Original Article

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Cytological Assessment of Barr Bodies in Buccal Mucosal Smears and Peripheral Smear in Sex Determination

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Abstract

Introduction: Barr body has been recognized as the cytological manifestation of the inactive X chromosome in interphase nuclei in a highly condensed state. *Aims and Objectives*: To assess the efficacy of sex determination between pap stained buccal mucosal scrapes and leishman stained drumstick appearance in peripheral smear. *Material and Methods*: Buccal mucosal smears and peripheral smears are collected from 100 individuals who visit K. R. hospital and Cheluvamba hospital of age ranging from 20-30 years. The buccal mucosal scrapes were stained with papanicolaou stain and peripheral smear of the same individual were stained with leishman stain. *Results*: In females and males drumstick appearance was more compared to barr body. *Conclusion*: Sex determination using buccal scrapes and peripheral smear stained with papanicolaou stain and leishman stain proved to be simple, accurate and cost effective method in our study.

Keywords: Barr Body; Drumstick; Papanicolaou Stain; Leishman Stain.

Introduction

Establishing the identity of an individual is of utmost importance in forensic science and sex determination is one of the first steps in identification of an individual in the field of forensic science and in athletes [1]. Some individuals have mosaicism, meaning that some of their cells are XX while others are XY. Depending on the proportion of the cells that are XX, the individual may appear phenotypically female. Sexual ambiguity has created difficulties in the area of sports for many years [2]. Sex determination or estimation by observation of the presence of barr body is a relatively simple, inexpensive technique that yields immediate results [3,4].Barr body has long been recognized as the cytological manifestation of the inactive X chromosome in interphase nuclei [5,6]. Barr body test is a test to determine the presence of multiple X chromosome that present in acell, only one X is allowed to remain active and any excess X chromosomes are randomly inactivated and condensed. These inactivated X chromosome are present adjacent to the nuclear membrane and is called barr body. The term sex chromatin in blood refers to the drumstick appearance of polymorphonuclear leukocytes or Davidson bodies. These drumstick appendages are found on 0.5% to 2.6% of neutrophils [7]. Demonstration of sex chromatin forms an important aspect of human genetics. It also establishes the interrelationship between sex chromatin and an inactive X-chromosome.

Aims and Objectives

To assess the efficacy of sex determination between pap stained buccal mucosal smears and leishman stained drumstick appearance in peripheral smear.

Materials and Methods

Buccal mucosal smears and peripheral smears are collected from 100 individuals who visit K.R. Hospital and Cheluvamba Hospital (50 males and

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50 females). Their age ranging from 20-30 years.

Method of Collection of Data

Consent is taken from the subjects and are asked to rinse their mouth with water. After this the buccal mucosa is scraped using a wooden spatula. The first scraping is discarded to avoid mucous and debris. The consecutive mucosal scrapes will be done by applying gentle and firm pressure to collect the mucosal cells. The collected scraping is then transferred onto clean slides and smears are made and examined under light microscope. A total of 100 cells per slide are counted for the presence of Barr bodies, under 100x magnification which appears as semidisc or triangle with flat side against the nuclear membrane. Doubtful cells areconsidered negative. The count of morphologically acceptable Barr bodies is expressed as percentage.

Tables showing the frequency of Barr body and Drumstick in both the sex

Table 1: Females

Sl. No	Study Findings	No of Cases	Percentage
1.	Increased barr body	08	16
2.	Increased drumstick appearance	30	60
3.	Both equal	12	24

Table 2: Males

Sl. No.	Study Findings	No of Cases	Percentage
1.	Increased BARR body	06	12
2.	Increased drumstick appearance	22	44
3.	Both equal	22	44

