Assessment of Barr Bodies in Oral Exfoliative Cells for Sex Determination

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Abstract

Introduction: Individual identity is an imperative aspect in any investigation procedure. At times it becomes necessary to determine the sex of a particular individual, like for deciding questions relating to legitimacy, divorce and paternity for some criminal offences. Objective of this study was to assess presence of Barr bodies in oral exfoliative cells of both sex for gender determination.

Aim & Objectives: To Assess Barr bodies in oral exfoliative cells or sex determination

Method: Smear prepared from 30 men and 30 women were stained by the Papanicolaou stain. Cells be served for Barr bodies under oil immersion with compound microscope and the percentage of Barr body-positive cells determined.

Results: Two non-overlapping ranges for the percentage of Barr body positive cells have been obtained for men and women. In the male samples, the percentage of Barr body positive cells ranged from 0-4%. Out of the 30 male samples observed, 18 samples showed 1-4% presence of Barr bodies. In the remaining 12 samples no Barr-body positive cells (0%) were observed. Mean value for barr body positive cells in male samples was 1.46. In the female samples, the percentage of Barr-body-positive cells ranged from 22-74% and all the samples showed the presence of Barr bodies. Out of 30 female samples, 25 female samples showed more than 35% presence of barr body. Mean value for barr body positive cells in female samples was 43.56

Conclusion: Presence of Barr body in exfoliated cells can be demonstrated with a fair degree of accuracy in sex determination.
Keywords: Lyonization, Barr body, Papanicolaou stain

INTRODUCTION

Individual identity is an imperative aspect in any investigation procedure. Sometimes it becomes necessary to determine the sex of a particular individual, for the purpose of simple identification in the living, where the individual of one sex carries the features of the opposite sex; when a person appears to possess the primary sex organs of both the sexes; for the purpose of deciding whether an individual can exercise certain civil rights reserved for one sex only; for deciding questions relating to legitimacy, divorce, paternity, affiliation and also to some criminal offences; simple identification of dead individuals in an advanced state of decay where primary sex organs are lost due to decomposition.

It becomes a challenging task when insufficient samples are obtained. In such conditions, Nuclear sex determination makes way for identification of the individual. Nuclear sex chromatin can be demonstrated by various methods, among these, one of the common method is **Buccal smear** method. In this method, sex of the individual is identified using nuclear sex chromatin method[1]

Various cytologic studies prove the presence of condensed deeply stained chromatin material in nuclei of female cats in 1940's which was later termed as Barr-body by Murray Barr. These cells found to be present only in females which can be used as a vital tool for determination of sex of the individual. Significance of presence of such densely stained material in nuclear material was given by Lyon, who suggested that inactivation of one of the X chromo some in each somatic cell occurs during the early embryonic development and named such process as Lyonization. Simple techniques such as determination of sex from buccal smear were given by Moore and Barr.[2]

Inactivated X-chromosomes seen in female somatic cells are called Barr-bodies which are present adjacent to the nuclear membrane. Barr bodies are small, dark stained mass of inactive X-chromosomes in females within the nucleus. " Mc.Barr" and "Bertram" were the two scientists to observe the deeply stained chromatin body in the nerve cell of female cat in the year 1943. Later they came to know that the chromatin body was found absent in male cat. This chromatin body is called as a sex chromatin or Barr body.[3] Male have one "X" and one "Y" chromosome and both are active. In female there are 2 "X" chromosomes (xx chromosomes), among which one is active and other one is inactive. This inactive "X" chromosome represents the Barr body. When the body inactivates extra X chromosomes to keep the dosage of genetic products equal it is called **dosage compensation**.[4]

