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Comparative differential leucocyte count and morphometrical analyses of black rats *Rattus rattus* (Linnaeus, 1758) and white rats *Rattus norvegicus* (Berkenhout, 1769)

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Abstract

This paper aims at a systematic approach to morphologically characterize of five types of white blood cells (WBC), and its nuclei from light microscopic image of blood samples. Hence, cellular and nuclei based geometric features are computed and analyzed statistically with *t*-test to show their discriminating potentiality among the species. In morphometry study, the length and breadth along with nucleus of leukocytes are compared between and within the species using one-way Analysis of Variance (ANOVA) followed by Tukey's pairwise comparison tests. In this study, the estimated values of *Rattus rattus* and *Rattus norvegicus* with respect to sex were compared. A total of 20 black and white rats (05 each from males and females) were collected. Blood samples were then collected from the caudal vein of anaesthetized rats. In differential leucocyte count, the parameters namely, lymphocyte, monocyte, neutrophil ($p < 0.001$) and eosinophil and basophil ($p < 0.05$) reveal significant difference. In morphometrical study, the cell length, breadth along with nucleus of lymphocyte, monocyte, neutrophil ($p < 0.01$) and eosinophil, basophil ($p < 0.05$) deviates significantly between and within the species.

Keywords: *Rattus rattus, Rattus norvegicus, Blood cells, differential leucocyte count, morphometry*

1. Introduction

In today's diagnostic paradigm, the technology of microscopic imaging has an immense contribution in generating fruitful medical images, which has essentially become the basis for medical experts for better decision and diagnosis. In practice, experts like radiologists and pathologists, visualize the abnormalities if any, in the images through microscope based on their subjective knowledge from the different point of view such as, intensity, morphology, and texture based features. Cytomorphometry is a quantitative description of geometrical structures in all dimensions (Baak, 1985; and 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

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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; and 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 about erythrocyte and lymphocyte counts (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. However, when the stipulated oscillations of haematological parameters are taken into consideration, care must be taken in interpreting altered results. Cell shape is a large-scale expression of many biological processes, controlled by interactions between the cytoskeleton, the membrane and membrane-bound proteins, and the extracellular environment (Pincus and Theriot, 2007). 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; and Rosioru et al., 2012).

Cytomorphology of blood cells, an important aspect of hematology, can reveal the physiological conditions of the organism. Investigations on the measurement of blood cells are well described in fishes (Homatowska et al., 2002), amphibians (Atatur et al., 1999; and Tok et al., 2009) and reptiles (Oros et al., 2010). The measurements of erythrocytes are available in case of fishes, amphibians, and reptiles (Hartman and Lessler, 1964). Blood cell morphology has been undertaken on several species of herpetofauna (Arikan and Cicek, 2010). In the case of wild black rats, and white rats the data of blood cell morphometry are scanty.

Moreover, according to the physiological condition such as, age, cell morphology also changes (Tadjalli et al., 2003). So, in this study, an attempt has been made to report sex-wise differences in cytomorphometry of erythrocytes and leukocytes of wild black rats and white rats. Morphometrical characterization of blood cells plays a significant role to detect the pathological conditions of animals. In most mammals, erythrocytes are enucleated and biconcave. Morphometry is used as the prognosis and diagnosis of diseases of animals. It enables analysis of changes to the entire cell, changes in cytoplasm and changes in the nucleus and structures of the nucleus (Dalton, 1992; and Russack, 1994). Since the cytomorphometrical data on rodents are inadequate, the particular study on the blood cells of two species of rats are morphometrically analyzed and interpreted.

2. Materials and 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, and white rat *R. norvegicus* (five from each sex) which were brought from the farm of Saha Enterprises, Kolkata. These were allowed to acclimatize to the 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 anesthetized by using chloroform in a jar, and two milliliters 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 the 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.

3. Statistical Analysis

The data were analyzed and presented as mean ± standard error (SE) by using statistical software Microsoft Office Excel 2007 and one-way Analysis of Variance (ANOVA) followed by Tukey's pairwise comparison

statistical tests to assess the significance of difference between and within the species by the help of Paleontological Statistics (PAST) version 2.17..

