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Original Article

Reliability of sexual dimorphism in blood

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Abstract

Background : Demonstration of sex chromatin forms an important aspect of human genetics. It also establishes the interrelationship between sex chromatin and an inactive X-chromosome. The term “sex chromatin” in blood refers to the “Drumsticks of polymorphonuclear leukocytes” or “Davidson’s bodies”. Objective: This correlative study evaluates the presence of these drumsticks quantitatively and also highlights the concept of blood chimaerism in humans. Method: Leishman-stained peripheral blood smears from 60 individuals (30 males and 30 females) were obtained and studied under bright-field microscope (40X) for presence of Drumstick appendages. Results and conclusion: On comparing mean numbers of Davidson’s bodies in females and males, an extremely significant correlation ($P<0.0001$) was seen. Hence, it could be surmised that the presence of appendages in neutrophils (Drumstick bodies) can be useful in gender differentiation.

Introduction

The “drumsticks” of the polymorphonuclear neutrophils were first identified by Davidson and Smith as nuclear appendages related to sex chromatin. These drumstick appendages are found on 0.5% to 2.6% of neutrophils. In their original observation, Davidson and Smith classified five types of nuclear appendages in mature neutrophils: drumsticks, sessile, nodules, small clubs, minor lobes and racket formation. They considered only ‘drumsticks’ to be related to the sex chromatin (1). However, Kosenow believed that sessile nodules are of equal sex-diagnostic significance (2, 3). The purpose of this study was validate the Davidson’s bodies as confirmatory guide towards sex identification at par with more studied, Barr bodies. Hence, it intends to highlight the use of Davidson bodies as a useful parameter for gender identification in a North Indian population subset to assess demographic variables, if any.

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Methods

60 subjects (30, males and 30, females) were chosen at random. Informed consent was obtained and peripheral blood smears were made. Slides were stained with Leishman’s stain. 100 well stained neutrophils were double-blindly studied in the tail-end of the smears under 100X magnification. Neutrophils were identified and drumstick appendages were identified and recorded under oil-immersion objective according to Davidson and Smith’s criteria (1). However, in this study neutrophils were classified into: Form A (Drumstick-containing) and Form B (Non-Drumstick containing).Observations pertaining

to evidence or absence of Drumstick bodies were done on coded sheets. On completion of microscopic evaluation, identity of coded sheets were disclosed as per gender criteria.

Inclusion criteria for subject selection were :

1. Total leukocyte count between 4000-10,000 cells/ cu.mm.
2. Normal quantitative distribution of blood cells.
3. At least 100 well-stained, non-shrunk neutrophils observed in the smear.
4. Identification of a classical 'drumstick' with its rounded head and a narrow stalk (4).

Exclusion criteria were :

1. Hormonal therapy.
2. Children, menstruating, perimenopausal and menopausal females.
3. Immunosuppressive therapy/conditions.
4. Any other systemic condition requiring medical intervention.

Mean±S.D. were calculated for microscopic evidence of Drumstick bodies for male and female genders and P values determined. Level of significance was set at 0.05. A P value of < 0.0001 was considered extremely significant.

Results

On comparison of mean Davidson's bodies in females and males, an extremely significant correlation (P<0.0001) was seen (Table I). Hence, it could be surmised that the presence of appendages in neutrophils (Drumstick bodies, Davidson's Form A) can be useful in gender differentiation.

TABLE I : Table depicting mean numbers, median, standard deviation and P values on comparison between Drumsticks in male and female peripheral blood smears.

