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Original Research Article

GENDER DETERMINATION USING PERIPHERAL BLOOD SMEAR

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ABSTRACT

Background: Sex chromatin is a chromatin mass of 1 micron size usually seen at the periphery of nucleus in females. The term sex chromatin comprises of two superficially dissimilar structures known as Barr body in epithelial cells, other tissue cells and as Drumstick appearance in polymorphonuclear leucocytes. Aim of the study is gender determination by drumstick appearance of neutrophils using Leishman's stain and Field stain.

Materials and Methods: The present study included 60 individuals ranging from 20-50 age group of both the sexes to observe the morphology of sex chromatin in neutrophil by using Leishman's and Field stain for the gender determination and comparison between the 2 staining procedure, using research microscope.

Results: Presence of drumsticks, sessile nodule and non specific appendages like racket, tag, hook, minor lobe, small club, were found in neutrophils. In males 37% of drumstick, 7% of sessile nodule, 20% of racket structure, 3% small club, 13% minor lobe, 7% hook, 3% tag, was seen and in females 10% of drumstick, 60% of sessile nodule, 17% of racket structure, 7% small club, 1% minor lobe, 0% hook, 3% tag, was seen and field's stain showed better results in appreciating the morphology of neutrophil.

Conclusion: Observation on morphology and comparison between 2 different stain presented a moderate agreement in gender determination.

KEY WORDS: Sex chromatin, drumstick, sessile nodule, non specific appendages.

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INTRODUCTION

Sex chromatin is an approximately 1 micron clump of chromatin seen usually at the periphery of female nuclei in certain tissues like corneal epithelium, buccal mucosa, oral and vaginal mucosa, fibroblasts etc. and as a drumstick in the blood smears [1-3]. Sex chromatin is derived from one of the two x chromosomes in the female which replicates its deoxyribonucleic acid much later than the other and is thus positively heteropyknotic. This process of inactivation of X chromosome is known as "Lyonization"[1].

The inactive X chromosome in neutrophils

appears in one of the three forms. They are drumsticks, racquet forms and sessile nodules. Davidson and smith are the first to identify and report the presence of neutrophil drumsticks and nonspecific appendages and their differences in sexes [1].

Peripheral blood film is a basic and a highly informative haematological tool at the clinician's disposal in screening, diagnosis and monitoring of disease progression and therapeutic response [4]. The present study is aimed in determining the gender using peripheral blood smear and comparsion between Leishman's and Field's stain with inter and intra observer variability.

MATERIALS AND METHODS

The present study was conducted at Department of Oral Pathology and Microbiology in Bapuji Dental College and Hospital, Davangere. This study included 60 subjects (30 female, 30 male) 2 study groups. It was blind study. The inclusion criteria in the study was, age group of 20- 50 years and total leukocyte count between 4000-10,000 cells/ cu.mm and at least 50 well-stained, non-shrunk neutrophils observed in the smear. The exclusion criteria was children, menstruating, pregnant and lactating females, those who are on hormonal therapy, immunosuppressive therapy/conditions and any other systemic condition requiring medical intervention were excluded. Subjects who fulfilled the eligibility criteria's were included in this study. In each gender, age was matching criteria for the study. The study was approved by the Institutional Review Board and the ethical clearance was obtained.

Blood smears are made by placing a well mixed drop of blood 1 to 2 mm in diameter 1/4 inch from the frosted edge of the slide. The drop were placed in the centre line approximately. The purpose is to get a region where the cells are spaced far enough apart to be counted and differentiated. The blood smear is then fixed with methanol for proper microscopic examination. The smear is further stained by leishman's stain and field stain to see the drumstick appearance of neutrophils for microscopic examination under the following technique.

Leishman's stain: The smear is flooded with undiluted stain and not to overflow with excess stain. Preferably 7-10 drops required to cover the film so that double the number of water can be added and adjust the incubation time of 1-2 minutes. Add twice the volume of water to dilute the stain, incubation time of 10-15 minutes and washed with water and dried.

Field's stain: Dip the fixed smear in field stain B (red stain) for 5-6 seconds wash in running tap water, dip smear into field stain A (Blue stain) for 10 seconds wash in running tap water and air dried. Counting of drumstick, sessile nodule and non specific appendages was done in "Z" pattern. A total number of 50 neutrophils were counted in each slide and observed under oil immersion in research microscope leica DMRB.

RESULTS

Table 1: Sex wise distribution of drumstick, sessile nodule presence in neutrophils.

Gender	Number of cases examined	Total number of cells counted in two different stain	Drumstick appearance	Sessile nodule appearance
Male	30	3000	37%	7%
Female	30	3000	10%	60%

Table 1a: Percentage of nonspecific appendages presence in neutrophils.

Gender	Minor lobe	Racket	Small club	Hook	Tag
Male	13%	20%	3%	7%	3%
Female	1%	17%	10%	4%	3%

Table 1b: number of unusual finding of drumstick and nonspecific appendages presence in neutrophils.

Gender	2 or more drumstick appearance in one lobe	Drumstick with racket appearance	Drumstick with hook appearance	Two racket appearance in one lobe	Unnamed lobe
Male	4	5	1	0	0
Female	1	2	0	2	1

Table 2: The distribution of sex chromatin in males and females.

Sex chromatin	Female	Male
Drumstick	10%	37%
sessile nodule	60%	7%
Racket	17%	20%
Small club	10%	3%
Minor lobe	1%	13%
Hook	4%	7%
Tag	3%	3%

A total of 6000 neutrophils were observed in blood smear collected from 30 males and 30

females of 20-50 years age for gender determination. Chi square test was used for comparing the drumstick appearance of neutrophils in both gender.

