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Comparative Evaluation of Hematological and Biochemical Parameters in Male and Female Wistar rats

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Abstract

In present study, several hematological and biochemical parameters are analyzed to see their significant difference between male and female Wistar rats. A total of 30 adult Albino wistar rats were selected randomly for determination of their hematological and biochemical profile. Rats were housed under normal conditions and received standard food and water *ad libitum*. An hour before experimentation, animals were brought and kept in the experimental room. The rats were weighed, marked and divided into two groups *viz.* Group A and Group B each consisted of 15 male and 15 female Wistar rats respectively. All blood samples were collected from jugular veins using 30 gauze needle, in EDTA (Ethylenediamin etetraacetic acid) vials and plain vials, for hematological and biochemical investigations respectively. It was seen that WBCs, Monocytes and ALP values were higher in male rats as compared to female rats and this difference was statistically significant. The values of RBC, HGB, Creatinine and Triglycerides were higher in female rats as compared to male rats and this difference was statistically significant. Therefore, it concludes that it is necessary to partition the reference intervals.

Keywords: Biochemical; hematological; profile; reference values; wistar rat

Introduction

Blood constitutes a highly dispersed organ system that includes widely heterogeneous population of cells, each having specialized functions in the total body system. Hematology provides insights into the understanding of pathologic processes involved in toxicity or safety profile of drugs and chemical compounds and complements information obtained from clinical signs, biochemical and urine analysis and gross and microscopic examinations. Baseline control data are established and available for comparison during the critical analysis of hematologic profile for possible disease or compound-induced or spontaneous changes in parameters. Variations are known to occur as a result of different analytical procedures, sampling sites, handling at the time of sampling (anesthetized versus non-anesthetized), type of anesthetic used, frequency of sampling, nutrition and environment. However, these key points can usually be identified and controlled. There appear to be few fundamental differences in hematologic parameters between different rat strains (Bailly and Duprat, 1990).

About 95 percent of all lab animals in medical researches are rats and mice bred specifically for research purposes. The reasons for their wide usage include their small size, ease of handling and housing, rapid reproduction, short life span and ability to observe several generations in a short period. Rats also share about 95 percent of the human DNA and hence are more or less susceptible to similar diseases to humans and respond to treatments in similar manner (Delwatta *et al.,* 2018).

Reference intervals are very important laboratory tools used to make medical diagnosis, therapeutic management decision, or other physiological assessment in clinical laboratory (Wayne, 2008). Hematology and biochemistry are commonly used to determine the biological significance of findings that cannot be detected by direct examination of organs and tissues in toxicity and safety studies. Hematological and biochemical reference values are critical for assessing the health and disease status associated with diagnosis of blood disorders, infectious diseases, immune system and lipoprotein metabolism, glucose regulation and liver and kidney function. When these parameters have deviated from their normal homeostatic state, the increase or decrease of their activities or concentrations could result in pathology (Everds, 2015).

In long history of medical research, animals have always been important investigation tools. The use of animals enables the researcher to carry out

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experiments on development of new pharmaceuticals, vaccines, new surgical materials and procedures, investigation of diseases, safety and toxicity testing of different substances, etc. Since humans cannot be used for most of these experiments, animals make a good substitute. In this context, hematological, biochemical and physiological parameters of rats are of significance to researchers as they are used to evaluate vital information about response of the body to different diseases and treatments. The parameters depend on various factors including age, nutrition, environment and genetic factors and changes in any of these conditions would affect reference values of above mentioned parameters (Delwatta *et al.,* 2018).

Several recent studies determined hematology and biochemistry reference intervals in Wistar rats (Boehm *et al.,* 2007). In present study, several hematological and biochemical parameters are studied/ analyzed to see their significant difference between male and female Wistar rats maintained at the Animal House of UP University of Medical Sciences, Saifai, Etawah.

Materials and Methods

Animals

In this study, a total of 30 adult Albino wistar rats of approximately 4 months age (body weight approximately 150-200 gms) of either sex were selected randomly for determination of their hematological and biochemical profile. Rats were procured from Animal Husbandry department of UP University of Medical Sciences (UPUMS), Saifai, Etawah (CPCSEA Registration no. 1087/ GO/ ReBi/ S/ReBi/L/ CPCSEA) and experiment was started after taking approval from Institutional Animal Ethics Committee of UPUMS (IAEC protocol approval no.- IAEC/02/AH/2019-20 dated 07/03/2020). Rats were housed under normal conditions and received standard food and water *ad libitum.* An hour before experimentation, animals were brought and kept in experimental room. The rats were weighed, marked and divided into two groups *viz.* Group A and Group B each consisted of 15 male and 15 female Wistar rats respectively.

