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Original Article

Cytomorphometrical Analyses of Blood of Black rat *Rattus rattus* (Linnaeus, 1758)

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Abstract

Quantitative microscopy has strengthened conventional diagnostic scheme through better understanding of microscopic features from clinical perspective. Towards this, analysis of pathological image analysis havegained immense significance among medical fraternity through visualization and quantitative evaluation of clinical features. Till date, pathological inspection of rodents' blood is solely dependent on subjective assessment which usually leads to significant inter-observer variation in grading and subsequently resulting in late diagnosis of certain diseases. This paper aims at a systematic approach to morphologically characterize of five types of white blood cells, and its nuclei from light microscopic image of blood samples. Hence, cellular and nuclei based geometric features are computed and statistically analyzed with t- test to show the discriminating potentiality of the species.

Keywords: *Rattus Rattus;* Blood Cells; Differential Leucocyte Count; Morphometry.

Introduction

Cytomorphometry is a quantitative description of geometrical structures in all dimensions (Baak 1985; Vandiest et al. 1991). Morphometry is the simplest form of image cytometry and refers to the evaluation of cells or tissues by measurement of various cellular features in a two-dimensional view. Abnormal and insufficient white blood cell function is most often reflected in modified cell morphology, and mathematical analysis of morphometrical cell characteristics is very useful for its estimation (Bins 1985). Furthermore, changes in morphometrical erythrocyte indicators have been detected in certain humans(Alexandratou et al. 1999; Manjunatha and Singh 2000) and dog ailments (Berezina et al. 2001). A complete blood count is an ideal indicator of general health, as stress and numerous illnesses can modify haematological parameters, especially with regard to erythrocyte and lymphocyte count (Hinton et al. 1982). A complete blood count is undisputedly the most important diagnostic method available to veterinarians, along with proper anamnesis and physical examination of the animal. The modern computerized geometric, and morphometric methods have been established as efficient tools to quantify differences in the cell shape or morphological structures in particular and can provide a better characterization in describing the complexity of anatomical structures (Grizzi and Chiriva- Internati 2005; Russ 2007; Rosioru et al. 2012).

Morphometry is used as the prognosis and diagnosis of diseases of animals.It enables analysis of changes in to the entire cell, changes in cytoplasm and changes in the nucleus and structures of nucleus (Dalton 1992; Russack 1994). Since thehaemocytomorphometrical data on rodents are inadequate, the particular study onthe blood cells of two species of rats are analyzed, and interpreted.

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Materialsand Methods

The investigation was conducted on wild black rats *R. rattus* (five from each sex) which were caught by wooden and wire net trapping in evening time from the backyard of the residential complex of the Utkal University campus, Bhubaneswar, Odisha. These were allowed to acclimatize to captive condition prior to experimentation and were carefully handled to minimize the stress. Trapped rats were kept at room temperature and were fed with paddy, cereals, and grains. After a week, the rats were anaesthetized by using chloroform in a jar and two milliliter of blood was drawn from the jugular vein with the help of a disposable syringe. Thin blood smears of peripheral blood were prepared from a small drop of fresh blood directly from the needle and were made for each sample to determine the differential blood count and morphometrical analyses of leucocytes and erythrocytes. The morphometry like cellular and nuclear length and breadth of erythrocytes, and leucocytes (monocytes, lymphocytes, neutrophils, eosinophils, and basophils) was undertaken. Cytomorphometry of cells and nuclei of blood of both the species with sexual dimorphism was carried out with the help of Microscope Eyepiece Digital Camera [CatCam130–1.3 Mega Pixel (MP), Code No. CC130, Catalyst Biotech, Maharashtra, India] attached to Hund Wetzlar Microscope [MICROSCOPE H 600 WILOZYT PLAN, Serial No. 1024980, Helmut Hund GmbH, Wetzlar-Nauborn, Germany] and computer.