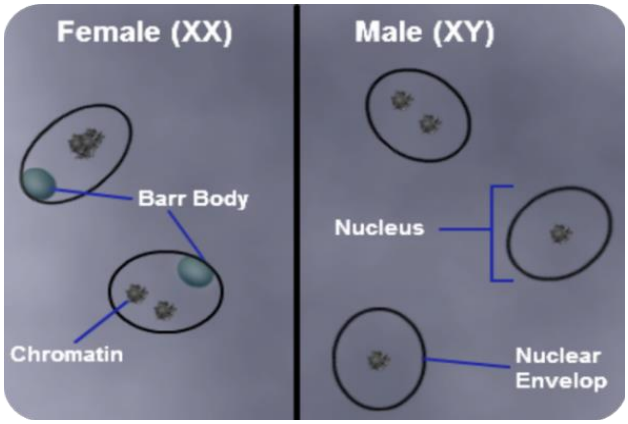

Figure 1a

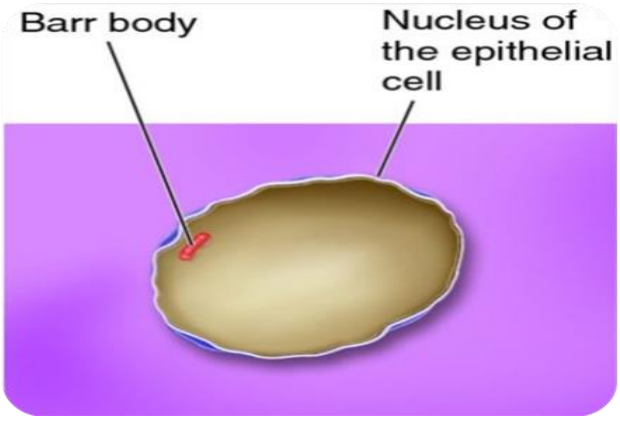

Figure 1b

Figure 1(a & b):Showing Barr body in female cells and no barr body in male cells

Aim & Objectives: To Assess Barr bodies in oral exfoliative cells or sex determination**.**

MATERIAL &METHOD

A cross-sectional study wasconductedamongst60 study subjects who reported to Department of Oral Maxillofacial Pathology, RUHS College of Dental Sciences, Jaipur between Dec 2015 and July 2016 . A simple random sampling by lottery method was done to select the study subjects for the purpose of the study . Ethical approval was obtained from the institutional ethical committee and declaration of Helsinki[5] followed . As per the study conducted by Reddy et al 72% positivity for females were found for assessment of Barr Bodies in oral exfoliative cells for sex determination so by taking 72% prevalence, 90 % power, 5% significance level with 95 % confidence interval and by using the equation 4pq/L2, sample size was calculated as 54. The present study was done on 60 study subjects which was divided into two groups consisting of 30 men and 30 women between the ages of 24 and 45 years. Participants were informed in their vernacular language regarding the procedure, and written informed consent was obtained.

In the present study, we have determined the sex of the individuals from exfoliative cytology. Smears prepared from 30 men and 30 women were stained by the Papanicolaou stain. Cells were observed for Barr bodies under oil immersion with compound microscope, and the percentage of Barr body-positive cells determined.

Samples of buccal mucosa smears from 30 men and 30 women were obtained by scraping with flat wooden sticks (exfoliative cytology).

- Patient's mouth rinsed thoroughly with tap water and followed with distilled water.
- Mucosa scraped gently from the deeper layer of mouth with the help of cleanly washed wooden spatula.
- Sample smeared over a small area on a clean dry slide and allowed for air drying.
- The smears were fixed in Zenker's fixative.
- The fixed slides then stained by using Papanicolou stain.
- Stained slides were mounted with cover slip using DPX solution.
- Morphological details of the exfoliative cells were studied with compound microscope under oil immersion (100X objective) lens and the microphotographs were taken.
- 100 cells were observed in each slide.
- Total number of Barr-body-positive cells was counted among these 100 cells.

Data Analysis

The data was entered in the MS EXCEL spread sheet, coded appropriately and cleansed for any possible typing error and then the data was analyzed by chi-square statistical test using SPSS 20 software as per study objective.

