ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 02 | February - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SEX DETERMINATION BY OBSERVATION OF BARR BODY & F BODY IN DENTAL PULP OF THE TEETH SUBJECTED TO DIFFERENT PHYSICAL CONDITIONS: A FLUORESCENT MICROSCOPIC STUDY

Dental Science

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ABSTRACT

Background: The importance of a single tooth for forensic analysis cannot be over-stressed in scenarios when the body has undergone post-mortem decomposition or mutilation beyond recognition. In forensic odontology, molecular techniques for sex determination include identification of Barr body in females & Fluorescent/ F body in males. However, researchers have a difference of opinion regarding the reliability of Barr bodies and F bodies in sex determination, when the compared for changes in temperatures & different physical conditions to which the teeth were subjected.

Aim: The present study was conducted to evaluate the reliability of Barr bodies & F bodies for sex determination in teeth subjected to different physical conditions and to assess the effectiveness of combined evaluation of Barr bodies and F bodies for sex determination.

Materials & method: The 90 freshly extracted teeth samples were divided into 3 groups- at room temperature, buried in garden soil for 15 days, and subjected to extreme heat. Two smears were made from cell suspension obtained from the dental pulp of each sample- one for acridine orange staining and second for quinacrine dihydrochloride staining. Each slide was studied under fluorescent microscope for demonstration of Barr bodies or F bodies.

Results: We observed that the females showed 6-36% Barr body-positive cells while males showed 0-9% Barr body positivity. It was seen that F body positivity in male samples was 8-37% and in females it was 0-2%. This indicates that Barr body and F body detection is reliable; however a combined evaluation is more accurate. These two fluorescent bodies reduce gradually, especially at high temperatures.

Conclusion: The present study put forth the notion that the efficacy of sex determination using dental pulp would increase if the samples were checked for both Barr bodies and F bodies.

KEYWORDS

Forensic odontology, Barr body, F body.

INTRODUCTION:

Sex, age, stature & ethnic background are the hallmarks of biological identity of an individual.[1] The standard methods of identification like visual recognition or fingerprint analysis are of no use when there is tissue destruction due to decomposition or charring.[2,3] The importance of a single tooth for forensic analysis cannot be over-stressed in scenarios when the body has undergone post-mortem decomposition, desiccation or mutilation beyond recognition.[4] In forensic odontology, sex determination can be done by morphological analysis or by molecular analysis. Morphological patterns could vary with time & external factors, the best-suited method of sex determination is molecular analysis.[5] DNA analysis using polymerase-chain-reaction (PCR) is known to give satisfactory and reproducible results.[6] But it is a time-consuming & technique-sensitive method that requires high-end facilities. Other techniques of molecular analysis for sex determination include identification of X chromosome, that is, Barr body in females & Y chromosome, that is, Fluorescent/ F body in males by using special stains.[7]

However, researchers have a difference of opinion regarding the reliability of Barr bodies and F bodies in sex determination. Therefore, the present study was conducted to assess the effectiveness of combined evaluation of Barr bodies and F bodies in teeth subjected to different physical conditions.

MATERIALS & METHOD:

A quantitative in-vitro study was conducted in the department after taking the Institutional ethics committee approval. Freshly extracted healthy non-carious teeth which were extracted for the purpose of orthodontic treatment were included in the study. Complete case history and patient consent was taken from each patient relating to the use of their tooth sample in the study.

The study comprised of 90 teeth samples out of which 45 were male teeth samples and 45 were female teeth samples. To prevent any observational bias, appropriate blinding was done; the samples were collected in separate containers and numbered. The 90 samples were divided into-Group I: 30 samples kept at room temperature, Group II: 30 samples buried in garden soil about 10cm deep for 15 days to simulate deep burial conditions, and Group III: 30 teeth samples subjected to extreme heat of 200°C (n=10), 300°C (n=10) & 400°C (n=10) in a dental furnace for 10 minutes to simulate higher temperature conditions as in cases of fire accidents.

The teeth were cleaned with sterile water to remove blood and organic debris. Root canal access cavity was prepared on each tooth and the dental pulp was extirpated using barbed broach. After adequate fixation of pulp tissue, a cell-suspension was obtained by centrifugation at 6000 rpm for 5 minutes. Two smears were made from each sample- one for acridine orange staining and second for quinacrine dihydrochloride staining. Each slide was studied under fluorescent microscope and evaluated for presence of Barr bodies in slides stained with acridine orange and for presence of F bodies in slides stained with Quinacrine dihydrochloride. The cells showing bright fluorescent spot attached to the nuclear membrane were counted as positive cells.

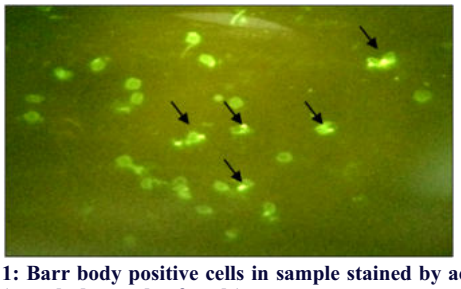

Figure 1: Barr body positive cells in sample stained by acridine orange (sample detected as female)

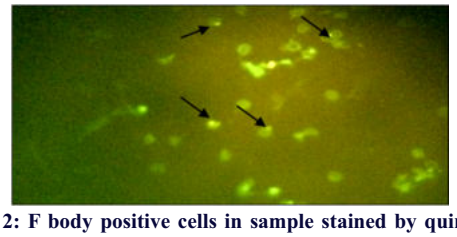

Figure 2: F body positive cells in sample stained by quinacrine dihydrochloride (sample detected as male)

Quantitative assessment of Barr body & F body positivity was done for each sample by counting the number of positive cells per five high power fields with a total of 100 cells counted per slide. The data collected was compiled on to a MS office excel sheet. The statistical analysis was done to evaluate the effectiveness of sex determination

based on presence of Barr & F bodies and to compare & evaluate the number of Barr & F bodies in the three groups.

