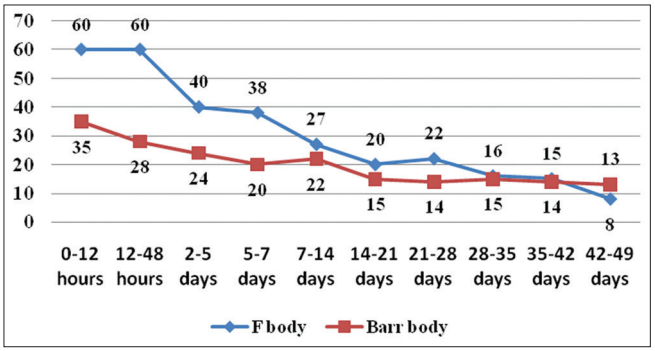
Sex chromatin	*T* test for equality of means			
	T	Df	*P* value	Mean difference
F-bodies	10.766	50.550	0.000	28.120
Barr-bodies	–13.214	65.211	0.000	–14.960

Table 4: Contingency table used for testing accuracy

	Gold standard		
Test	Female	Male	Total
Female	50	00	50
Male	00	50	50
Total	50	50	100

Table 5: Comparison of the incidence of the sex chromatin (Barr bodies and F-bodies) in the present study with that of the other studies

Study	Number of Barr-bodies in females		Number of F-bodies in males	
	Mean±SD	Range	Mean±SD	Range
Present study	20.12±7.4	10-41	30.68±18.32	7-69
Das *et al*.	24.92±3.74	0-52	35.64±6.59	0-82
Suazo *et al*.	20.4±0.44	-	-	-
Yunis and Chandler	30	15-40	-	-
Veeraraghavan	-	-	33.43	5-71
Seno and Ishizu	-	-	30	-
Whittaker *et al*.	-	-	17.4-51.2%	-
Duffy *et al*.	9-28%	-	37-75%	-



Graph 1: Effect of time interval on sex chromatin (F and Barr bodies)

As per Das *et al*.[12] and WhittakerWhittaker *et al*.,[16] the accuracy of sex determination was 100% up to 4 weeks and 5 weeks respectively. With a decreased accuracy, the sex could be determined up to 10 weeks (Whittaker *et al*.), which is in contrast to Seno and Ishizu[17] and Veeraraghavan *et al*.,[15] who in their respective studies could observe fluorescent FB (5-22 bodies) even up to 5 months. Ionesiï[18] found that there was a reduction in the ability of sex chromatin staining in cases of teeth, which were stored at room temperature for 1 year, as compared with that of the freshly extracted teeth. Dange *et al*.[19] stored samples of permanent teeth for 4 years and still could discriminate the sex through

their cells, when analyzed. The discrepancy between the various studies can be due to geographical factors as weather, temperature, and humidity. Das *et al*.[12] made an attempt to study the effect of environment, i.e., the role of temperature and humidity on pulp tissue after the extraction of the tooth and concluded that the process of decomposition by bacterial action generally reduces the possibility of clear observation of chromatin.

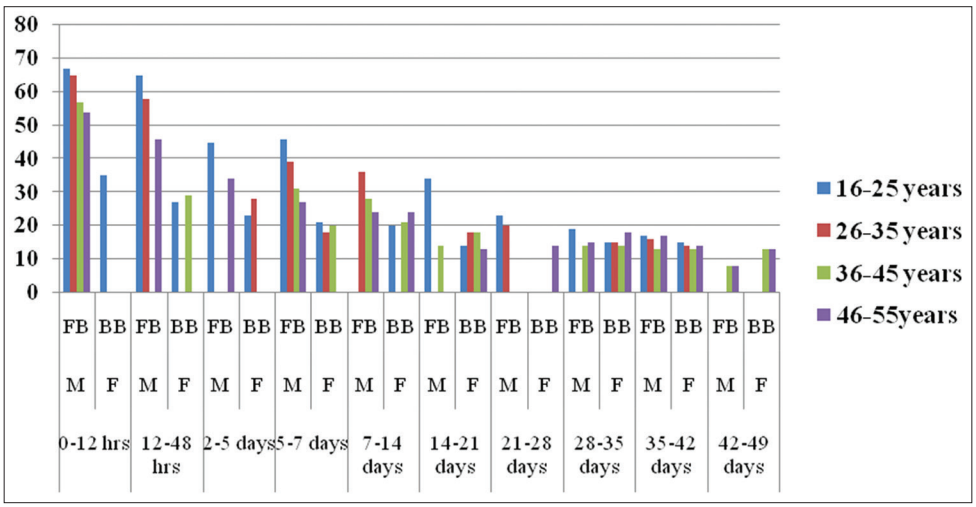
Effect of age of the subject on the percentage of the sex chromatin

