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.

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

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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-shrunken 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

<table>
<thead>
<tr>
<th>Females</th>
<th>Males</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean numbers</td>
<td>7.6</td>
<td>0.8</td>
</tr>
<tr>
<td>S.D.</td>
<td>3.15</td>
<td>1.8</td>
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<tr>
<td>Sample size</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Standard error of means</td>
<td>0.5760</td>
<td>0.3301</td>
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<tr>
<td>Low 95% confidence limit</td>
<td>6.489</td>
<td>0.1249</td>
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<tr>
<td>Upper 95% confidence limit</td>
<td>8.845</td>
<td>1.475</td>
</tr>
<tr>
<td>Median (50th percentile)</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

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 the presence of appendages in
neutrophils (Drumstick bodies, Davidson’s Form A)
can be useful in gender differentiation.

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).
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). Osztovis 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 in leukocytes 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 had drumsticks 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).

References