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TECHNICAL NOTE

Sex determination from buccal mucosa scrapes

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Abstract Establishing individuality is an imperative aspect in any investigation procedure. At times, it becomes necessary to determine the sex of the individual to establish identity, and saliva stains found at the scene of crime are of major help in such cases. In the present study, we have determined the sex of the individual from buccal mucosal scrapings. Buccal smears prepared from 100 men and 100 women were stained by the Papanicolaou staining method. Cells were observed for Barr bodies under oil immersion with a compound microscope, and the percentage of Barr-body-positive cells was determined. It was observed that 1.14% of buccal mucosal cells in men (range=0–4%) and 39.29% of buccal mucosal cells in women (range=20–78%) showed Barr bodies. Inferences from the study show that the presence of Barr body in buccal mucosal cells can be demonstrated with a fair degree of accuracy using Papanicolaou staining. The sex of the individual can be determined accurately, as two non-overlapping ranges for the percentage of Barr-body-positive cells has been obtained for men and women. This method not only proves to be accurate but is also simple and economic.

Keywords Sex · Buccal mucosa · Papanicolaou · Barr body

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Introduction

Establishing individuality is an imperative aspect in any investigating procedure. There are numerous means and ways to do so in human beings (either alive or dead) when a human body is in its entirety but very few when only part(s) is available. Human specimens, such as blood, semen, hair, and saliva stains containing buccal mucosal cells, found at the scene of crime or on a lethal weapon, are of major help in solving criminal cases. 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. Often, it becomes necessary to determine the sex of the individual from whom these stains originate. Determination of sex helps in criminal investigations 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 such instances, buccal smears could help in detecting the sex and thereby establishing the identity.

Demonstration of nuclear sex plays a vital role as far as sexing of the individual is concerned. Nuclear sex can be demonstrated by the study of:

Karyotyping: direct study of type of sex chromosome in the cell by culture of the cell. This is expensive and is not feasible in all institutions.

Fluorescent body (Y chromatin): a demonstration of nuclear fluorescent bodies indicates male. This requires special stain and fluorescence microscope.

Polymerase chain reaction: polymerase chain reaction to amplify DNA sequences on the sex chromosome. This is similar to karyotyping as it is not feasible in all institutes. It is expensive and inferior to karyotyping but not superior to the chromatin test.

Barr bodies (X-chromatin): in contrast, the study of Barr bodies is advantageous in that it can be studied under an ordinary compound microscope with simple staining techniques. The easily available material for Barr body studies is the buccal mucosa, which can be obtained without inflicting trauma on the subject [6, 9]. The buccal smear technique to identify sex was developed by Moore and Barr in 1955 [2].

Barr bodies are known to arise from inactivation of X chromosome in a female cell. This process of inactivation is known as lyonization. Barr bodies are named after the scientist Murray Barr who first described them [2]. Barr bodies are feulgen positive, heteropyknotic, basophilic, intranuclear structures, seen in mammalian cells during interphase. Since they are nuclear structures and all nuclear structures are known to fluoresce, Barr bodies also fluoresce. Most often, they are noticed as densely stained condensed chromatin masses adjacent to the nuclear membrane. In some cells (especially neurons), they can be observed adjacent to the nucleolus or even free in the nucleoplasm. They can be plano-convex, biconvex, triangular, spherical, or rectangular in shape when observed under ordinary microscope in oil immersion or high power. Sometimes, they resemble the letter V, W, S, or X under electron microscope. They measure about 0.8 to 1.1 μm in diameter [6, 9].

In 1961, Lyon [7] outlined the X-inactivation or what is commonly known as the Lyon hypothesis. It states that (1) only one of the X chromosomes is genetically active, (2) the other X of either maternal or paternal origin undergoes heteropyknosis and is rendered inactive, (3) inactivation of either the maternal or paternal X occurs at random among all the cells of the blastocyst on or about the 16th day of embryonic life, and (4) inactivation of the same X chromosome persists in all the cells derived from each precursor cell. Thus, the great preponderance of normal women are in reality mosaics and have two populations of cells, one with an inactivated maternal X and the other with an inactivated paternal X. Herein lies the explanation of why women have the same dosage of X-linked active genes as have men. The molecular basis of X inactivation is just beginning to be understood. It 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 [13].

Materials and methods

This study has been reviewed by the institutional ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

A total of 100 men and 100 women were selected for the study after obtaining informed consent.

