Cytological Determination of Condensed Sex Chromatin Body in Buccal Smear and Peripheral Blood Film Using Various Nuclear Staining Techniques

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Abstract

Background *Buccal smear and blood film examination for identification of condensed sex chromatin body is still being requested in hospitals in Nigeria for various reasons. Different hospitals use different staining techniques to aid in the identification.*

Objectives: *This study aimed to identify the staining techniques that best identify the presence of sex chromatin among some commonly available dyes and also the usefulness of the method to the clinician.*

Method: *One hundred and sixty – two apparently healthy female medical and medical laboratory students were recruited for the study. Buccal smear and blood film from the students were stained with cresyl violet, giemsa, papanicolaou and Haematoxylin and Eosin staining and leishman techniques. The slides were examined for the purpose of identifying the presence of sex chromatin body using light microscopy. The total number of barr bodies and drumsticks seen were recorded for each stain.*

Results: *The highest number of barr bodies were seen in 47 (29%) slides out of the 162 slides stained with cresyl violet and examined for the presence of barr bodies, while slides stained with papanicolaou stain, Giemsa stain and Haematoxylin and Eosin show the present of barr bodies in 41 (25.3%), 35 (21.6%) and 10 (6.2%) slides respectively. One hundred and twenty one blood films were examined with drum sticks seen in 50 (30.9%) of the slides examined.*

Conclusion: *None of the stains used can best identify the presence of barr body in buccal smear. The usefulness of this test to the clinician is doubtful. A combination of buccal smear with blood film is therefore necessary to improve the diagnosis where other advance method of diagnosis is not available.*

Keywords: Sex chromatin, Buccal smear, Barr bodies, Drum sticks, Stains

Introduction

Sex chromatin is a condensed mass of chromatin that represents an inactivated X chromosome. It is also called barr body or drum stick depending on whether it is found within the squamous epithelium or the neutrophils in a peripheral blood film. It is frequently observed in normal female cells and

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not in normal male cells. The X chromosome that constitutes the sex chromatin remains tightly coiled and visible while the other X chromosome is uncoiled and not visible [1, 2]. Lyonization is the process of inactivation of the X chromosome and in this process all but only one X chromosome are inactivated during embryogenesis[1,2,3]. The inactivation of the X chromosome is initiated at the X inactivation centre (Xic) near the centromere[4, 5].

The presence of sex chromatin mass indicates that the individual has at least more than one X chromosome, while the absence indicates that the individual has just one X chromosome.

The main reason why nucleus is examined for the presence of sex chromatin in our environment is for the determination of genetic sex in the case of ambiguous genitalia, delayed menarche and delayed puberty. Occasionally sex chromatin number is also determined for some other genetic abnormalities. In developed society sophisticated DNA diagnostic method like PCR is employed[6].

The presence of sex chromatin in genetic female is usually demonstrated cytologically using different nuclear staining techniques. The use of nuclear stains to demonstrate the presence of sex chromatin in squamous epithelium of the oral cavity is a diagnostic test for certain genetic abnormalities and gender verification. The difficulty in demonstrating the sex chromatin using the routine cresyl fast violet account for the search for a commonly available nuclear stain that best demonstrate sex chromatin

Materials and Method

The study was carried out using buccal smear and blood film. One hundred and sixty–five female medical students of the University of Benin, female medical students of Delta State University, Abraka and female Medical Laboratory students of University of Benin Teaching Hospital volunteered to participate in the study. After rinsing the mouth with clean water to prevent the presence of food particles the buccal cavity of each volunteer was gently scraped with the rounded end of ayre's spatula and a smear made on a plain glass slide and fixed immediately in 95% methanol. The student was also made to relax and blood sample was collected by venopuncture from the mid cubital vein. Blood film was made immediately and stained with Leishman stain. For the buccal smear the following nuclear staining techniques were deployed using standard procedure: Cresyl Fast violet, Papanicolou, Giemsa and Haematoxilyn and Eosin. For the blood film, only leishman stain was carried out.

The stained slides were examined for the presence of barr body in the buccal smear or drum stick in the case of the blood film.

Results

A total of 162 out of 168 (96.4%) buccal smear were analysed. Where the slide was not satisfactory, a repeat smear was carried out from the same student. However 6 students whose slides were not adequate for analysis were not included in the study because the students were not available for a repeat smear. Only 121 (74.7%) of the students consented to venopuncture for blood film examination. The highest number of barr bodies were seen in 47 (29%) slides out of the 162 slides stained with cresyl violet and examined for the presence of barr bodies, while slides stained with papanicolaou stain, Giemsa stain and Haematoxylin and Eosin show the present of barr bodies in 41 (25.3%), 35 (21.6%) and 10 (6.2%) slides respectively. Examination of the blood film shows the presence of drum sticks in 50 (30.9%) of the slides examined.