RESULTS

Table 1: DETECTION OF BARR-BODY POSITIVE CELLS IN MEN AND WOMEN

SEX	RANGE %	STANDARD DEVIATION
MALE	4	1.5024
FEMALE	74	11.8806

All the data were calculated and statistically analyzed with SPSS 20.0 computer software package for windows (SPSS IN., Chicago, IL.USA). The data expressed as mean+SEM.

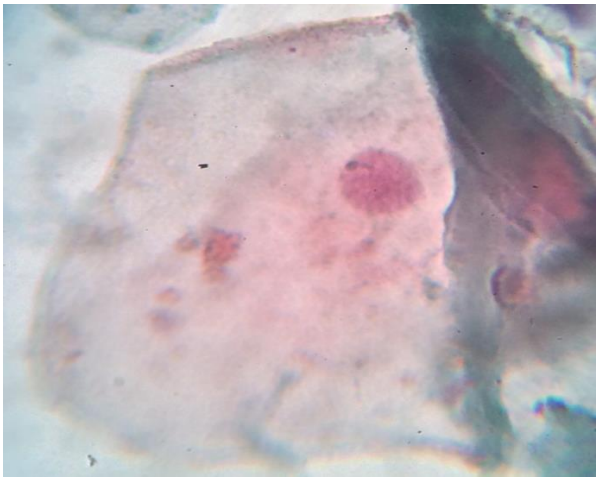

Figure 2a

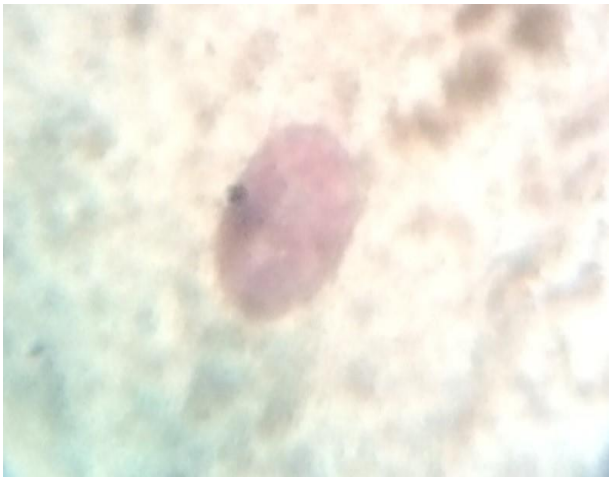

Figure 2b

Figure 2 (a, b) showing Barr body (100x under oil immersion)

In the present study, the following results from the buccal smears of men and women were obtained. Two non-overlapping ranges for the percentage of Barr body positive cells have been obtained for men and women. In the male samples, the percentage of Barr body positive cells ranged from 0-4%. Out of the 30 male samples observed, 18 samples showed 1-4% presence of Barr bodies. In the remaining 12 samples no Barr-body positive cells (0%) were observed. In the female samples, the percentage of Barr-body-positive cells ranged from 22-74%and all the samples showed the presence of Barr bodies. Out of 30 female samples, 25 female samples showed more than35% presence of barr body.

DISCUSSION

Determination of individual's sex becomes an imperative aspect in any investigation procedure. Apart from criminal investigations, disputes related to legitimacy, divorce, paternity and affiliation also demands determination of individual's sex.

There are two methods for Sex determination analysis; morphological analysis orby molecular analysis. Morphological analysis can be done on hard tissues (odontometric, orthometric etc.) of para oral and oral regions or soft tissue analysis (Cheiloscopy, Rugoscopy etc.). Molecular analysis involves the study of DNA from extracted pulp, skin, cartilage, hair, buccal mucosa, epithelium attached to denture .[7]

Sex determination by buccal epithelial cells is one of the commonly used method in the determination of individual's sex. In this method, sex of the individual is identified using nuclear sex chromatin method. In this method we need to identify the presence of Barr Body in the nucleus of buccal epithelial cells.

