

SEXUAL DIMORPHISM IN MORPHOLOGY AND MORPHOMETRY OF NEUTROPHIL DRUMSTICKS

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ABSTRACT

Introduction: Sex chromatin is a chromatin mass of 1 micron size usually seen at the periphery of nucleus in females. In the literature majority reported its absence in males while few reported its low incidence in males. The term 'sex chromatin' comprises two superficially dissimilar structures the "Barr body" present in epithelial and other tissue cells and the "Drumstick" of the polymorphonuclear leucocytes.

Materials and methods: The present study was conducted to observe the morphology, morphometry and percentage incidence of Drumsticks in the blood neutrophils of 110 individuals ranging from 17-30 age group and both sexes using a calibrated ocular/eye piece micrometer.

Results: The percentage incidence of drumsticks including non-specific appendages as well as the total number of true drumsticks in females exceeds that in males. Four different types of nonspecific appendages-sessile nodules, racket structures, minor lobes and small clubs were found in the blood neutrophils along with the drumsticks. A higher percentage of non-specific appendages i.e. minor lobes (46.2%), racket structures (42.3%), and small clubs (11.5%) were observed in males and sessile nodules were found only in females.

Conclusion: Observations on morphology, morphometry and percentage incidence of polymorphonuclear drumsticks presented a valuable data on sex differences.

KEY WORDS: Sex chromatin, Drumstick, Non-specific appendages, Head, Stalk.

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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". 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 non-specific appendages and their differences in sexes.

A drumstick consists of a small nuclear mass of

about 1.5 μ in diameter, attached to the body of the nucleus by means of a thin stalk and the incidence of drumsticks varied between 1% to 17 % with an average of 2.9% [1]. In the literature there was no single study on percentage incidence of true drumsticks and non-specific appendages their morphology and morphometry. The present study is the first on these aspects of drumsticks and forms the data base for future reference.

MATERIALS AND METHODS

This work was carried out in the department of Anatomy with the co-operation of Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupathi, Andhra Pradesh, India. Peripheral blood smears were prepared for each of 110 individuals of 17-30 years age and both sexes after informed consent. The physical parameters like sex, age, height, weight, thyroid enlargement (if any), menstrual history i.e., cycles and age of attaining menarche (in females) were recorded. The blood smears were stained with Leishman's stain. 100 neutrophils in each blood smear were observed for presence of drumsticks and their number and shape on the surface of the lobes of neutrophils under oil immersion using binocular light microscope that is in turn connected to a LCD monitor. The measurement such as size of the head and length of the stalk of drumstick were measured using a calibrated eye piece micrometer. Counting of drumsticks was done from the starting point of the selected / marked area on the slide and continued until the lens reaches the other end of the marked area i.e., from left to right. After reaching the other end of the marked area the slide was adjusted in such a way that the lens shifts down by 05 divisions of the ocular micrometer. The procedure is repeated from right to left in the "Z" pattern. A total of 100 neutrophils were counted in each slide to calculate the percentage incidence of drumstick.

RESULTS

A total of 11,000 neutrophils were observed in blood smears collected from 47 males and 63 females of 17-30 years age for sexual differences in percentage incidence, morphology and morphometry of true drumsticks and non-specific appendages.

The percentage incidence of drumsticks including non-specific appendages in females was higher than in males (Table.1). The true drumsticks presented a rounded head and a thin stem (Fig.1). The various types of non-specific appendages observed were sessile nodules (Fig.2), minor lobes (Fig.3) racket structures (Fig.4) and small clubs (Fig.5). The percentage incidence of minor lobes, racket structures and small clubs were more in males when compared to females and sessile nodules were not observed in males (Table.2). Combinations of drumstick and sessile nodule presented higher incidence in females whereas combinations of drumstick and racket structures/small clubs/minor lobes were high in males (Table.3) Three cases of tri (three) combination of drumstick-racket-minor lobe, two cases of drumstick-racket-club and one case of drumstick-minor lobe-small clubs were observed in males but none in females. Other three combinations i.e., two cases of drumsticks-racket-sessile, five cases of drumsticks-minor lobes-sessile, one case of drumsticks-clubs-sessile were seen in females with no incidence in males.