Comparing the values of cresyl violet with that of the leishman stain and assuming equal variance, it shows a significant level of 0.06 at 95% confidence interval. Though Haematoxilyn and Eosin staining technique could demonstrate the normal structure of epithelial cells; it could not demonstrate satisfactorily the presence of Barr bodies. Figures 1, 2, and 3 shows the present of barr bodies in different stains.

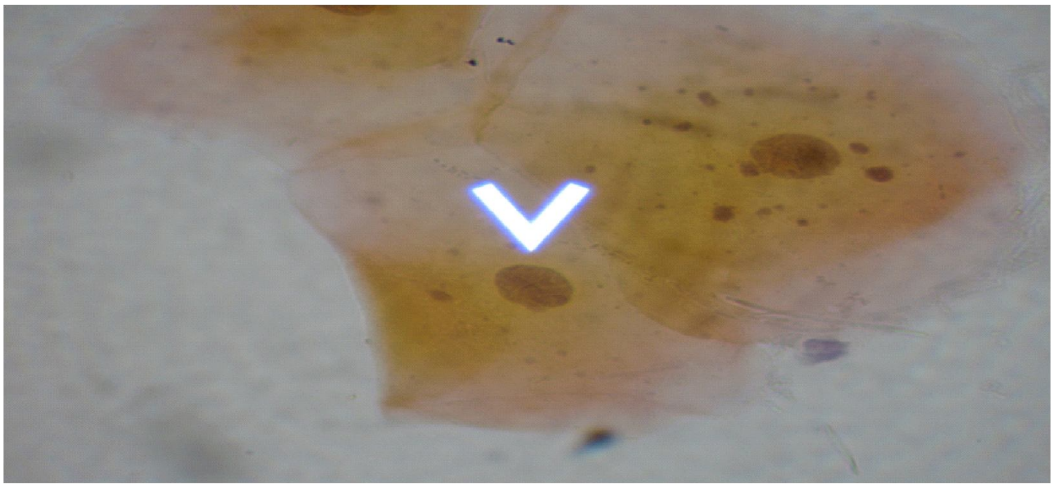

Fig. 1. Barr body in the nucleus of squamous epithelial cell stained with Papanicolou staining technique

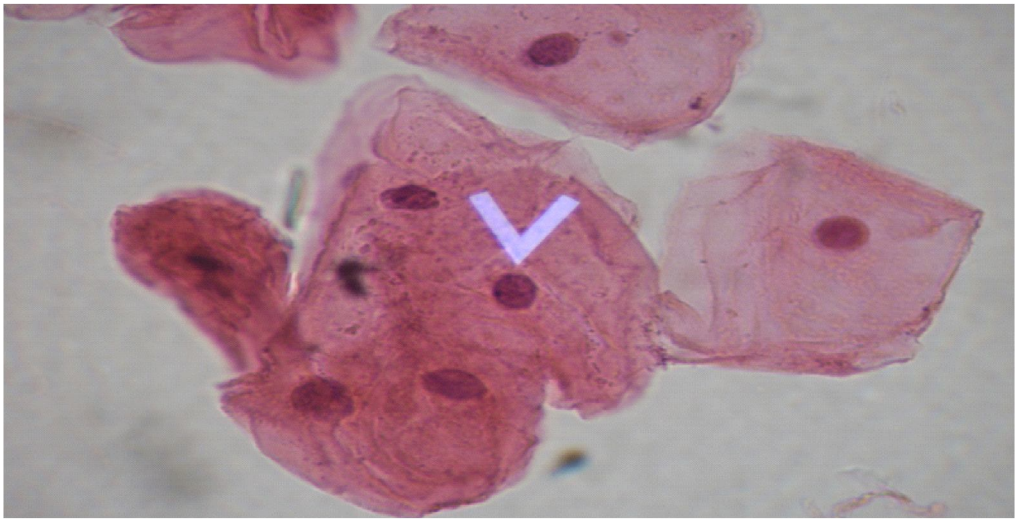

Fig. 2. Demonstration of barr body in the nucleus of squamous epithelial cell using Haematoxylin and Eosin staining technique

