Research Article

Reliability of neutrophilic nuclear appendages in morphological sex differentiation

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ABSTRACT

Background: The inactive X-chromosome in neutrophils appears in one of the three forms. They are drumsticks, racquet forms, and sessile nodules. **Objective:** A correlative study based on the presence of "drumstick and other nuclear appendages" in polymorphonuclear neutrophils to determine the morphological sex. **Materials and Methods:** Sixty-eight randomly selected blood smears (34 males and 34 females) were stained with Leishman's stain. One hundred well-stained neutrophils were double-blindly studied in the tail-end of the smears and classified into four groups based on Kosenow's formula as drumstick (Form A), sessile nodule (Form B), and other pedunculated nuclear projections such as tag and hooks (Form C). **Results:** Significant correlation of neutrophilic nuclear appendage of Form A ($P = 0.00001$) and Form C ($P = 0.00016$) was obtained for females and males, respectively. Difference of A-C gives a positive value in females and a negative value in males. **Conclusion:** Neutrophilic nuclear appendages which include true drumsticks, sessile nodules, and racket structures form a useful tool for morphological sex differentiation.

KEY WORDS: Neutrophils; Drumstick; Sessile Nodule; Morphological Sex; Non-specific Appendages

INTRODUCTION

Sex chromatin seen as darkly staining mass at the nucleus of all non-dividing cells of genotypically females represents the heterochromatin of the inactivated X-chromosome. It is also found in polymorphonuclear leukocytes of normal females as a drumstick-shaped mass attached to one end of the nuclear lobes.

Leukocyte test for the diagnosis of sex chromatin depends on the identification of specific nuclear formations which was first recognized as important by Davidson and Smith in 1954. They described neutrophilic nuclear appendage that becomes separated from the main nuclear lobe in females, a drumstick which has a solid round head attached to one lobe of the nucleus by a single, chromatin strand.[1]

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Drumsticks are present in peripheral blood of individuals carrying more than one X-chromosome in at least part of their granulocytes. The original description of drumsticks notes that they had to be distinguished from sessile nodules, small clubs, minor lobe, and tags, all of which can be observed in male neutrophils.

Studies have shown that neutrophilic nuclear appendages are genetically determined, constituted of sex chromatin, but the frequency and the distribution were also influenced by other factors including hormones, granulocyte metabolism, cell proliferation, and age. Recording of sex chromatin has gained importance in human genetics for establishing the relationship with X-chromosome and determination of the sex of individual has great medico-legal importance to solve many criminal and civil problems.[2-4]

Although earlier studies state that typical or true drumstick appendage are rarely seen in normal males, later observation

in literature suggests that they can be seen in males but with lesser percentage compared to females.[5-8]

The present study is aimed to identify the morphological sex of the individual by screening neutrophils for the percentage of various forms nuclear appendages.

MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, The Oxford Medical College, Hospital & Research Center, Bengaluru. Sixty-eight individuals (34 males and 34 females) were randomly chosen from outpatient and inpatient patients of various departments of our institute, submitted in the Department of Pathology for complete blood count with the following criteria for subject selection. Total leukocyte count between 4000 and 11000/cumm. Distribution of cell was within normal limits. At least 100 well-stained non-shrunken neutrophils should be observed in the smear. Neutrophils should not show toxic granules. There should not be shift to left. Ethical clearance was obtained from Institutional Ethics Committee.

Venous blood samples collected into ethylenediaminetetraacetic acid tubes and thin tongue-shaped smears were made, labeled, and stained with Leishman's stain. Automated hematology analyzer (Sysmex XP-100) was used to determine neutrophil counts. Peripheral smears were examined under the light microscope in oil immersion. One hundred well-stained neutrophils were double-blindly studied in the tail-end of the smears and classified into four groups based on Kosenow's formula: Drumstick as Form A, sessile nodule as Form B, and different types of nuclear projections such as small club, hook, and tags as Form C. The difference of A-C was calculated and "cytological sex" defined as female in positive value and as male in negative value.

For statistical evaluation, continuous variables were summarized as mean ± standard deviation. Comparisons between continuous variables were using the Student's *t*-test. $P = 0.05$ was used as a threshold for significance.

RESULTS

Peripheral smears collected from 34 males and 34 females, with age range of 20-83 years and 22-60 years respectively, were observed for sexual differences in percentage incidence and morphology of true drumsticks and non-specific neutrophilic nuclear appendages. The true drumstick nuclear appendage presented a rounded head and a thin stem (Figure 1a). The various types of non-specific appendages observed were sessile nodules (Figure 1b), tags (Figure 1c), and hook-like forms (Figure 1d).

The number of true drumsticks was found to be significantly higher in females, with a mean of 5.4, whereas among males, incidence of Form A appendages was lower with a mean of 2.1. Form B: Sessile nodules were seen in slightly higher number among females. Form C: Tag and hooks were seen in increased frequency among males with mean of 7.4 (Table 1).