Fig. 1: Neutrophil: Drumstick with head connected by a narrow filamentous stalk.

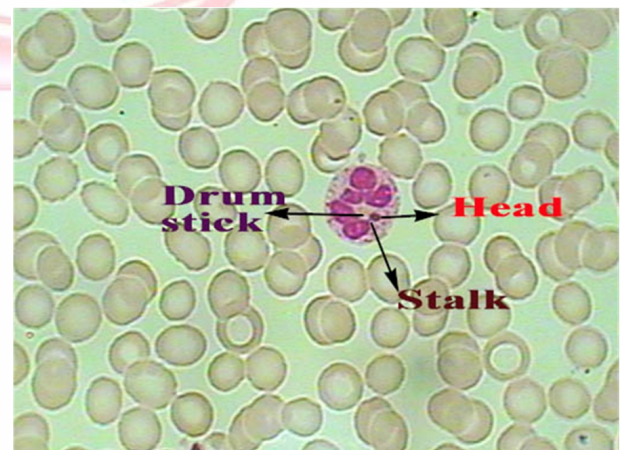


Fig. 2: Neutrophil: Showing sessile nodule with chromatin.

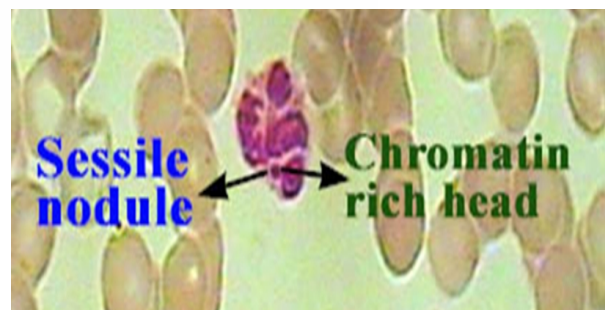


Fig. 3: Neutrophil Drumstick: Non- specific appendage (Minor lobe) - head connected by two stalks.

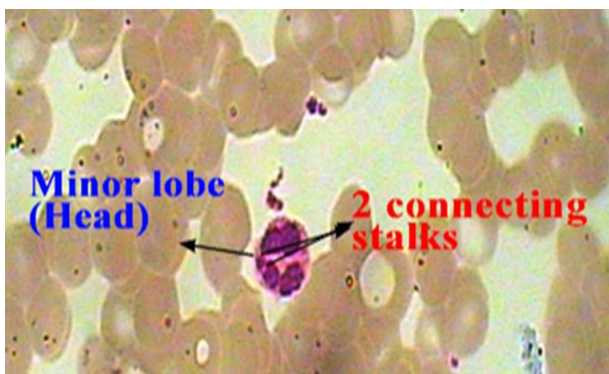


Fig. 4: Neutrophil: Racket structure showing pale central portion of head connected by a stalk.

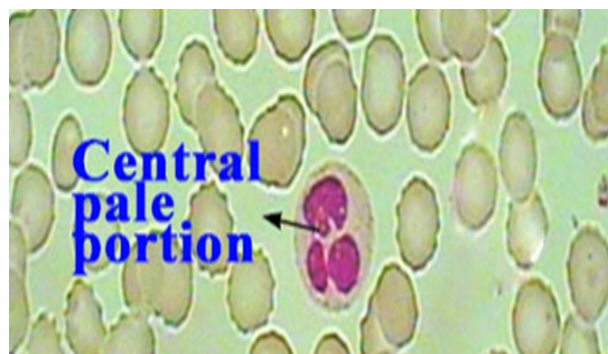


Fig.5: Neutrophil: Small club shaped Non-specific appendage.