Materials

Various materials used in the study include metal spatula, glass slides (all frosted), one Coplin jar of fixative, cover slips, mouth wash, sterile cotton swabs, compound microscope, and Papanicolaou stain [which consists of Harris's hematoxylin (without acetic acid), Orange G6 (OG 6) and Eosin-Azure 36 (EA 36 or EA 50) stains].

Methods

The subject was asked to rinse the mouth with mouthwash and then with water. A sterilized metal spatula was drawn along the buccal surface of the cheek. The cellular material was quickly smeared on the slide and was gently flattened with a cover slip. The cover slip was drawn in a horizontal motion, and the slide was dropped immediately into a Coplin jar containing 95% ethyl alcohol.

For Papanicolaou staining, the smears were fixed in 95% ethyl alcohol for 15–30 min, rinsed in distilled water, and stained in Harris's hematoxylin for 4 min. The slides were washed under tap water for 1–2 min, differentiated in acid alcohol, blued in tap water or 1.5% sodium bicarbonate, and rinsed in distilled water. Now they were transferred to 70% and then 95% alcohol for a few seconds. After staining in OG 6 for 1–2 min, they were rinsed in three changes of 95% alcohol for a few seconds each and then stained in EA 36 for 1–2 min. They were rinsed again in three changes of 95% alcohol for a few seconds each. Finally, they were dehydrated in absolute alcohol, cleared in xylol, and mounted in dibutyl phthalate and xylene.

The Papanicolaou-stained slides were then examined under oil immersion of a compound microscope. One hundred cells were observed in each slide. Out of these 100 cells, the total number of Barr-body-positive cells (cells which showed the presence of a Barr body) were counted. As 100 cells were observed, this number became the percentage of Barr-body-positive cells. The data was entered into Microsoft Excel, and the mean and standard deviation were determined for the percentage of Barr-body-positive cells in all individuals.

Results

From the observations on the buccal smear after Papanicolaou staining, the following results were obtained.

In the male samples (Fig. 1), the percentage of Barr-body-positive cells ranged from 0% to 4% (Table 1). Out of the 100 samples observed, 67 showed the presence of Barr bodies. Among these, 41 had only 1% Barr-body-positive cells, while 26 had 2–4% Barr-body-positive cells. No Barr bodies were observed in the remaining 33 samples. None of the male samples had more than 4% Barr-body-positive cells.

In the female samples (Fig. 2), the percentage of Barr-body-positive cells ranged from 20% to 78% (Table 1), and all showed the presence of Barr bodies. The majority of the samples (84) had a lower percentage of Barr-body-positive cells (20–50%) and in only a few (16) was this figure greater than 50%. None of the women showed less than 20% Barr-body-positive cells.

In both men and women, a co-relation between the percentage of Barr-body-positive cells and the age of the individual does not exist.

Discussion

The results of the present study show that it is possible to identify Barr bodies in buccal mucosa in both men and women. The men in this study had 0–4% Barr bodies, and women showed 20–78% Barr bodies in buccal mucosal cells. Strict criteria of selection for typical Barr bodies was taken into account without considering any influencing factors on the mean frequency. In addition to these, an attempt was made in the present study to find out the relationship, if any, between the percentage of Barr-body-positive cells and the age of the individual, but this does not seem to be the case.

Table 1 Detection of Barr-body-positive cells in men and women

Sex	Range %	Mean	Standard deviation
Male	0–4	1.14	1.17
Female	20–78	39.29	13.57

In the present study, the percentage of Barr bodies in men can be compared to other similar studies [1, 11]. Manjulabai et al. [8] did not report any Barr-body-positive cells in men.

However, there seems 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. Few studies [1, 11, 14] reported a higher range and mean values, whereas others [5, 8, 10] found lower levels compared to the present study. Obi and Ikerionwu [12] also reported lower values of Barr-body-positive cells among Nigerians. This difference may be due to different staining materials and methods used. A comparison cannot be made with some other studies [3, 15, 16] because only the range and not the mean was available.