4. 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 and cytomorphometry datas are tabulated in detail (Tables 1 and 2).

Table 1: Differential leukocyte count of *R. rattus* and *R. norvegicus* (per sex n = 05)					
S. No.	**Leukocytes**	**Rattus rattus**		**Rattus norvegicus**		**F-value**
		Male	**Female**	**Male**	**Female**	
1.	Lymphocyte	36.8 ± 1.48^a	32.0 ± 0.59	12.2 ± 0.59^a	28.2 ± 1.59^a	83.75^{***}
2.	Monocyte	12.6 ± 0.61^a	12.8 ± 0.44^b	22.6 ± 0.77^a	17.3 ± 1.03^{abc}	39.48^{***}
3.	Neutrophil	48.5 ± 1.21^a	50.6 ± 0.85^b	64.6 ± 1.58^{ab}	56 ± 1.17^a	33.81^{***}
4.	Eosinophil	3.0 ± 0.33^{NS}	3.0 ± 0.33^{NS}	2.8 ± 0.24^{NS}	2.1 ± 0.23^{NS}	2.47^{**}
5.	Basophil	0.7 ± 0.15	0.6 ± 0.16^b	1.4 ± 0.16^b	1 ± 0.25	3.60^{**}

Note: Mean ± SE with similar superscripts (a, b) in the same row differ significantly at $p < 0.001$; Significant at $^{***}p < 0.001$; NS: Not significant.

Table 2: Morphometry of blood cells of *R. rattus* and *R. norvegicus* (per sex n = 05)							
S. No.	**Types of Cells**	**Cell/Nucleus**	**Parameters**	**Rattus rattus**		**Rattus norvegicus**		**F-value**
				Male	**Female**	**Male**	**Female**	
1.	Erythrocyte	Cell	Length	19.81 ± 3.12^a	14.10 ± 0.33^{ab}	6.04 ± 0.08^{ac}	5.85 ± 0.06^{abc}	117.5^{***}
			Breadth	10.80 ± 0.32^a	7.91 ± 0.19^a	5.59 ± 0.08^{ac}	5.31 ± 0.05^{ac}	163.3^{***}
2.	Lymphocyte	Cell	Length	8.14 ± 0.80^a	6.82 ± 0.23^b	7.53 ± 0.36	6.82 ± 0.23^b	6.29^{**}
			Breadth	8.07 ± 0.83^a	6.54 ± 0.19^b	6.32 ± 0.25^b	6.02 ± 0.15^a	7.08^{**}
		Nucleus	Length	6.92 ± 0.29	5.27 ± 0.19^a	6.62 ± 0.36	6.28 ± 0.32^a	3.34^{**}
			Breadth	6.35 ± 0.30	5.22 ± 0.15	6.56 ± 0.34	5.61 ± 0.27	NS
3.	Monocyte	Cell	Length	10.88 ± 0.40^a	11.19 ± 0.32^{ab}	7.85 ± 0.23^{ab}	8.00 ± 0.26^a	27.49^{**}
			Breadth	9.38 ± 0.41^a	10.14 ± 0.29^{ab}	7.62 ± 0.23^{ab}	7.35 ± 0.15^{ab}	45.97^{**}
4.	Eosinophil	Cell	Length	10.85 ± 0.46^a	10.61 ± 0.44^{ab}	9.15 ± 0.21^{bc}	7.08 ± 0.27^{bc}	21.46^{**}
			Breadth	10.60 ± 0.33^a	9.31 ± 0.28^{ab}	8.70 ± 0.11^{ab}	6.48 ± 0.28^{ab}	13.84^{**}
5.	Neutrophil	Cell	Length	9.21 ± 0.32^a	10.78 ± 0.39^{ab}	8.33 ± 0.38^{ab}	8.04 ± 0.32^{ab}	11.85^{**}
			Breadth	9.18 ± 0.31^a	10.14 ± 0.45^{ab}	7.38 ± 0.25^a	7.00 ± 0.24^{ab}	11.71^{**}
6.	Basophil	Cell	Length	10.25 ± 0.33^a	9.54 ± 0.30^{ab}	7.35 ± 0.39^b	7.18 ± 0.43^b	7.09^{**}
			Breadth	9.72 ± 0.26	9.24 ± 0.38	6.75 ± 0.36	6.91 ± 0.39	NS

Note: Mean ± SE with similar superscripts (a, b, c) in the same row differ significantly at $p < 0.001$; Significant at $^{***}p < 0.001$; NS: Not significant.

