



SEX DETERMINATION BY EVALUATION OF BARR BODIES IN BUCCAL SMEARS: A NORTH INDIAN STUDY

Oral Pathology

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ABSTRACT

Aim: A mammalian somatic cell from a normal (XX) female typically has one fully active X chromosome. The other X chromosome is transcriptionally inert, heterochromatic, late replicating and termed Barr body. Present study was conducted to assess the accuracy of Barr body in sex determination.

Study Design: A blind study was conducted over 50 male and 50 females. Buccal scrapings were taken with wooden spatula and a thin smear was prepared followed by staining with Hematoxylin and Eosin. In each slide, 100 cells having vesicular nuclei and distinct cellular outline were counted and percentage of sex chromatin was calculated.

Result: Among females, mean % of Barr body count was significantly higher ($p < 0.001$) as compared to that of males. It was found to be 88% sensitive, 74% specific and overall diagnostic accuracy of 81%.

Conclusion: Buccal smear evaluation has enabled chromosomal sex to be determined with considerable accuracy.

KEYWORDS

Barr Body; Sex Determination; Sex Chromatin; Lyon Hypothesis.

INTRODUCTION

Determination of sex and establishing individuality are important and necessary in any forensic investigating procedure. It helps in criminal investigators for identification of the person which can help in solving many cases of assault, theft, and sexual offences, etc. In train and aircraft accidents and also in natural disasters, it becomes difficult to identify the bodies.

In cases of sexual offences, the buccal mucosal cells along with saliva stains are found in various parts of the body and also at the scene of crime. Saliva stains may be present on cigarette butts and also on cups and glasses which criminals have used.¹

A sex difference in the morphology of intermitotic nuclei was first described as a feature of nerve cells in the female cat by Barr and Bertram (1949). Further studies have shown that sex chromatin may be demonstrated, not only in many tissues of the cat, but also in the cells of other species, including man.²

Demonstration of nuclear sex plays a vital role as far as sexing of the individual is concerned and it can be demonstrated by karyotyping, fluorescent body (Y chromatin), polymerase chain reaction, Barr bodies (X-chromatin) examinations.¹

According to the Mary Lyon, every female is a mosaic, since some of her cells use her maternal X to make proteins and some use her paternal X. The inactive X chromosome is also called the Barr body and presents as a planoconvex mass attached to the nuclear membrane.³

Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp. After death it persists for variable periods depending upon the humidity and temperature in which tissue has remained.⁴ The buccal smear technique to identify sex was developed by Moore and Barr in 1955.⁵

The purpose of this study was to assess the accuracy of Barr body detection in sex determination and to estimate and compare the mean Barr body count in healthy normal males and females.

MATERIAL AND METHODS

A blind study was performed on 50 male and 50 female patients in the Department of Oral Pathology and Microbiology, Vananchal Dental College and Hospital, Jharkhand, India.

Subjects were first made to rinse mouth with water. Buccal scrapings were collected under firm pressure with the help of wooden spatula. Thin smear were prepared on clean, dry glass slide and then the slides were numbered. The smears were fixed with 95% alcohol (spray fixative) followed with staining with Haematoxylin & Eosin (H & E). The slides were then scanned under oil immersion microscope (X100). Cells with sex chromatin along the inner side of nuclear membrane were scored positive. (Fig. 1) Hundred cells with vesicular nuclei & distinct borders were counted. Cells with shrunken, folded and pyknotic nuclei were discarded.

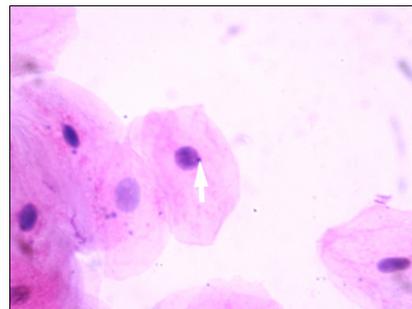


FIG 1: Barr body in female buccal smear as densely stained intranuclear condensed chromatin mass (H & E stain, X 100)

RESULTS AND STATISTICS

TABLE 1: Comparison of mean Barr body counts between two genders

Actual Gender	N	Mean	Std. Deviation	Std. Mean Error
Male	50	4.10	7.98	1.13
Female	50	33.58	11.82	1.67

TABLE 2: Agreement between predicted and actual gender

Predicted Gender	Actual Gender			Total
	No.			
Male	44	13	57	
	%	88.0%	26.0%	57.0%
Female	6	37	43	
	%	12.0%	74.0%	43.0%
Total	50	50	100	
	%	100.0%	100.0%	100.0%

The mean comparison of % Barr body count in two genders is shown in Table 1. It was observed that among males the mean % of Barr body count (4.10 ± 7.98) was significantly lower ($p < 0.001$) as compared to that of females (33.58 ± 11.82).