The mean number of drumsticks including non-specific appendages and true drumsticks were found to be significantly higher in females (Table.4). The mean number of non-specific appendages was found to be comparatively higher in males compared to that in females (1.10 vs 0.84) though the difference is not statistically significant(Table.4). An analysis of different types of non-specific appendages reveals that the mean number of almost all types of non-specific appendages is found to be higher in males than in females except sessile nodules. However the statistical significance was found with regard to racket structures in males and sessile nodules in females.

Table 1: Sex wise distribution of Drumsticks and non-specific appendages.

Sl. No	Sex	Number of cases examined	Total number of cells counted	Number of cells an containing drumsticks and non-specific appendages	Number of cells containing true drumsticks (percentage incidence)	Number of cells containing non-specific appendages (percentage Incidence)
1	Male	47	4700	235 (5%)	183(3.9%)	52 (1.1 %)
2	Female	63	6300	532 (8.4%)	479 (7.6%)	53 (0.8 %)
3	Total	110	11,000	767(7.0 %)	662 (6.0%)	105 (1.0 %)

Table.2: Percentage incidence of non-specific appendages-Sex wise.

Sl. No	Sex	Various shapes of non-specific appendages			
		Sessile nodules (Fig. 2)	Minor lobes (Fig. 3) No.	Racket structures (Fig. 4) No.	Small clubs (Fig. 5) No.
1	Male	--	24 (46.2%)	22 (42.3%)	06 (11.5%)
2	Female	33 (62.3%)	15 (28.3%)	03 (5.7%)	02 (3.7%)
3	Total	33 (31.4%)	39 (37.1%)	25 (23.9%)	08 (7.6%)

Table 3: Sex- differences in incidence of combination of drumsticks and non-specific appendages.

Sl.No.	Sex	Number of cases with drumsticks and non-specific appendages							
		drumsticks- sessile nodules		drumsticks-minor lobes		drumsticks-racket structures		drumsticks-small clubs	
		No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)
1	Male	--	--	10	58.80%	14	93.30%	3	75%
2	Female	13	100%	7	41.20%	1	6.70%	1	25%
3	Total	13	100	17	100	15	100	4	100

Table 4: Mean number of various types of Drum sticks by Sex.

Type of drum stick cell	Sex		P value and significance
	Male Mean (95% CI)	Female Mean (95% CI)	
Total no. of drum sticks	5.00 (4.69 – 5.31)	8.41 (8.01 – 8.81)	<0.001; S
Total no. of Non-specific Appendages	1.10 (0.81 – 1.39)	0.84 (0.54 – 1.14)	0.22; NS
Total no. of true drumsticks	3.89 (3.66 – 4.12)	7.57 (7.30 – 7.84)	<0.001; S
No. of minor lobes	0.51 (0.25 – 0.77)	0.24 (0.10 – 0.38)	0.051; NS
Number of Racket structures	0.46 (0.25 – 0.67)	0.05 (0.01 – 0.10)	<0.001; S
No. of small clubs	0.12 (0.01 – 0.23)	0.03 (0.01 – 0.07)	0.09; NS
No. of sessile nodules	0.00 (0.00 – 0.00)	0.52 (0.31 – 0.73)	<0.001; S
Drum stick head diameter	1.11 (1.10 – 1.12)	1.08 (1.07 – 1.09)	<0.001; S
Drum stick stalk length	1.10 (1.09 – 1.11)	1.07 (1.06 – 1.08)	0.006; S

Table 5: Sex wise distribution of varying sizes of heads of drumsticks and non-specific appendages.