The age of the subject did not show any significant effect on the percentage of FB observed in male-dental pulp and BB observed in female dental pulp [Table 6, Graph 2]. As the age increases the teeth undergo several regressive changes as attrition, abrasion, thermal/mechanical/chemical trauma. These factors have a certain effect on the tooth as deposition of secondary dentin, reversible/irreversible pulpitis. In the present study, along with the healthy teeth, teeth with exposed pulp, or with attrition/abrasion were also included. It was observed that the sex chromatin could be efficiently recognized and counted even in teeth with acute and chronic inflammatory processes, and also in those with the regressive alterations as in attrition and abrasion. These teeth did not give any discrepancy as in the percentage of FB or BB as compared to the healthy teeth in their respective age groups. In their respective studies, Barr *et al*.[20] and Larson and Knapp[21] have concluded that the Barr chromatin could be differentiated in polymorphonuclear neutrophils in teeth with acute and chronic inflammatory processes. In the present study, the percentage of FB has also proved to be unaffected by inflammation or regressive alteration.

Technique

In the study of BB, Suazo GI[13] embedded the pulp tissue in the paraffin wax block to obtain the histological sections of 5 μm thickness and examined for the BB. As per Suazo *et al*. the appearance of BB in histologic section may be less frequent as the BB tend to hide behind or in front of the nucleoplasm and may not be very evident. In the present study, the method given by Das *et al*.[12] was followed wherein the cells are floating freely in the supernatant. The mean percentage of BB did not show much discrepancy among the three studies [Table 5].

To consider a positive Barr chromatin test, one cell in the sheet has to form chromatin condensation at the nuclear periphery, which gets stained intensely [Figure 1]. Certain cells, wherein the intensely stained chromatin condensation fails to lie against the nuclear membrane, tend to get counted as negative for BB. Unlike FB, which can be easily identified by their fluorescence, identification and counting of BB seems to be difficult. Thus, it is stated that the frequency of the BB will be definitely more per hundred cells than reflected in our results.



Graph 2: Effect of time interval and age on sex chromatin (FB - F body; BB - Barr bodies)

Table 6: Effect of age on the incidence of sex chromatin (FB and BB)

Age range in years	0-12 h		12-48 h		2-5 days		5-7 days		7-14 days		14-21 days		21-28 days		28-35 days		35-42 days		42-49 days	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB
16-25	67	35	65	27	45	23	46	21		20	34	14	23		19	15	17	15		
26-35	65		58			28	39	18	36			18	20			15	16	14		
36-45	57			29			31	20	28	21	14	18			14	14	13	13	8	13
46-55	54		46		34		27		24	24		13		14	15	18	17	14	8	13

M: Male, F: Female, FB: F body, BB: Barr body

Table 7: Barr body level abnormalities in certain conditions

Condition	Sex chromosomes	Number of Barr bodies
Normal male	XY	None
Normal female	XX	One
Trisomy X	XXX	Two
Turner's syndrome	X	None
Kleinefelter's syndrome	XXY	One

Limitations

BB is found only in those cases in which more than one X chromosome is present[22] and thus it is not found in male cells. In normal men, no BB are reported, and in 46XX women, one BB in cell nuclei is observed. However, in certain individuals with abnormal chromosomal levels [Table 7] gender cannot be correctly identified using the BB technique; for example 47XXY men will have a BB, and 47XXX women will have two.[23]

The procedure for quinacrine dihydrochloride staining is very technique sensitive, with each step right from the separation of pulp from the dental hard tissue until the counting of the brightly fluorescent spots attached to the nucleus. It is necessary to get a homogenous monolayer of cells from the suspension on the slide; thus, preventing the masking of the fluorescent FB by fluorescent debris or by another cell. Further, the bacteria, dead cells, and putrefied cellular debris may give false positive fluorescence particularly in case of female cells.[12,14]

Further, as the number of both FB and BB decrease with time interval [Graph 1] a cutoff value cannot be given either for FB or BB. Taking into consideration these limitations and the overlap in the count of X and Y chromatin, a combined search for the presence or absence of BB and FB should be made to establish the sex from human pulp tissue.