Table 2 shows that using % Barr Body score criteria, it was observed that out of 50 males, 44 (88%) could be differentiated correctly while out of 50 females, 37 (74%) could be differentiated correctly. Table 2 also describes the agreement between actual and predicted gender. As per % Barr body score criteria 57 specimen were predicted as males and 43 were predicted as females, but out of 57 specimen identified as males only 44 (77.19%) were found to be males whereas out of 43 samples predicted as females only 37 (86.05%) were observed to be females. The agreement between predicted and actual findings was assessed using kappa statistic. A value of 0.62 indicated a good agreement between two.

Efficacy of the criteria was also assessed in terms of sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy. It was found to be 88% sensitive, 74% specific with 77.2% positive predictive value, 86.0% negative predictive value and overall diagnostic accuracy of 81%.

DISCUSSION

Barr and Bertram (1949) first described a sex difference in the morphology of intermitotic nuclei in nerve cells. This difference is characterized by a mass of chromatin which, from its intimate relationship to the nucleolar membrane in nerve cells, was named the sex chromatin.³ Since the original observation of Barr and his colleagues, there have been many reports on the morphological sex difference in the nuclei of cells from males and females.^{6,7}

For the 1968 Olympics, Barr body detection was introduced and was widely proclaimed to be the solution to gender misrepresentation in sports. This reportedly 'simpler, objective and more dignified' test involved the cytological analysis of a buccal smear. Ewa Klobukowska, a Polish sprinter, became the first woman to be disqualified from sport following the introduction of the Barr body test.⁸

It has already been established that there is a well-recognized difference between the nuclei of male and female epithelial cells. In the present study it was observed that among males the mean % of Barr body count was 4.10 ± 7.98 and it was significantly lower ($p < 0.001$) as compared to that of females 33.58 ± 11.82 .

The small percentage of Barr bodies obtained in men in this study, can be compared to other similar studies of Nagamori H et al and Agarwal NK et al.^{9,10} However, Dixon AD, Torr JB found no Barr body in male smears whereas the majority of female smears contained a sex chromatin mass.² Similarly, Manjulabai et al also did not report any Barr-body-positive cells in men.¹¹ This difference may be due to different staining materials and methods used.

There seems to be a variation in the mean percent of Barr bodies among women in the present study as compared to other studies. Marberger E et al reported the average incidence of sex chromatin in females to be 45.6%, while Moore KL et al found sex chromatin in the nuclei of cells from female skin in 52% to 85%, with an average of 64%, a greater difference than that found in oral mucosa.⁶ Few other studies also reported a higher range and mean values.^{9,10,12}

However, Nakadate KH in his study found the average incidence of Barr body in females to be 19.73%.⁶ Obi and Ikerionwu also reported lower values of Barr body- positive cells among Nigerians.¹³ Similarly, some other studies found lower levels.^{11,14,15}

The variation in values for the percentage of female cells possessing sex chromatin in different studies would appear to be due to differences

in the type of cells counted and also to the fact that some estimations have included cells in which the mass of chromatin was not in an absolutely peripheral position in the nucleus. Unless it is in such a position it is partially obscured by the substance of the nucleus and cannot be positively identified. This probably explains why, in a proportion of cells in any smear from females, the sex chromatin is not visible.¹⁶

Oh YK et al studied 100 Koreans for sex determination using the sex chromatin of epithelial cells from the oral mucosa. The percentage of sex chromatin in the nuclei of epithelial cells showed a remarkable difference between the male and the female of 23.6%.¹⁶

The results of present study were in accordance with the previous studies, as two non-overlapping ranges for the percentage of Barr-body positive cells have been obtained for men and women and this emphasise the accuracy of Barr body in gender differentiation. Therefore, we suggest the inclusion of the study of Barr bodies in saliva for gender identification to further strengthen the evidence.

CONCLUSION

The examination of buccal smears of 100 individuals (50 males and 50 females) from North Indian population, representative of a wide age and both genders, has enabled their chromosomal sex to be determined with considerable accuracy as two non-overlapping ranges for the percentage of Barr-body positive cells have been obtained for men and women. With increasing experience in assessment the diagnosis becomes progressively more accurate.

This technique is very economic, simple in its application and if necessary may be readily repeated. It can also be extended to other cells like pulp tissue and hair follicles during natural calamities.

Conflicting Interest (If present, give more details): Nil

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