Results

In this investigation, the differential leucocyte count and cytomorphometrical analyses are taken into account. The findings of this study reveal the effect of sex on blood parameters. The results of differential leucocyte count are tabulated in detail (Table 1). The number of neutrophils is found to be 36.8±1.48 in males and 32±0.59 in females. The number of monocytes is 12.6±0.61 in males and 12.8±0.44 in females. The concentration of lymphocytes is 48.5±1.21 in males and 50.6±0.85 in females. The number of eosinophils is 3±0.33 in males and 3±0.33 in females. The number of basophils is 0.7±0.15 in males and 0.6±0.16 in females.

The morphometrical parameters like length and breadth of both RBC and five categories of WBC such as monocyte, lymphocyte, neutrophil, eosinophil and basophil are measured and expressed in micron metre (µm) (Table 2). The cell

Table 1: Differential leukocyte count of *R. rattus* (per sex n= 05) in number

Sl. No.	Parameters	*Rattusrattus*	
		Male	Female
1	Neutrophil	36.8±1.48	32±0.59
2	Monocyte	12.6±0.61	12.8±0.44
3	Lymphocyte	48.5±1.21	50.6±0.85
4	Eosinophil	3±0.33	3±0.33
5	Basophil	0.7±0.15	0.6±0.16

Table 2: Morphometry of blood cells of *R. rattus* (per sex n=05) in micron

Sl. No	Types of cell	Cell/ Nucleus	Parameters	*Rattusrattus*	
				Male	Female
1	Erythrocyte	Cell	Length	19.8±3.12	6.57±0.11
			Breadth	10.80±0.32	5.91±0.10
2	Monocyte	Cell	Length	4.47±0.36	6.34±0.27
			Breadth	3.90±0.34	5.91±0.24
3	Lymphocyte	Cell	Length	8.14±0.80	5.72±0.29
			Breadth	8.29±0.83	5.22±0.30
		Nucleus	Length	6.34±0.76	6.30±0.73
			Breadth	5.36±0.68	5.31±0.63
4	Neutrophil	Cell	Length	10.59±0.94	5.88±0.39
			Breadth	9.59±0.91	5.19±0.39
5	Eosinophil	Cell	Length	7.93±0.70	4.5±0.32
			Breadth	7.78±0.65	4.08±0.27
6	Basophil	Cell	Length	7.45±0.86	4.39±0.37
			Breadth	6.59±0.86	6.14±2.05

length of erythrocyte is 19.8±3.12, and6.57±0.11 in males and females, respectively. The cell breadth of erythrocytes is 10.80±0.32 in males, and 5.91±0.10 in females. The length of monocytes is 4.47±0.36, and 6.34±0.27 in males and females, respectively. The breadth of monocytes is 3.90±0.34 in males and 5.91±0.24 in females. The cell length of lymphocyte is 8.14±0.80, 5.72±0.29 in males and females, respectively. The cell breadth of lymphocytes is 8.29±0.83, and 5.22±0.30 in males and females, respectively. The nucleus length of lymphocytes is 6.34±0.76 in males and 6.30±0.73 in females. The nucleus breadth of lymphocytes is 5.36±0.68, 5.31±0.63 in males and females, respectively. The cell length of neutrophils is 10.59±0.94, 5.88±0.39 in males and females, respectively. The cell breadth of neutrophils is 9.59±0.91 in males and 5.19±0.39 in females, respectively. The cell length of eosinophil is 7.93±0.70, and 4.5±0.32 in males and females, respectively. The breadth of eosinophils is 7.78±0.65 in males and 4.08±0.27 in females. The cellular length of basophils is 7.45±0.86, and 4.39±0.37 in males and females, respectively. The breadth of basophils is 6.59±0.86 in males and 6.14±2.05 in females.