RESULTS:

The mean ± SD of Barr body-positive cells in female samples for groups I, II & III were 31.67 ± 2.498, 29.87 ± 2.997 and 18.07 ± 5.106 respectively (Table 1). There was a statistically significant difference seen for the mean values between the groups, with higher values seen in group I. Tukey's Post Hoc Test revealed that the difference was statistically significant for Barr bodies between groups I & III, and groups II & III.

Table 1: One way ANOVA test analysis for comparison of mean number of Barr bodies & F bodies in female samples among three groups

Female samples		Mean	P value	Significance
Barr body positive cells	**Group I**	31.67	0.000**	Highly significant
	Group II	29.87		
	Group III	18.07		
F body positive cells	**Group I**	0.40	0.062#	Non-significant
	Group II	1.00		
	Group III	1.87		

The mean ± SD of F body-positive cells in male samples for groups I, II & III were 31.07± 3.990, 22.60 ± 3.582 and 17.80 ± 4.039 (Table 2). There was a statistically significant difference seen for the values between the groups for F bodies with higher values at Room temperature. Tukey's Post Hoc Test revealed that the difference was statistically significant between the groups I & II, groups II & III, and groups I & III.

Table 2: One way ANOVA test analysis for comparison of mean number of Barr bodies & F bodies in male samples among three groups

Male samples		Mean	P value	Significance
Barr body positive cells	**Group I**	0.33	0.114#	Not significant
	Group II	1.00		
	Group III	1.87		
F body positive cells	**Group I**	31.07	0.000**	Significant
	Group II	22.60		
	Group III	17.80		

In our study, 72% female samples showed at least a small percentage of F body positivity and remaining 28% females showed only Barr body positive cells. Similarly, 70% male samples showed few Barr body positive cells while rest of them could be clearly detected as male because of only the F body positivity. Therefore, our study confirmed the effectiveness of combined evaluation of Barr bodies and F bodies for sex determination from teeth subjected to different physical conditions. This technique could decrease the chances of false positive or false negative results.

DISCUSSION:

Literature shows that various researchers had tried to identify Barr bodies found in females using a range of nuclear stains such as hematoxylin and eosin, Feulgen, thionine, Papanicolaou (PAP), fluorescent stain like acridine orange whereas F bodies in males were identified using derivatives of quinacrine dye.[8,9,10,11,12,13,14] However, very few researchers have tried to compare the effectiveness of observation of both Barr bodies and F bodies in given teeth samples.[15]

In our study, the females showed 6-36% Barr body-positive cells while males showed 0-9% Barr body positivity (Table 1). Our results were in accordance with the studies done by Khorate et al, Duffy et al, Suazo et al, Ravishankar et al and Herrmann & Davis.[4, 10, 15, 16,17] There was no significant decrease in number of Barr body positive cells in the samples which were subjected to deep burial conditions. There was a statistically significant decrease in the Barr body positivity in the female samples subjected to extreme heat which was similar to studies done by Reddy et al and Suazo et al.[2,8] Our study results were contradictory to those by Murugesan and his associates.[11] They noticed almost 50% reduction in Barr body positivity with increase in temperature. However, the use of two fluorescent stains and our methodology of cross-checking for presence of F bodies in the same sample aided the observers to reliably determine the correct sex with sensitivity & specificity of 97.78%.

In our study, F body positivity in male samples was 8-37% and in females it was 0-2% (Table 2). Our findings were comparable to those of Khorate et al, Seno et al and Das et al.[7, 12, 15] On comparing the sensitivity & specificity of the test between groups I & II, we did not get any difference. The efficiency of the test remained 100% which was in accordance with the results of Das et al and Veeraraghavan G et al.[7,13] Hence, our results showed that there was no significant difference in the effectiveness of combined evaluation of Barr body & F body in all three groups, even though the number of Barr body-positive cells & F body-positive cells reduced gradually.

Presence of Barr body is usually sufficient enough to prove the female sex as males lack Barr bodies. However, individuals with certain chromosomal abnormalities can yield false positive or false negative results. Further, the presence of bacteria, dead cells, and putrefied cellular debris might give false positive results. Similarly, demonstration of F bodies in dental pulp is reliable method for sex determination, but the sensitivity & specificity of the test decreases due to post-mortem decomposition. Whitaker et al observed significant reduction in number of F bodies after a period of 6-10 weeks.[18] Therefore, in our study we subjected each smear sample to test for both Barr bodies as well as F bodies. This was the way of cross-checking and confirming the sex of the individual to rule out false positive and false negative results.

CONCLUSION:

The present study put forth the notion that the efficacy of sex determination using dental pulp would increase if the samples were checked for both Barr bodies and F bodies. Further studies with larger sample size and set up for exact simulation of burial and fire as occurring in real-life situations are needed to validate the study results.

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