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A Comparative Study of Drum Stick pattern in the Polymorpho-Nuclear Neutrophil Leucocytes in Common Mammals

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ABSTRACT

Barr and Bertram announced in 1949 that sexual dimorphism existed down to cellular level (at the nuclear level. Davidson and Smith were the first to identify the peculiar nucleus projections in neutrophils and define their relationship with sex chromatin of the cells. Blood of following mammals were obtained for the study.Primate (Human) – 10 (5 males & 5 females), Lagomorpha Rabbit (Lepus cuniculus) - 10 (5 males and 5 females), Rodentia albino mouse (Mas masculus L.) - 10 (5 males & 5 females), Artiodactyla sheep (Ovis aries indicus) - 10 (5 males & 5 females). The present study helps to establish a simple and reliable method for diagnosis of difficult, abnormal and confusing cases of the genetic abnormalities Percentage incidence of polymorphonuclear drumsticks presented a valuable data on sex differences.

KEYWORDS Sex chromatin, Drumstick, neutrophil, leucocyte

INTRODUCTION

Barr and Bertram announced in 1949 that sexual dimorphism existed down to cellular level (at the nuclear level). 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). Davidson and Smith were the first to identify the peculiar nucleus projections in neutrophils and define their relationship with sex chromatin of the cells. (2) Because of its characteristic shape they called it as drum stick. Bamford classified the drum stick as follows: Large drum stick and Normal drum stick. (3) Other non specific nuclear appendages noted in polymorpho nuclear neutrophil leucocytes are named as 1) Racket 2) club, hooks &Rods 3) Sessile nodules (4). Though it was stated in the literature that drumsticks are never seen in males [5] but in our study and some other studies [4.6] suggested that true drumsticks are also present in males though their percentage incidence is less. The present study helps to establish a simple and reliable method for diagnosis of difficult, abnormal and confusing cases of the genetic abnormalities.

MATERIALS AND METHOD

Mammals required for present study are as following:

1. Primate (Human) – 10 (5 males & 5 females)
2. Lagomorpha Rabbit (Lepus cuniculus) - 10 (5 males and 5 females)
3. Rodentia albino mouse (Mas masculus L.) - 10 (5 males & 5 females)
4. Artiodactyla sheep (Ovis aries indicus) - 10 (5 males & 5 females).

Blood of above mentioned mammals were obtained as follows:

Primate -Human: Relatives and friends.

Lagomorpha- Rabbit, Rodentia- albino mouse, Artiodactyla- sheep: from Veterinary College, Bikaner.

All the mammals under study were physically and mentally healthy. A drop of peripheral blood was taken from each mammal for making the blood film and then stained with Wright's stain and haematoxylin stain. Some slides of haematoxylin stain were also counterstained with 1 % eosin.

Staining with Wright's stain:

1. Place the blood films on a staining trough and cover it with the Wright's stain solution.
2. Keep it for one minute and then dilute the stain with equal volume of distilled water.
3. Keep the diluted stain for 5 minutes.
4. Wash the film in a stream of distilled water so that no traces of stain remain on the film. Dried the film and examined.

Staining with Harris's Haemotoxylin stain:

Fixed and dried films were dipped in to a capling jar containing the solution of Harris's Haemotoxylin and kept for one minute. Then the stain was developed by placing the slide under running tap water for ten minutes. Dried the film and examined.

Staining procedure with Eosin stain:

In some cases the stained and developed films from haematoxylin stain were counter stained with 1 % aqueous solution of eosin for 15 seconds. After that films was washed rapidly under tap water and then film was dried and examined.

Examination of stained films:

Stained blood films were examined under high power (10x X 45x) and oil immersion lens (10x X 100x). Examination was carried out from the left to right corner of the film. Then film was moved a little forward and examined from right to left. In each film 500 polymorph nuclear neutrophil leucocytes were examined for typical drum stick. The polymorph nuclear neutrophil cells were considered typical when it contained an oval or rounded chromatin mass nearly 1 to 1.5 micron remained separated from the main lobe of the nucleus by a thin chro-

matin thread. Drum stick recorded till 500 cells were screened. Thus 1000 cells were examined from two films of a same mammal to minimize possible error.