Sl.No	Sex	Head size- (Range:<1.0->1.2) in microns									
		Category 1 (<1.0)		Category 2-1		Category 3 (>1.0)		Category 4-1.2		Category5 (>1.2)	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	Male	6	75%	44	20.80%	114	33.10%	65	36.50%	2	20%
2	Female	2	25%	167	79.20%	231	66.90%	113	63.50%	8	80%
3	Total	8	100	211	100	345	100	178	100	10	100

The mean number of drumsticks was found to be higher in 15-19 years followed by 20-24 years and 25 & above age groups. The mean number of non-specific appendages however did not show any specific pattern. Either the true drumsticks or non-specific appendages did not show any statistical difference in terms of mean number of cells.

The mean diameter of head and mean stalk length of the drumsticks were measured in both sexes for both true drumsticks and non-specific appendages. Sex wise distribution of varying sizes of heads of true drumsticks and non-specific appendages were represented in

Table.5. The size of head in majority of cases in both the sexes falls into the category 3 (>1.0 micron). When the head size of true drumsticks and non-specific appendages are analysed separately (Table. 6 and 7) it pointed out that less than 1.0 micron diameter head size was observed in non-specific appendages group only. Table 6 predicts the higher percentage of true drumstick head size being concentrated in category 2 (1.1) among both the sexes. Table.7 shows the incidence of head size of non-specific appendages in males and females which was found to be high in category 3(24-57.2% and 18-42.8%).

Table 6: Percentage incidence of varying sizes of heads of true drumsticks-Sex wise.

Sl.No	Sex	Head size - (Range:1.0->1.2) in microns							
		Category 1 -1		Category 2 -1.1		Category 3 -1.2		Category 4 -1.25	
		No.	%	No.	%	No.	%	No.	%
1	Male	34	18.10%	90	29.70%	58	37.40%	--	0
2	Female	154	81.90%	213	70.30%	97	62.60%	4	100%
3	Total	188	100	303	100	155	100	4	100

Table 7: Sex wise percentage incidence of varying size of heads of non-specific appendages.

Sl.No	Sex	Head size - (Range:<1.0->1.2)microns									
		Category 1 -0.5		Category 2 -1		Category 3 -1.1		Category 4 -1.2		Category 5 (>1.2)	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	Male	6	75%	10	43.50%	24	57.20%	7	30.50%	2	33.40%
2	Female	2	25%	13	56.50%	18	42.80%	16	69.50%	4	66.60%
3	Total	8	100	23	100	42	100	23	100	6	100

Table 8: Sex wise distribution of length of stalks of true drumsticks and non-specific appendages.

Sl.No	Sex	Stalk length -(Range:<1.0->1.2) in microns									
		Category 1 (<1.0)		Category 2 -1		Category 3 (>1.0)		Category 4 -1.2		Category 5 (>1.2)	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	Male	--	0	64	27.80%	108	31.50%	62	37.30%	--	0
2	Female	9	100%	166	72.20%	235	68.50%	104	62.70%	1	100%
3	Total	9	100	230	100	343	100	166	100	1	100

Sex wise distribution of varying lengths of stalks of true drumsticks and non-specific appendages were represented in Table.8. Majority of both the sexes fall into the category 3 (>1.0 micron) followed by category 2 (1.0 micron). When the lengths of stalks of true drumsticks and non-specific appendages are separately analysed (Table. 9 and 10) category 3 (1.1 microns) shows higher incidence in both the sexes for length of stalk of true drumsticks while that of category 2 and 4 showed high incidence in females compared to males (Table.9). In cases of non-specific appendages Category 2, 3 showed higher proportions in females compared to males and category 4 showed a high proportion in males (Table.10).

Table 9: Sex wise calculation of Percentage incidence of varying length of stalks of true drumsticks.

Sl.No	Sex	Stalk length -(Range:<1.0->1.2) in microns									
		Category 1 (<1.0)		Category 2 -1		Category 3 (>1.0)		Category 4 -1.2		Category 5 (>1.2)	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	Male	--	0	64	27.80%	108	31.50%	62	37.30%	--	0
2	Female	9	100%	166	72.20%	235	68.50%	104	62.70%	1	100%
3	Total	9	100	230	100	343	100	166	100	1	100

Table 10: Percentage incidence of varying lengths of stalks of non-specific appendages Sex-wise.