Fig. 1: Black rat in wire net cage

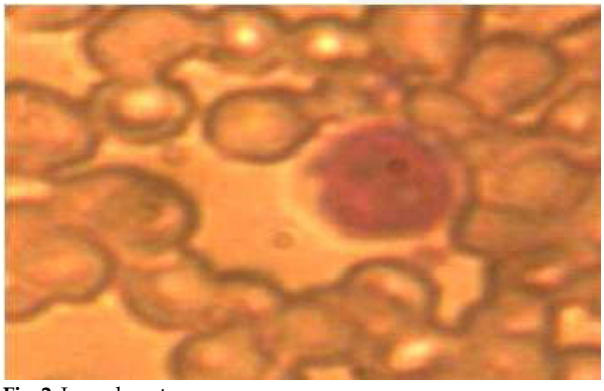

Fig. 2: Lymphocyte

Fig. 3: Neutrophil

Fig. 4: Eosinophil

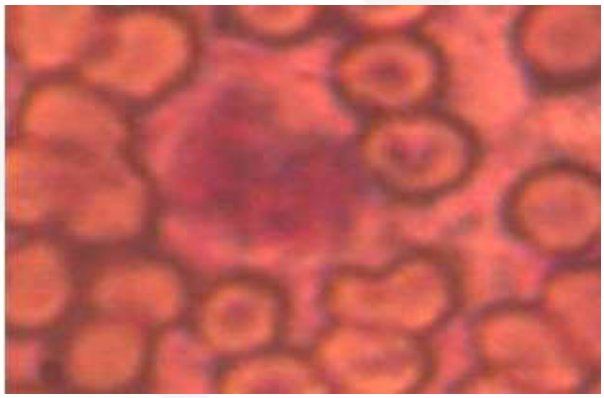

Fig. 5: Basophil

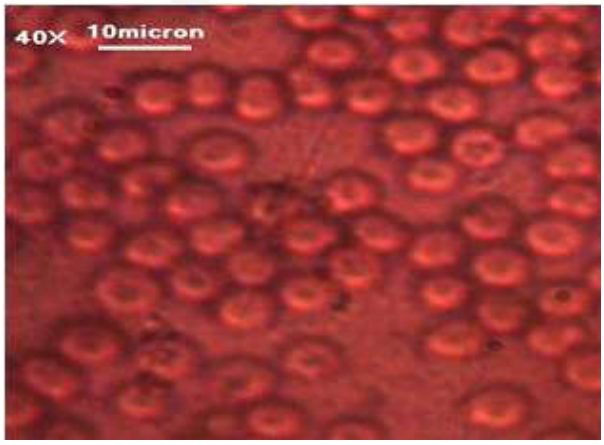

Fig. 6: Erythrocyte

Fig. 7: Monocyte

Discussion

The findings of this study reflect the effect of sex on the size of erythrocytes and leucocytes (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) of *R. rattus*. In this investigation, the cytomorphometrical parameters of blood of healthy black rats have been observed. The findings of cytomorphometrical analyses of *R. rattus* can be helpful in clinical investigation and interpretation. In differential leucocyte count, the highest number of lymphocytes is found in males than females and shows significant difference at $p < 0.01$ which is due to variation in species. It is observed that the percent of monocytes increases in females and males which is due to variation in sexual dimorphism. The neutrophils are found to be more in females in comparison to males which is possibly due to some pathogenic infections. The number of eosinophils is same for both males and females of *R. rattus*. The basophils are observed to be more in males than females which may due to inflammatory responses such as certain types of skin inflammation, asthma or parasite infections, settings in which basophils may appear in the affected tissues.The parameters like monocytes, neutrophils, eosinophils, and basophils do not show any significant difference.

The length and breadth of leukocytes do not vary much due to their round shape. Among agranulocytes (lymphocytes and monocytes), lymphocytes are larger than monocytes.The cellular length and breadth of erythrocytes are noted to be more in males than females which deviates significantly sex wise at $p<0.001$. The cell length and breadth along with nucleus of lymphocytes have more value in males whereas less infemales. The parameters show sex wise significant difference at $p<0.01$. The morphometric parameters of neutrophils are found to be higher value inmalesand lower in females and deviate significantly sex wise at $p<0.001$. The morphometric parameters of eosinophils are observed to be higher in males than females anddeviate with respect to sex which is significant at $p<0.01$, and basophil cell breadthdoes not show significant difference.

Conclusion

The study reveals that as a whole, effect of sex on morphometry of blood cells of black rat but,certain genetic and non-genetic factors such as, onset of maturity, sexual dimorphism, breeding, and environment are believed to affect the shape and size of blood cells. So, it is equally important to consider these factors and detailed investigation as to the stated aspects is suggested to arrive at accurate clinical and physiological interpretations.

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