Conclusion

Gender determination from the human pulp is independent of the age of an individual. The percentage of FB and BB gradually decrease as the time interval increases. In certain disasters such as high impact accidents, explosions and fragmentation of thermal trauma, natural calamities wherein traditional methods like determination of sex from skeleton fail, this method based on dental pulp may give a more confirmatory result; as the teeth are a stable part of skeleton and the pulp tissue is well-protected.

Acknowledgments

We wish to thank Ms. Pallavi Nachinolkar Lecturer in Biostatistics, Department of Community Dentistry, Goa Dental College and Hospital, for the valuable assistance in the statistical analysis of the data.

References

1. Sweet D. Why a dentist for identification? Dent Clin North Am 2001;45:237-51.
2. Barr ML, Bertram LF, Lindsay HA. The morphology of the nerve cell nucleus, according to sex. Anat Rec 1950;107:283-97.
3. Pearson PL, Bobrow M, Vosa CG. Technique for identifying Y chromosomes in human interphase nuclei. Nature 1970;226:78-80.
4. Nogami H, Tsutsumi H, Komuro T, Mukoyama R. Rapid and simple sex determination method from dental pulp by loop-mediated isothermal amplification. Forensic Sci Int Genet 2008;2:349-53.
5. Veeraraghavan G, Lingappa A, Shankara SP, Mamatha GP, Sebastian BT, Mujib A. Determination of sex from tooth pulp tissue. Libyan J Med 2010;5:10.
6. Barr ML, Bertram EG. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature 1949;163:676.
7. Moore KL, Graham MA, Barr ML. The detection of chromosomal sex in hermaphrodites from a skin biopsy. Surg Gynecol Obstet 1953;96:641-8.
8. Duffy, J. An appraisal of the stability of sex chromatin and the H-Y molecule in forensic contexts. Thesis (M.A.), Simon Fraser University; 1991 http://www.sfu.ca/archaeology/graduate/theseslist/thesisduffyj.html.
9. Vernino DM, Laskin DM. Sex chromatin in mammalian bone. Science 1960;132:675-6.
10. Tepliz RL. Sex chromatin of cone cells of human retina. Science 1965;150:1828-9.
11. Dixon AD, Torr JB. Sex chromatin in oral smears. Br Med J 1956;2:799-800.
12. Das N, Gorea RK, Gargi J, Singh JR. Sex determination from pulpal tissue J Indian Forensic Sci 2004;26:50-4.
13. Suazo GI, Roa HI, Cantin LM. Sex chromatin in dental pulp. Performance of diagnosis test and gold standard generation. Int J Morphol 2010;28:1093-6.
14. Caspersson T, Zech L, Modest EJ, Foley GE, Wagh U, Simonsson E. Chemical differentiation with fluorescent alkylating agents in Vicia faba metaphase chromosomes. Exp Cell Res 1969;58:128-40.
15. Zech L. Investigation of metaphase chromosomes with DNA-binding fluorochromes. Exp Cell Res 1969;58:463.
16. Whittaker DK, Llewelyn DR, Jones RW. Sex determination from necrotic pulpal tissue. Br Dent J 1975;139:403-5.
17. Seno M, Ishizu H. Sex identification of a human tooth. Int J Forensic Dent 1973;1:8-11.
18. Ionesiĭ AG. Possibility of the cytological diagnosis of sex by the teeth. Sud Med Ekspert 1980;23:27-8.
19. Dange AH, Malvankar AG, Madiwale MS. Determination of the sex origin of teeth. Arch Kriminol 1978;162:115-9.
20. Barr ML. Dysgenesis of the seminiferous tubules. Br J Urol 1957;29:251-7.
21. Larson JS, Knapp SJ. Sexual dimorphism in beaver neutrophils. J Mammal 1971;52:212-5.
22. Parikh CK. Parikh's Text Book of Medical Jurisprudence and Toxicology. 5th ed. Bombay: Medicolegal Centre; 1990. p. 38-9.
23. Kelly TE. CIinical Genetics and Genetics Counselling. 2nd ed. Chicago: Year Book Medical Publishers; 1986. p. 125-58.

How to cite this article: Khorate MM, Dhupar A, Ahmed J, Dinkar AD. Gender determination from pulpal tissue. J Forensic Dent Sci 2014;6:107-12.

Source of Support: Nil, **Conflict of Interest:** None declared