The percentage of drumsticks, sessile nodule and nonspecific appendages like racket, tag, hook, minor lobe, small club, were found in neutrophils. The 37% of drumstick, 7% of sessile nodule, 20% of racket structure, small club 3%, minor lobe 13%, hook 7%, tag 3%, was seen in males and 10% of drumstick, 60% of sessile nodule, 17% of racket structure, small club 7%, minor lobe 1%, hook 0%, tag 3%, was seen in females. Combination of drumstick and nonspecific appendages percentage was more in males than in females and combination of drumstick and sessile nodule percentage was comparatively more in females than in males and there was also presence of double drumstick appearance.

DISCUSSION

Davidson and Smith [1] are the first to identify and report the presence of neutrophil drumsticks and non-specific appendages and their differences in sexes. Brahmi et.al., [4] observed 200 neutrophils from 74 blood smears of both sexes (35 females and 39 males) and reported 3 types i.e. neutrophils with type A (drumstick), type B (sessile nodule) and type C (tag or hook). In literature there was no mention about the percentage of nonspecific appendages like sessile nodules, racket structures, minor lobes and small clubs in blood neutrophils along with drumsticks. Morphology and comparison between two different stains were dealt in the present work, Field's stain showed clear appearance of nuclear lobe of neutrophils, which helped for understanding the morphology of sex chromatin and non-specific appendages. Though it was stated in literature that drumsticks are never seen in males [5]our study and several other studies [4.6] in literature suggests that true drumsticks are also present in males though their percentage incidence is less.

According to literature [7] typical drumsticks are rarely seen in normal men but, in the present study 37% of true drumsticks was observed in males and the number of true drumsticks in females was 10% .

The percentage of non-specific appendages showed sex differences in the present study. In which males showed a higher proportion of non-specific appendages racket structures (20%), small clubs (3%) and minor lobes (13%), hook (7%), tag (3%) and Sessile nodules were 7%. Females showed a higher proportion of sessile nodule 60% and less proportion of non-specific appendages racket structures (17%), small clubs (7%) and minor lobes (1%), hook(0%), tag(3%) and drumstick were 10%.

Single cell with 2 drumsticks reported in a patient with XXX sex chromosomes [8,9]. Kesaree and Woolley [10] reported single cell nuclei with a combination of true drumstick and a non-specific appendage among the controls. R Shankar [11] reported 40% of sex chromatin was seen in 50 females and 0% in males. Another study conducted by Vikram [12] reported that male pulpal tissue did not show the presence of barr bodies.

In present study also there was combination of drumsticks and racket structures and was seen among males and 2 drumstick in single cell and drumstick with hook was also present in males and very few 2 drumstick in single lobe and drumstick with racket appearance was seen in females and one new different lobe was identified in females. The present study showed moderate agreement in determination of gender using kappa static.

CONCLUSION

The present study compared between 2 different stain for studying the morphology of neutrophils that which help in identifying the sex chromatin in which Leishman's stain could not provide the good nuclear clarity, and the stain gradually fades but Field's stain showed best results by providing the good nuclear clarity and retaining the stain for longer duration, less time consuming which helped in better identifying the sex chromatin in the neutrophils. So field's stain is best for studying the morphology of neutrophils.

According to the literature, they considered only 'drumsticks' to be related to the sex chromatin. However, Kosenow believed that sessile nodules are of equal sex-diagnostic significance, but in

the present study even non-specific appendages are of equal in gender determination.

Conflicts of Interests: None

REFERENCES

[1]. Davidson W.M, Smith D.R. A morphological sex difference in the polymorphonuclear neutrophil leucocytes. Brit Med J 1954;2:6.

[2]. Miles CP. Peripheral Position of Sex Chromatin. Nature 1961; 191: 626 – 627.

[3]. Levine H. 'Clinical Cytogenetics'. 1971; pp. 77-81. Churchill: London.

[4]. Brahimi M, Adda A, Lazreg H, Beliali H, Osmani S, Bekadja M.A. Can sex be determined from a blood smear. Turk J Hematol 2013;30:53-57.

[5]. Briggs D.K. The individuality of nuclear chromatin with particular reference to polymorphonuclear neutrophil leukocytes. Blood 1958;13:986-1000.

[6]. Tomonaga M, Matsuura G,Watanabe B, Kamochi Y, Ozono N. Leukocyte drumsticks in chronic granulocytic leukemia and related disorders. Blood 1961;18:581-591.

[7]. Mittwoch U. Frequency of drumsticks in normal women and in patients with chromosomal abnormalities. Nature 1964;201;317-319.

[8]. Jacobs PA, Baikie AG, Court BrownW.M., MacGregor TN,Maclean N, Harnden DG. Lancet,1959; ii: 423.

[9]. Wondergem MJ, Ossenkoppele GJ. Genotyping by morphology. Blood 2011; 117:2566.

[10]. Kesaree N,Woolley PV Jr. A phenotypic female with 49 chromosomes, presumably. A case report. J Pediatr. 1963;63:1099-1103.

[11]. R.Shankar,P.Brahmaji Master, L.C.Obulesu, Y.K.C.Rangiah. A Study of Sex Cheromatin Form Buccal Smear. Journal of Evolution of Medical and Dental Sciences 2015; 4(83):14497-14503.

[12]. Reddy AVS, Prakash AR, Killampalli LK, Rajinikanth M, Sreenath G, Sabiha PB. Gender determination using barr bodies from teeth exposed to high temperatures. Journal of Forensic Dental Sciences. 2017;9(1):44.

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