To Determine the Reliability & Sensitivity of Giemsa Stain by Barr Body Test for Accurate Gender Determination in Buccal Mucosal Cells.

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Abstract

Sex determination in forensic odontology can be done by either on morphological analysis or molecular analysis. Molecular analysis involves the study of DNA from extracted pulp, cartilage, hair, skin. Buccal mucosa, epithelium attached to denture and toothbrush. Barr bodies, F-bodies, SRY gene, AMEL gene can be studied to determine sex from these samples.

Key words: Barr body, giemsa stain, gender determination, buccal mucosal cell

INTRODUCTION

Deeply stained X chromatin and intra-nuclear structure in females is also known as Barr body as it is first discovered by Barr and Bertam 1949. It is present as a mass usually lying against the nuclear membrane in the females.[4] The chromatin or Barr body is a condensation of chromatin present at the nucleus of the cells in female individuals. Their observation is possible in different cell types and is used for rapid diagnosis of biological gender.[1]

Giemsa is economical and yields prompt result. There were few studies have been done with Giemsa, therefore, this study aimed to assess the effectiveness of buccal smears in sex determination. [2]

MATERIALS & METHOD

Source of data: The study sample consisted of Buccal smears of 120 patients which were collected from the Department of Oral and Maxillofacial Surgery of Career Post Graduate Institute of Dental Sciences & Hospital Lucknow.

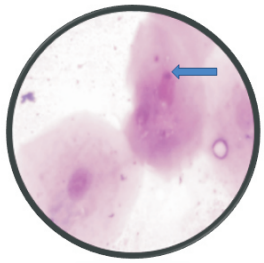

Figure 1: The arrow denoting the Barr body in cell that appeared as dark spot lying against the nuclear membrane (staining with Geimsa stain)

Sample For The Barr Body Test:

a) Buccal mucosa smear were obtained from 120 subjects (Males and Females combined). 60 were taken from male subjects and 60 were taken from female subjects.
b) The subjects sample were divided into six groups of 20 each.
c) Each group of 20 subjects were further divided into 2 subgroups a and b, with 10 subjects in each subgroups (5 male & 5 female).

Buccal Smear Preparation:

a) The subject was asked to rinses the mouth with normal water.
b) A sterilized disposable cytological brush was drawn along with buccal surface of the cheek.
c) The cellular material was quickly smeared on the clean glass slide.
d) A thin film of cells smeared over the slideand kept them for air-drying.

Fixing & Staining of the Cells:

a) After air-drying at room temperature, a few drops of Methanol was added to fix the mucosal cells.
b) After natural evaporation of Methanol, Giemsa stain was poured over the slide and allowed to stand for15 minutes.
c) The slide was then washed with tap water for 2-3 minutes to remove excess stain.

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d) A drop of DPX was added and a cover slip will be placed on the top of smear avoiding trapping of any air bubbles.

Specimen Observation:

The specimen were observed under oil immersion lens (100x) of compound light microscope for Barr-body. Those cells that appeared as dark spot usually lying against the nuclear membrane was counted as positive.

Inclusion Criteria

- Subject who was with healthy mucosa.

Exclusion Criteria

- Subjects with any clinical sign/symptoms of any obvious pathology associated with the concerned tooth / teeth and oral mucosa.

RESULT

Table 1: Gender Comparison of G.S. for Barr Body in group 1

Corelation	Male	Female
Positive	0	9
Negative	10	1
Fisher's Exact Test P Value	0.000119*	

*p-value significant

Table 2: Gender Comparison of G.S. for Barr Body in group 2

Corelation	Male	Female
Positive	0	8
Negative	10	2
Fisher's Exact Test P Value	0.000714*	

*p-value significant

Table 3: Gender Comparison of G.S. for Barr Body in group 3

Corelation	Male	Female
Positive	0	8
Negative	10	2
Fisher's Exact Test P Value	0.000714*	

*p-value significant

Table 4: Gender Comparison of G.S. for Barr Body in group 4

Corelation	Male	Female
Positive	0	6
Negative	10	4
Fisher's Exact Test P Value	0.010836*	

*p-value significant

Table 5: Gender Comparison of G.S. for Barr Body in group 5

Corelation	Male	Female
Positive	0	5
Negative	10	5
Fisher's Exact Test P Value	0.032508*	

*p-value significant

Table 6: Gender Comparison of G.S. for Barr Body in group 6

Corelation	Male	Female
Positive	0	6
Negative	10	4
Fisher's Exact Test P Value	0.010836*	

*p-value significant

Table 7: Overall Gender Comparison of G.S. for Barr Body

Corelation	Male	Female
Positive	0	43
Negative	60	17
Fisher's Exact Test P Value	<0.0001*	

*p-value significant

Table 8: Sensitivity and specificity for G.S. for Barr Body in gender prediction

		Gender Prediction	
G.S. for Barr Body		**Male**	**Female**
	Positive	60	1
	Negative	17	42
Sensitivity (%)	77.92%		
Specificity (%)	97.67%		
Positive Predictive Value (%)	98.36%		
Negative Predictive Value (%)	71.19%		

Table 8 shows sensitivity of 77.92 %, specificity of 97.67%, Positive Predictive Value 98.36% and Negative Predictive Value 71.19% for G.S. for Barr Body in gender prediction.

DISCUSSION

Ekanem VJ et al. (2012)[3] showed that the number of Barr bodies were seen in 35 (21.6%) Giemsa-stained slides which was found to be congruent to our study.

Another similar study was done by Khorate MM et al. (2014)[4]who determined 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. The range of BB in male subjects was found to be 0-14 with a mean of 5.16 ± 3.05. 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%. The authors suggested that 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.

Our study was found to be in accordance to the results obtained by Htun KS et al. (2017)[2] who showed that the Giemsa-stained slides after examination revealed that the percentage of Barr bodies in females ranged from 2 – 14 (mean 9.04 ± 3.583) and in males, it ranged from 0 – 3 (mean 0.22 ± 0.616). The sensitivity of Giemsa was 96% and the specificity was 100% for detecting sex accurately. The overall accuracy for Giemsa was 98% (95% CI) which was seen to be almost similar to the results of the present study. .In the present study it was observed that there was sensitivity of 77.92%, specificity of 97.67%, Positive Predictive Value 98.36% and Negative Predictive Value 71.19% for G.S. for Barr Body in gender prediction with Fisher's exact test p value <0.0001 (significant)

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. The advantages of Giemsa are the relative simplicity, cost effectiveness and time effectiveness.

Limitations of the Study:

BB is found only in those cases in which more than one X chromosome is present 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 gender cannot be correctly identified using the BB technique; for example 47XXY men will have a BB and 47XXX women will have two.

CONCLUSION

The visualization of Barr body with Giemsa is more than with other stains. However, there is a great advantage, rapid diagnosis easily accessible when prompt diagnosis is essential. The slides do not spent duration for several days or weeks. The fine nuclear details were effortlessly observed using Giemsa staining method and Barr bodies were easily appreciated. So, the final suggestion is that it is more preferable method using Giemsa staining method for gender determination purpose.[2,5]

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