While the present study has focused only on Indians, this study on Barr bodies has also been researched by other authors on subjects from various countries. Interestingly, in one study [12], although the value of Barr bodies in the Nigerian women was different from the present study, the range and mean of Barr bodies among Caucasian women is comparable to the present study. Our study was conducted among Indians who are mainly Caucasoid in origin. Though our study is separated by many years and different geographical locations from the study by Obi and Ikerionwu [12], this similarity in the range and mean percent of Barr bodies among the Caucasian women emphasizes a possible relationship between race and Barr bodies. Since the number of Caucasians in the study by Obi and Ikerionwu [12] was only 17, a future study comprising a larger number of subjects with different

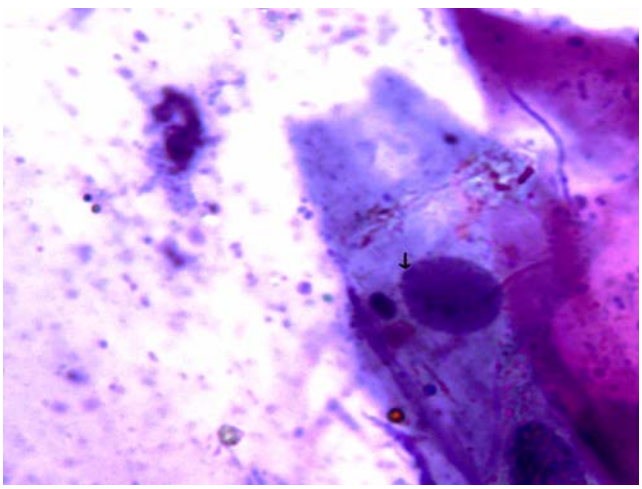

Fig. 1 Barr body (*pointed arrow*) in a male buccal smear (Papanicolaou stain, ×1,000)

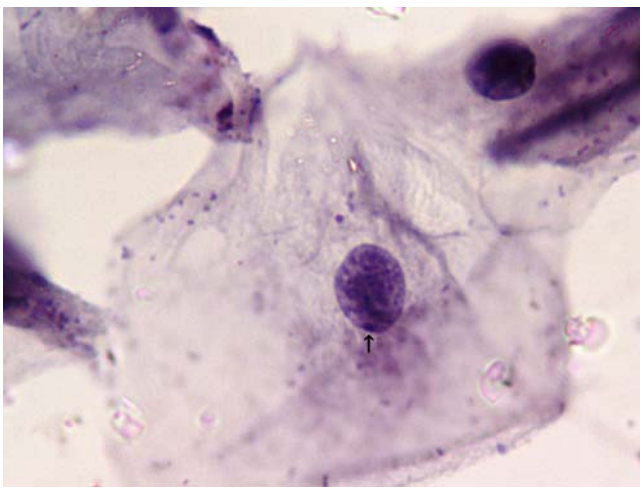

Fig. 2 Barr body (*pointed arrow*) in a female buccal smear (Papanicolaou stain, ×1,000)

racial background may help in finding out if such a relationship exists.

In many forensic cases, sex identification is absolutely essential in rape cases where there is possibility of contamination of DNA from both the victim and culprit [17]. Amelogenin sex determination by pyrosequencing of short polymerase chain reaction products might be an advantage when dealing with degraded DNA [18]. The difference in the size of the amelogenin gene on the X and Y chromosome is utilized for sexing in forensic casework and prenatal diagnosis. However, one study on the reliability of the amelogenin gene for gender identification found a deletion of Y chromosome-specific amelogenin in five (1.85%) Indian men [17]. To overcome this limitation, the authors of the above-mentioned study have suggested the inclusion of additional Y chromosome markers. However, Y typing alone cannot provide a sufficiently resolved DNA fingerprint for court convictions. Similar to amelogenin system, DXYS156 offers a positive control for sex testing [4].

Considering the fact that the future of an individual is based on the reliability of these tests, we therefore suggest the inclusion of the study of Barr bodies in saliva for gender identification to further strengthen the evidence. Saliva could be detected by the use of five stable RNA markers [19].

Conclusion

The sex of the individual can easily be identified by determining the percentage of Barr-body-positive cells, as two non-overlapping ranges for the percentage of Barr-body-positive cells have been obtained for men and women. The presence of Barr bodies in buccal mucosal cells could be determined with a fair degree of accuracy using the Papanicolaou staining technique. This technique is very simple and economic, can be used routinely and also be extended to other cells like pulp tissue and hair follicles during natural calamities. The technique can be used even in rural areas as it requires just an ordinary compound microscope.

Sex determination is usually performed in the course of DNA profiling which lacks a positive confirmation of female individuals (a missing amelogenin signal for Y is interpreted as female) the idea of typing Barr bodies might be interesting in special cases where there is a discrepancy between a genetic profile and the witness account.

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