5. Discussion

Some investigations are reported in the literature that WBC is the cell of the immune system, which is originated in the bone marrow and blood. Since the number of WBC indicates certain diseases, the count of different classes of WBC (differential count) plays a pivotal role in the determination of patients' health in different stages such as diagnosis, treatment, and follow up (Beucher and Meyer, 1992). Blood is a universal transport mechanism of chemical substances and cells with a definite function, so it is used as a reliable criterion for assessing the state of the body through a number of tests. The results of this experiment were compared with respect to sex for the purpose of determining possible differences between male and female rats of two species. The sex-wise variation (Table 2) of various types of blood cells of *R. rattus* and *R. norvegicus* are recorded in micrometer (μm) to establish a crystal clear comparative difference as well as the magnitude of difference.

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* and *R. norvegicus* (Figure 1). In this investigation, the cytomorphometrical parameters of the blood of healthy black and white rats have been observed. The findings of cytomorphometrical analyses of both the species can be helpful for clinical experimentation and interpretation. In differential leucocyte count, the highest number of lymphocytes is found in *R. rattus* and lowest in *R. norvegicus* and shows a significant difference at $p < 0.001$ in males and females of both the species of rat which may be due to variation in species. It is observed that the percent of monocyte increases in white rat and decreases in black rat and shows a significant difference at $p < 0.001$, which is possibly due to
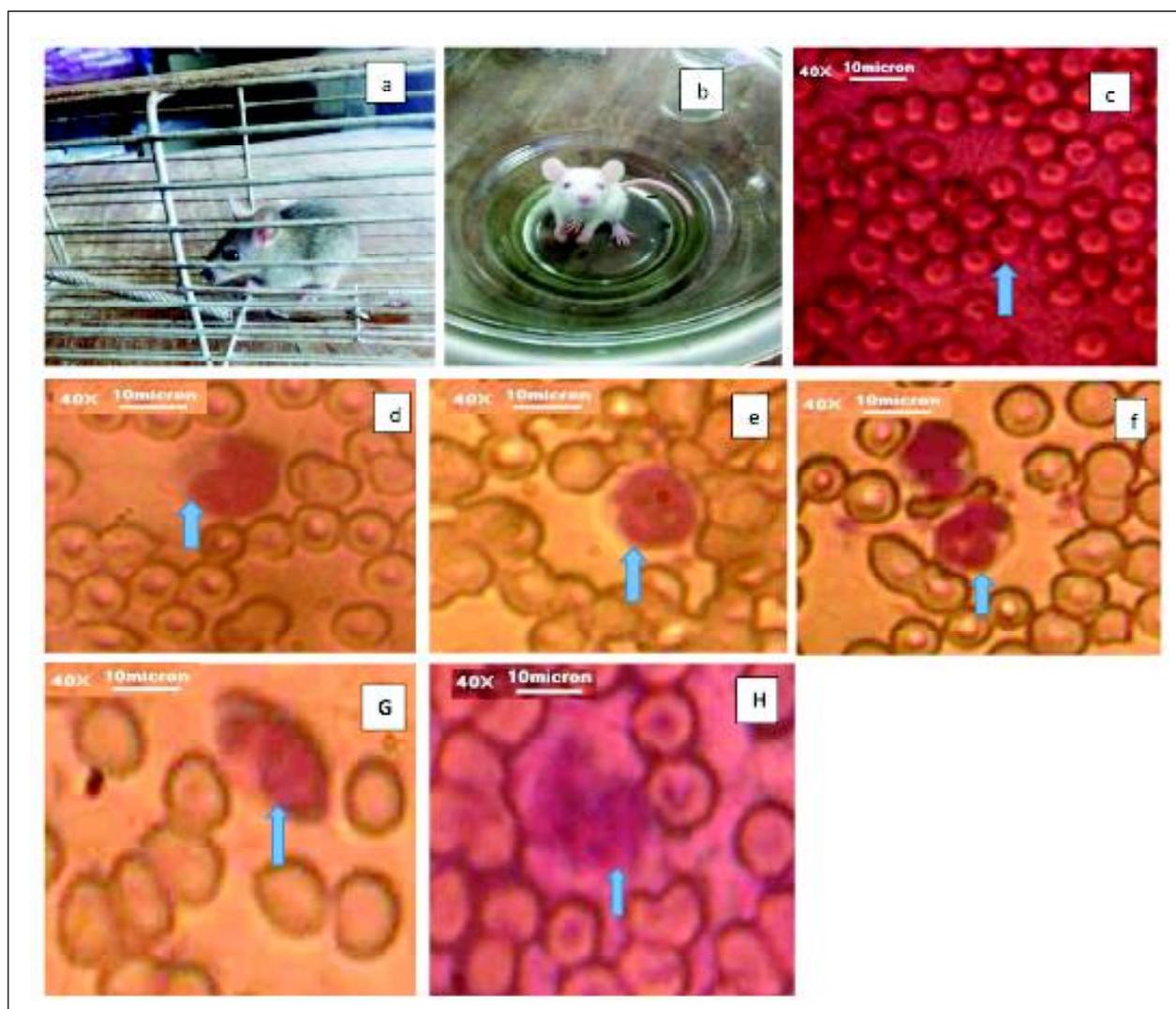