OBSERVATION

Table 1: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of primate human male.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	5	4	0.9%
2.	5	5	1.0%
3.	3	2	0.5%
4.	2	2	0.4%
5.	5	4	0.9%

Mean percentage: 0.74%

Table 2: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of primate human female.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	15	17	3.2%
2.	18	17	3.5%
3.	24	27	5.1%
4.	10	14	2.4%
5.	11	13	2.4%

Mean percentage: 3.32%

Table 3: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of Lagomorpha Rabbit males.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	4	5	0.9%
2.	5	5	1.0%
3.	4	3	0.7%
	2	2	0.4%
5.	3	2	0.5%

Mean percentage: 0.7

Table 4: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of Lagomorpha Rabbit females.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	44	44	8.8%
2.	35	34	6.9%
3.	37	35	7.2%
4.	38	37	7.5%
5.	32	35	6.7%

Mean percentage: 1.74%

Table 5: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of albino mouse male.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	15	14	2.9%
2.	10	12	2.2%
3.	14	14	2.8%
4.	13	12	2.5%
5.	14	15	2.9%

Mean percentage: 2.66%

Table 6: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of albino mouse female.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	15	52	10.1%
2.	10	51	10.5%
3.	14	54	11.1%
4.	13	51	10.2%
5.	14	67	13.2%

Mean percentage: 11.02%

Table 7: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of Artiodactyla sheep male.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	4	5	0.9%
2.	1	2	0.3%
3.	4	4	0.8%
4.	5	5	1.0%
5.	4	3	0.7%

Mean percentage: 0.74%

Table 8: Showing the frequency percentage of drum stick in polymorph nuclear neutrophil leucocytes counted in 500 cells of film of Artiodactyla sheep female.

Animal Number	No. of drum stick in first 500 neutrophils	No. of drum stick in second 500 neutrohils	Average percentage of drum stick
1.	40	41	8.1%
2.	43	42	8.5%
3.	45	44	8.9%
4.	29	33	6.2%
5.	37	41	7.8%

Mean percentage: 7.9%

RESULT & DISCUSSION:

1) Primate Human:

Briggs et al reported that the recognisation of drum stick is

only single polymorph nuclear neutrophil leucocytes is enough to say that individual belongs to female sex.

Mittwoch U et al (7) noted average incidence 3.66% in normally healthy human females.

By present study frenquency of drum sticks in polymorph nuclear neutrophil leucocytes are observed 2.4% to 5.1% with mean 3.32% in primate human females (Table 2) & 0.4% to 1.0% with mean 0.74% in private human males (as in Table 1).

2) Lagomorpha Rabbit:
De Castro (8) performed an extensive work on the frequency variation of the drum sticks in polymorph nuclear neutrophil leucocytes in the eripheral bloob of rabbit. In normal female rabbit the frequency variation of the drum sticks was found to be 6.5%, a much higher value than that of in human female and not mentioned at all about its existence in males.

Frequency of drum sticks by present study was observed 6.7% to 8.8% with mean 7.42% in female rabbit and 0.4% to 1.0% with mean 0.7% in male rabbits as shows in Table 3 & 4.

Rodentia Albino Mouse:
Regaeding albino mouse no literature is available for establishment of nuclear sex by examining the peripheral blood smear for drum sticks in polymorph nuclear neutrophil leucocytes. By present study, the frequency in female albino mouse varied from 10.1% to 13.2% with mean 11.02% while this frequency noted in male albino mouse 2.2% to 2.9% with mean 2.66% as shown in 5 & 6 Table no. Total mammals studied for present study are 40 (5 males and 5 females for each four species). Among these mammals the highest incidence of drum sticks 13.2% noted in female albino mouse.

Artiodactyla Sheep:
Colby & Colhoum (9) studied the sex dimorphism in peripheral blood of sheep with some othe animals like cow, dog, goat, horse, cat and pig. In every case they found that drum sticks were present in polymorph nuclear neutrophil leucocytes of female animals only. By present study the Table no.7 shows frequency of drum sticks in male sheep is observed 0.3% to 1.0% with mean 0.7% and in female sheep it varied from 6.2% to 8.9% with mean 7.9%, (as shown in Table no. 8) showing the sex dimorphism. If incidence of drum sticks more than 1.0% then diagnosis of female sheep should be made as concluded by present study.

CONCLUSION
Frequency of drum sticks in polymorph nuclear neutrophil leucocytes is higher in females than males of same species.

Percentage incidence of polymorphonuclear drumsticks presented a valuable data on sex differences.

Screening of large number of polymorph nuclear neutrophil leucocytes for drum sticks helps in minimizing the error for diagnosing sex.

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