Moore and Barr in 1955, first introduced the buccal smear technique to identify sex. Barr bodies are Feulgen-positive, hetero-pyknotic, basophilic, intra-nuclear structures, seen in mammalian cells during interphase. They are noticed as densely stained condensed chromatin masses adjacent to the nuclear membrane. They can be plano-convex, biconvex, spherical, rectangular or triangular in shape. Sometimes, they resemble the letter V, S, W or X under an electron microscope. They measure about 0.8 - 1.1 μmin diameter.[8]

Barr bodies are known to arise from inactivation of the X chromosome in a female cell. This process of inactivation is known as Lyonization. Only one of the X chromosomes is genetically active, the other X of either maternal or paternal origin under goes hetero-pyknosis and is rendered inactive. The molecular basis of X Inactivation involves a unique gene called Xist, whose product is a non-coding RNA that is retained in the nucleus, where it "coats" the inactive X chromosome and initiates a gene-silencing process by chromatin modification and DNA methylation. The Xist allele is turned off in the active X.[9]

Recent work has provided insight into how the travel of X inactivation is brought about. The XIST/Xistgene, located at the X-inactivation center, encodes a large untranslated RNA that coats the inactive X chromosome. By knockouts and transgenes, the Xistgene has been shown to be necessary and sufficient for X inactivation.[9]

The Barr body positive males are due to the inheritance of males to carry primary sex organs of both the sexes, as seen in Kleinfelter's syndrome. Though, inactivation process is not completely understood, but it has been suggested that it is under the control of inactivation center, located at Xq13.XIST, a gene which is transcribed from the inactive X, is necessary for initiation and propagation of X inactivation and does this by coating the inactive X chromosome. [10]

Inactivation of either the maternal or paternal X occurs at random among all the cells of the blastocyst about the 16th day of embryonic life. Manjula Bhai KH et al.[11]did not report any Barr-body-positive cells in men, this is in contrast to our study. However, there seemed to be a difference in the range and also the mean percent of Barr bodies among women in the present study as compared to other studies. Considering the fact that the future of an individual identification is based on the reliability of tests such a same logenin sex determination, DXYS156 tests etc. The inclusion of the study of Barr bodies in saliva for gender identification is also suggested to further strengthen the evidence**.**[12]

Any nuclear stain can be used for the demonstration of the Barr Bodies; most commonly used ones are hematoxylin and eosin (H and E), Papanicolou stain, Feulgen stains, guard stains, Cresyl violet, Carbolfuschin and fluorescent stains. In H and E and Papanicolou stains, the bacteria stains heavily, hence the Barr Bodies are not noticed prominently. Orcein also stains bacteria. Bacterial artifacts can be minimized with acid hydrolysis and thionine staining. Feulgen and guard stains are ideal but need to be standardized every time. Fluorescent stains being more confirmatory for the detection of the Barr Bodies but is expensive. In this study, Papanicolou stain was preferred as it stains Barr Bodies more prominently with fewer artifacts and is cost-effective.[2]

It has been observed that the frequency of Barr body is decreased during pregnancy and as well as in women who are on oral contraceptives.[13]

Reactivation of X chromosome was observed whenever the body was under physiological stress**.**[14]

Low frequency suggestive of reactivation of inactive X chromosome is associated with malignancy and is confirmed by enhanced glucose-6-phosphate dehydrogenase activity.[15]

Determination of sex by assessment of barr bodies have few limitations as well. Males with Klinefelter's syndrome tend to show one Barr body in each of their cells due to XXY and females with Turner's syndrome don't show any Barr body due to XO. In such cases the results may be wrong in that particular individual where the test would identify the individuals as females for men with Klinefelter's syndrome, and women with Turner's syndrome would test as men.[16]

CONCLUSION

The study showed that the assessment of Barr body in buccal mucosal cells can be demonstrated with a fair degree of accuracy using Papanicolou staining, to determine sex of individual, as two non-overlapping ranges for the percentage of Barr-body-positive cells had been obtained for men and women.

The sex of the individual can be determined accurately with the added advantages offered, such as the rapidity of processing and screening a specimen that results in saving of time.

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