Figure 1: (a) *Rattus rattus*, **(b)** *Rattus norvegicus*, **(c) Erythrocyte, (d) Monocyte, (e) Lymphocyte, (f) Neutrophil, (g) Eosinophil, (h) Basophil**

variation in sex or species. The neutrophils are found to be more in *R. norvegicus* than *R. rattus*. The significant statistical difference with respect to sex is recorded in neutrophils at $p < 0.001$. The highest number of eosinophil is found in black rats and lowest in white rats which may be due to the variation in species or sex. The eosinophils vary significantly with respect to sex and species which is recorded at the level of $p < 0.05$. The basophils are observed to be more in white rats and less in black rats. Sex-wise significant difference is calculated in basophils at $p < 0.05$. The higher percentage of basophils in norvegicus than rattus is possibly due to the variation of species or sexual dimorphism.

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 cell length and breadth along with the nucleus of lymphocytes do not show significant differences between, and among males and females. The cellular length and breadth of erythrocytes are noted to be more in *R. rattus* and less in *R. norvegicus*, which deviates significantly species-wise at $p < 0.001$. The cell length and breadth along with the nucleus of lymphocytes have more value in *R. rattus* whereas lower in *R. norvegicus*. The parameters show species-wise significant difference at $p < 0.05$. The morphometric parameters of neutrophils are found to be a higher value in black rats and lower in white rats and deviate significantly species-wise at $p < 0.05$. The morphometric parameters of eosinophils in both the species are observed to be higher in black rats than white rats and deviate regarding sex and species which is significant at $p < 0.05$. It is accounted that the length and breadth of basophils are more in black rats in comparison to white rats and species wise different and significant at $p < 0.05$, but cell breadth of basophil does not exhibit any significant difference between the species. This appears due to the difference in species or sexual dimorphism. This report corroborates with the findings of some researchers (Barros et al., 2017; and Ventura and Lopez-Fuster, 2016).

6. Conclusion

The study reveals that as a whole, the effect of sex or species on morphometry of blood cells of black and white rats but, certain genetic and non-genetic factors such as an 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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Conflicts of Interest

Authors declare that they have no conflicts of interest.

Ethical Approval

The approval of the Institutional Animal Ethics Committee (IAEC) of the Utkal University has already been taken to undertake this particular investigation for Ph.D.

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