On comparison of nuclear appendages in females and males, Student's *t*-test results mentioned in Table 1 shows that neutrophilic appendages were seen in both the sexes. However, Form A is much more common in females ($P = 0.00001$) and Form C is frequently seen in males ($P = 0.00016$). Difference of A-C gives a positive value in all females and a negative value in all males.

DISCUSSION

In the present study, age range of male and female subjects was 20-83 years and 22-60 years, respectively. Average number of true drumsticks (Form A) seen in polymorphs among female was 5.4 and in male was 2.1. Non-specific neutrophilic appendages, sessile nodule (Form B) was observed with an average of 0.6 in females and 0.3 in males, whereas tag and hook forms (Form C) were seen with a mean of 2.9 in females and 7.4 in males. Difference of Form A-C showed a positive value (mean: +2.5) for female subjects and negative value (mean: −5.3) for male subjects.

In this study, neutrophilic nuclear appendages were seen in nearly same frequency both in female and male subjects (Table 1), comparable to a study by Brahimi et al., which showed that the leukocytes of both sexes bore nearly the same frequency of appendages.[2] Frequency of Form A varied from 2 to 13 in females and 0-7 in males. Similar observation is made by Tomonaga et al.' s examination of 50 blood smears belonging to male subjects which found the frequency of Form A from 0 to 6.[9] Although Briggs stated

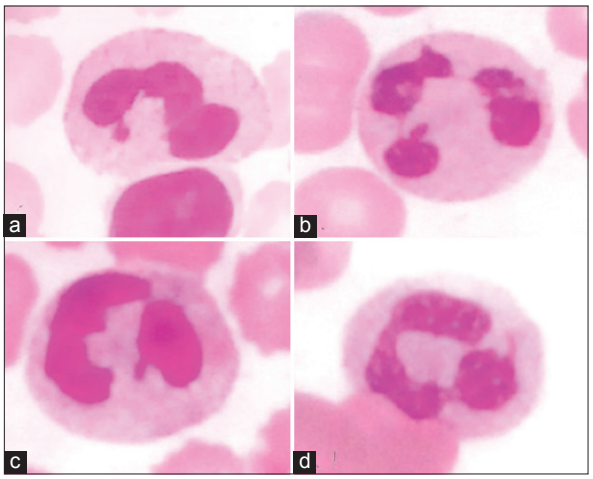

Figure 1: Forms of neutrophilic nuclear appendages: (a) Form A: Drumstick, (b) Form B: Sessile nodule, (c) Form C: Tag, (d) Form D: Hook

Table 1: Comparison of nuclear appendage of neutrophils in female and male peripheral smears

Appendages	Female		Male		Student *t*-test	
	Range	Mean±SD	Range	Mean±SD	T-score	*P* value
Form A	2-13	5.4±2.6	0-7	2.1±1.8	5.858927	0.000010
Form B	0-5	0.6±1.2	0-3	0.3±0.7	1.482269	0.071667
Form C	0-9	2.9±2.3	2-35	7.4±6.3	3.807599	0.000162
A-C	0-9	2.5±1.9	(−1)-(−31)	−5.3±5.7	2.629515	0.005384
A+B + C	2-21	9.1±4.9	2-42	9.7±7.9	0.468044	0.320711

SD: Standard deviation

that drumsticks are never seen in males, the present study and many other investigators in their literature suggested that the true drumstick appendages can be seen in males.[5-8] In a normal women, 6 or more drumsticks/500 cells can be seen. According to Mittwoch on an average, <3/100 neutrophils can show drumstick appendages. Other studies have shown drumsticks in up to 17% of neutrophils in the peripheral smear of a healthy women.[10-12]

Form B was seen with slightly higher frequency among females whereas Form C was observed with increased frequency among males with average of 7.4 compared to females' average of 2.3. The difference of A-C gives a positive value in females and negative value in males. Tupakula et al. and other investigators also found that true drumsticks can be seen in males though their percentage incidence is less and combination of Form A and C can be seen in significant number.[2,7,8] Méhes study suggested that appearance of nuclear appendages other than Form A and B depends on the aging, segmentation, and metabolism of neutrophils.[13]

Although the study brings an important observation with respect to various forms of neutrophilic nuclear appendages, limitations of our study include relatively small sample size and study was from a single institution; thus, it cannot be considered wholly as a reflection of entire population.

CONCLUSIONS

In observation from the present study, while using leukocyte test for the diagnosis of sex chromatin, it is essential to consider all forms of neutrophilic nuclear appendages which include true drumsticks, sessile nodules, and racket structures for morphological sex differentiation. This method is easy, reliable, less time-consuming, and cost-effective.

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