Sl.No	Sex	Stalk length -(Range:<1.0->1.2) in microns									
		Category 1 -0.5		Category 2 -1		Category 3 -1.1		Category 4 -1.2		Category 5 -1.25	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	Male	--	0	48	24.40%	86	29.10%	51	35.00%	--	0
2	Female	7	100%	149	75.60%	210	70.90%	95	65.00%	1	100%
3	Total	7	100	197	100	296	100	146	100	1	100

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 the literature there was no mention about the percentage incidence of nonspecific appendages like sessile nodules, racket structures, minor lobes and small clubs in the blood neutrophils along with the drumsticks. Morphology, morphometry and incidence of these non-specific appendages were dealt in the present work. Though it was stated in the 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.

The total count of drumsticks including non-specific appendages and true drumsticks outnumbered in females than in males (8.4% vs 5.0%) in the present study. According to literature [7] typical drumsticks are rarely seen in normal men but, in the present study 3.9% incidence of true drumsticks was observed in males. The number of true drumsticks was higher in females when compared to males (7.6% vs 3.9%) in the present study agreeing with that reported in literature [1,4].

The percentage incidence of non-specific appendages did not show sex differences in the present study and is in agreement with that reported by brahmi et.al., in literature

According to Brahmi et.al., and Tomonaga et.al., [4,6] other than sessile nodule (tag and hook shape) are more in males. Males showed a higher proportion of non-specific appendages-racket structures (42.3%), small clubs (11.5%) and minor lobes (46.2%). Sessile nodules were totally absent in males and were seen in females (62.3%) only. According to Mehes and Jobst [8] increase in high androgen level in male is responsible for small clubs and hooks.

Single cell with 2 drumsticks reported in a patient

with XXX sex chromosomes [9,10]. Kesaree and Woolley [11] reported single cell nuclei with a combination of true drumstick and a non-specific appendage among the controls. Combination of drumsticks and racket structures was higher among males in comparison to the other three combinations (drumstick-sessile nodule, drumstick-minor lobes, and drumstick-small clubs) in the present study. Tri combination of drumsticks showed differences in their combinations between the sexes and were found to be high in males.

The morphometric observations on head and stalk of true drumsticks and non-specific appendages could not be compared as there were no studies on these parameters in literature. The head size and stalk length of true drumsticks and non-specific appendages measured in both the sexes showed a value of 1.1 μm and this type of detailed analysis on morphometric parameters as was done in the present study was not reported in literature.

The present study forms a data base for morphological and morphometric parameters of drumsticks and suggests that true drumsticks, sessile nodules and racket structures are sex specific.

Conflicts of Interests: None

REFERENCES

1. Davidson WM, Smith DR. 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. Mohamed Brahimi, Affaf Adda, Hassiba Lazreg, Hedjer Beliali, Soufi Osmani, Mohamed Amine Bekadja. Can sex be determined from a blood smear. *Turk J Hematol* 2013;30:53-57.
5. Briggs DK. 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. Ursula Mittwoch. Frequency of drumsticks in normal women and in patients with chromosomal abnormalities. *Nature* 1964;201:317-319.

8. Mehes K, Jobst K. Hormonotherapy effect on rat neutrophil leucocytes chromatin sex. *Morph Ig Orv Szle* 1962; 2:34.
9. Jacobs PA, Baikie AG, Court Brown WM., MacGregor TN, Maclean N, Harnden DG. *Lancet*, 1959; ii: 423.
10. Wondergem MJ, Ossenkoppele GJ. Genotyping by morphology. *Blood* 2011; 117:2566.
11. Kesaree N, Woolley PV Jr. A phenotypic female with 49 chromosomes, presumably XXXXX. A case report. *J Pediatr*. 1963;63:1099-1103.

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