	Females	*Males*	*P value*
Mean numbers	7.6	0.8	< 0.0001 (extremely significant)
S.D.	3.15	1.8	
Sample size	30	30	
Standard error of means	0.5760	0.3301	
Low 95% confidence limit	6.489	0.1249	
Upper 95% confidence limit	8.845	1.475	
Median (50^{th} percentile)	7	0	

Discussion

Neutrophilic projections, excluding the genetically determined drumsticks and sessile nodules are nonspecific leukocytic pseudosegments. Their appearance depends on the segmentation, aging and metabolism of neutrophils. These features are also influenced by hormonal effects (5, 6). Mehes observed that androgens might induce an increase in the numbers of nuclear appendages (7). Estrogens produce only an initial increase in drumstick count and subsequent continued treatment, neither increases nor decreases the drumstick counts significantly (5). Although the appearance of these figures may be associated with certain disease, it should not be regarded as a specific sign for a single pathologic or physiologic condition. Davidson and Smith demonstrated sexual dimorphism of leukocytes (cytological sexual characteristics) by means of presence or absence of drumsticks. Leukocytic drumsticks are stalked, rounded chromatin appendages, 1.5 microns in diameter, projecting from the neutrophilic nuclei of female subjects only. In this study, 100% of female subjects and 0.2% of males exhibited Drumstick bodies. Few male subjects in this study demonstrated cellular chimerism due to the presence of neutrophilic drumsticks. This finding was reported earlier by Davidson implying that the cells ancestors can be grafted in initial stages of embryogenesis due to the influence of female sex hormones. Thus, affecting the cellular lineages (5).

According to Kosenow, the neutrophilic nuclear appendages can be classified as: "Forms A": drumsticks; "Forms B": Sessile nodules and "Forms C": Leukocytes with other pedunculated nuclear projections that are easily distinguished from forms "A and B" and appear as small clubs, hooks, tags etc (8). Harnack and Strietzel were first to demonstrate that all three forms were more frequent in children than in adults, irrespective of the gender (9). Mehes experimented by hydrolyzing leukocytes with 5N HCl, followed by staining and observing under

phase microscope. He surmised that Forms A and B are heterochromatic while form C is euchromatic (7). This indicated that forms C were not specific bodies of the leukocytes while drumsticks and sessile nodules might be regarded as equivalents of sex chromatin, thus, confirming Muller's hypothesis that sessile nodules are preformed drumsticks (10). Osztonis and Focher found that incidence of forms C was below normal in patients with pituitary hypofunction, and above normal in pre-puberty pituitary gland. According to these authors, the nuclear configuration of the leukocytes is influenced by pituitary basophils (11). Present study, found form A as a predominant feature in identification of cytological sex in accordance with observations recorded by Brahimi et al. Variations in X-chromatin frequency of female cells have been reported under different circumstances such as: during various hormone treatments, extensive burns, during menstrual cycle and during pregnancy.

The term 'sex chromatin' primarily encompasses two structures: 1) Barr body, present in epithelial cells; 2) Drumstick of the polymorphonuclear leukocytes. A drumstick comprises of a small nuclear mass, about 1.5 µ in diameter, attached to the body of nucleus by means of a thin filament. It is now accepted fact that the drumstick is an expression of an X-chromosome in cells and that the drumsticks and Barr bodies are equivalent structures. Individuals with chromosomal abnormalities for example, Klinefelter's syndrome have an incidence of drumsticks lower than that of normal females whereas the frequency of Barr bodies is not decreased. Haque et al in their study concluded that neutrophil nuclear drumsticks and mucosal cell Barr are independent variables related to maturation and nuclear configuration factors (12).

Present study exhibited a strong correlation between the observation of Davidson's bodies and gender differentiation. Hence, corroborating their value as independent variable for the identification of sex chromatin inaccordance withBrahimi et al (13, 14, 15).

Briggs surmised that drumsticks cannot be observed inleukocytes of males (4). However, this notion is in contradiction with ourfindings and those of many other investigators. In our series, inaccordance with the findings of others, 5 men out of 30 haddrumsticks in 0.2% of their polymorphs (3, 11, 13). This finding is corroborated by Tomonaga et al' s examination of 50 blood smears belonging to male subjects. They foundthat the frequency of form A varied from 0 to 6 per 1000polymorphonuclear neutrophils (3).

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