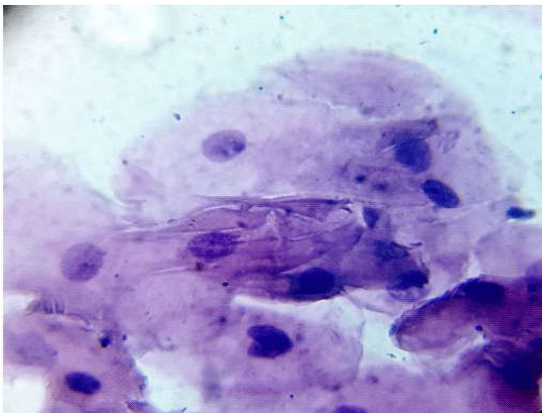

Fig. 1: Barr body appearance in buccal scrape

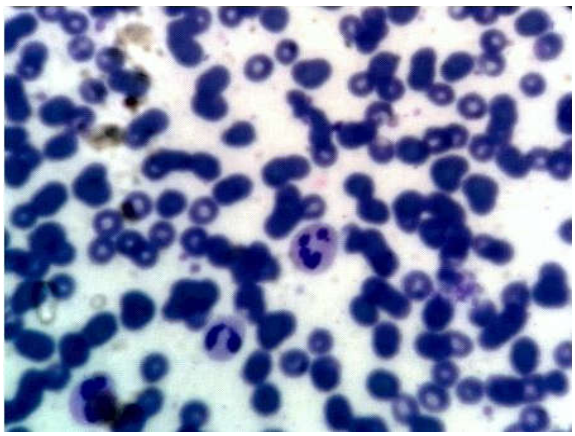

Fig. 2: Drumstick in peripheral smear

The peripheral smear of the subjects will be studied simultaneously for drumstick appearance of the neutrophils under 100x magnification.

The slides are analysed without referring to the recorded clinical data. Subjects with a chromatin count < 5% are recorded as male and those with > 5% are recorded as female. The sex as determined by microscopy will be compared with known sex of the subjects.

Inclusion Criteria

Subjects with age ranging between 20-30 years are included in the study.

Exclusion Criteria

Slides containing mucosal debris and bacterial contamination are excluded in this study.

Ethical clearance has been obtained from the institution.

Results

A total of 100 cases were analysed and the age of both the study groups ranged from 20-30 years with

50 cases of male and female each. In the present study we observed in femalesbarr body accounting to 5% - 16% and drumstick 3%-16%, where as in male it was 0% - 4% and 0%- 6% respectively. Also observed that in females highest number of barr bodies is found in 8 cases and increased number of drumstick appearance in neutrophils in 30 cases. Barr body and drumstick appearance in neutrophils are equal in 12 cases. In male subjects barr body is more in 6 cases and drumstick appearance in neutrophils is more in 22 cases and both equal in 22 cases as shown in the tables.

Discussion

Since the discovery of barr bodies, it has been employed in nuclear sexing to differentiate between female and male cells. The mean percentage of barr bodies in an individual varies not only with the sex of the person [8,9,10,11] but also as a function of ethnicity [8,10], age [12,13] and in females the phase of menstrual cycle [14]. The frequency of these sex chromatids is aberrant in patients suffering from Down's syndrome, klienfielter's and other chromosomal abnormalities [15]. In our study, only those barr bodies which were attached to the nuclear membrane were included. It is necessary to exert firm pressure to secure suitable cells for patches of cells with different types of nuclei occur. The patches are apparently derived from three different epithelial strata.

1. The superficial layer contains pyknotic degenerating nuclei which are often contaminated by bacteria.
2. The intermediate layer provides nuclei suitable for sexing. These have a smooth nuclear membrane and finely granular nucleoplasm in which the sex–chromatin body, if present is deeply stained.
3. The deep layer has nuclei with prophasic chromosomes nd prominent nucleoli. Squashing of the nuclies with subsequent enlargement, permits study of its fine structure. Regardless of the obscure nature of the very early embryological events as observed in pseudohermaphroditism can be diagnosed with sex chromatin pattern and analysis of leukocytes metaphase chromosomes. The mean percentage ofbarr bodies were consistently higher in females than in males, which is concordance with other studies Manjula BhaiK Hetal, Mittal Tetal, and Aggrawal NK etal. Leukocytic drumsticks are stalked, rounded chromatinappendages, 1.5 microns in diameter, projecting from the neutrophilic nuclei of female subjects only and are found to be more in females than in males in our study.

Conclusion

Sex determination using barr bodies in buccal scrapes is a simple method providing 100% accuracy; this makes it a significant accessory to other methods of sex determination. Identification of drumstick in peripheral smear is easier which don't need any expertisation. Papaniculou stain method is recommended for rapid nuclear sexing which helps in screening oligophrenic subjects at the primary health care units for early diagnosis of sexual ambigutity which is needed in the field of sports. Because of the simplicity of the procedure and coast effectivity this method can be adopted.

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