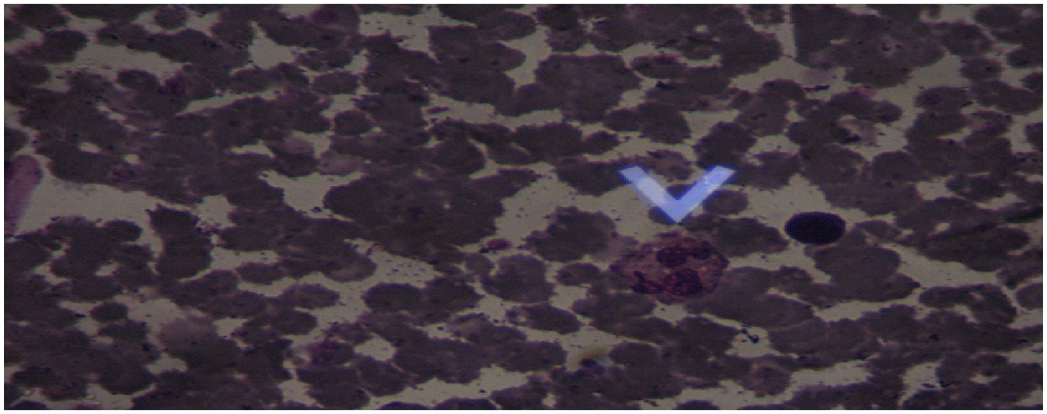

Fig. 3. Blood film stain with leishman and demonstration a drum stick attached to a lobe of nucleus in Neutrophil

Table 1: Frequency distribution for various stains used for the determination of the present of sex chromatin condensation in buccal smear and blood film

	Cresyl Violet			
	Frequency	Percent	Valid percent	Cumulative percent
Barr Body not Seen	115	71	71	71
Number of Barr bodies seen	47	29	29	100
Total	162	100	100	
	Giemsa Stain			
	Frequency	Percent	Valid percent	Cumulative percent
Barr body not seen	121	74.7	74.7	74.7
Number of Barr bodies seen	41	25.3	25.3	100
Total	162	100	100	
	Papanicolaou			
	Frequency	Percent	Valid Percent	Cumulative percent
Barr body not seen	127	78.4	78.4	78.4
Number of Barr bodies seen	35	21.6	21.6	100
Total	162	100	100	
	Haematoxylin and Eosin			
	Frequency	Percent	Valid Percent	Cumulative Percent
Barr body not seen	152	93.8	93.8	93.8
Number of Barr bodies seen	10	6.2	6.2	100
Total	162	100	100	
	Leishman Stain			
	Frequency	Percent	Valid Percent	Cumulative percent
Drum Stick not seen	71	43.8	58.7	58.7
Drum stick seen	50	30.9	1.3	100.0
Total	121	74.7	100.0	
No Venopuncture	41	25.3		
Total	162	100.0		

Discussion

The cytological determination of sex chromatin (Barr bodies/drum sticks) using different nuclear staining techniques is a very useful study aimed at identifying the most suitable techniques that best demonstrate the presence of sex chromatin which aids proper diagnosis of genetic disorders involving sex chromosome. This is particularly important in the developing society and rural areas where facilities for more superior diagnostic techniques like PCR is lacking.

In our study the highest number of "barr body" was recorded when cresyl violet staining technique was used (29%). This was followed closely by Giemsa and by papanicolaou stains. These different stains are commonly available in our environment. The findings in this study is however at variance with earlier study carried out by Marb Gee et al and Moore *et al*[7,8]. The difference may be due to the type of fixative and the staining technique used by them. They used a feulgen staining technique which is a more complex method. Care was taken in this study to ensure that nucleoli, lymphocytes or any artifact lying close to the nuclear membrane were not misinterpreted as sex chromatin body.

Drumstick is a small mass that is attached to the nucleus by a thin filament measuring about 1.5ì in diameter[9,10]. Drumstick on neutrophils should be interpreted with caution as one of the lobes may resemble a condensed chromatin body. This caution was applied in this study.

Perhaps that accounted for the relatively low percentage of 30.9% found on blood film examination. The possibility also is that some of the chromatin bodies might have been folded inside and covered by the nucleus of the neutrophils. The interpretation of drumstick has also given rise to difficulties that are not usually encountered with barr bodies. For example klinefelter's patients (47XXY) usually have a lower incidence of drumstick in their blood film compared to those found in normal females while the frequency of barr bodies is not affected[9].

There is argument whether sex chromatin determination is useful to the clinician or not. This argument is based on the several reasons. First is the fact that buccal smear examination do not detect all cases of abnormalities.[11] This assumption tallies with our finding in which only a small percentage (less than 31%) of chromatin condensation was identified in all the stains used for this study. It is also argued that even when the sex chromatin has been identified, there is still need to carry out full chromosome analysis.[11]

Request for identification of barr body in buccal smear is still being received in laboratories of most of the teaching hospitals in Nigeria. These requests are made for several reasons. Some of the reasons are: the absence of menstrual flow after puberty in supposedly females together with phenotypic abnormalities, cases of ambiguous genitalia in children and other abnormalities. This study has clearly shown that the identification of sex chromatin in buccal smear may not be very helpful to the clinician since there is a high percentage of false negativity. In addition it also showed that none of the stains used can best identify the presence of barr body in the smear. A combination of buccal smear with blood film is therefore necessary to improve the diagnosis where other advance method of diagnosis is not available. However attempts should be made by various hospitals in Nigeria to acquire materials and man power for genetic and molecular diagnosis.

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