Blood Samples

All blood samples were collected from jugular veins, using 30 gauze needles, in EDTA (Ethylenediaminetetraacetic acid) vials and plain vials for hematological and biochemical investigations respectively. The blood samples collected in plain vials were then centrifuged at 4000 rpm for 10 minutes and serum was collected (Singh *et al.,* 2021).

Hematological Studies

Blood samples in EDTA vials were used to assay hematological parameters. These were sent to Central Lab. Hematological investigations for parameters like WBC (White Blood Cells {X10^3/ìl}), RBC (Red Blood Cells {X10^6/ìl}), HGB (Hemoglobin {g/dl}), Hematocrit (%), MCV(Mean Corpuscular Volume {fl}), MCH (Mean Corpuscular Hemoglobin {pg}), MCHC (Mean Corpuscular Hemoglobin Concentration {g/dl}) were undertaken by using 3-Part Automated Hematology Analyzer.

For determining the number of platelets (PLT {X10^3/ìl}) and Differential Leucocyte Count (DLC) *i.e.* the percentage of Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils, the blood smear was made and stained with May-Grunwald-Giemsa (MGG) stain. Finally, the neutrophils, lymphocytes, eosinophils, monocytes and basophils were counted under light microscope.

Biochemical Studies

Blood samples were sent to Central Lab within an hour of collection and after collecting serum, serum biochemical parameters- Random blood sugar (RBS) (mg/dl), Urea (mg/dl), Creatinine (mg/dl), Total Cholesterol (mg/dl), Triglycerides (mg/dl), HDL (mg/dl), Total Bilirubin (mg/dl), Direct Bilirubin (mg/dl), Total proteins (g/dl), Albumin (g/dl), Globulin (g/dl), Serum Albumin Globulin Ratio (SAGR), Aspartate Amino Transferase (AST in IU/L), Alanine Amino Transferase (ALT in IU/L) and Alkaline Phosphatase (ALP in IU/L) were determined by using Fully Auto Biochemistry Analyzer Selectra ProXL.

Statistical Analysis

For statistical analysis, data were analyzed by student t-test using Statistical Package of Social Sciences (SPSS) software version 25 for windows. The results were expressed as mean ± standard error (SE). Statistical significance was accessed at 95 percent confidence interval and $P<0.05$ was considered statistically significant (Singh *et al.,* 2021).

Results

The results of hematobiochemical studies of male and female Wistar rats are listed in Table 1. While comparing different parameters of male and female rats, it was seen that WBCs, Monocytes and ALP values were higher in male rats as compared to female rats and this difference was statistically significant (P=0.047, P=0.031 and P=0.001 respectively). The values of RBC, HGB, Creatinine, and Triglycerides were higher in female rats as compared to male rats and this difference was statistically significant (P=0.038, P=0.004, P=0.003 and P=0.029 respectively). The statistical difference in the values of HCT, MCV, MCH, PLT, neutrophil, lymphocyte, eosinophil, basophil, RBS, Urea, Total cholesterol, HDL, Total bilirubin, direct bilirubin, total protein, albumin, globulin, SAG ratio, AST and ALT were P=0.098, P=0.097, P=0.159, P=0.139, P=0.205, P=0.726, P=0.643, P=0.264, P=0.11, P=0.55, P=0.636, P=0.869, P=0.726, P=0.399, P=0.904, P=0.291, P=0.439, P=0.514, P=0.886, P=0.947, P=0.082 respectively.

Discussion

In present study, it is seen that WBCs and monocyte values are significantly higher in male rats as compared to female rats and creatinine value is significantly higher in female rats as compared to male rats. These findings are in agreement with findings of He *et al.* (2017), who conducted a study using Sprague Dawley rats in which it was observed that most hematologic and biochemical analytes were significantly influenced by sex. Also, the significantly higher mean values of WBC and monocytes in males as compared to females were in agreement with studies in C57BL/J mice, C3H/HeJ mice and Wistar rats (Kampfmann *et al.,* 2012; Petterinom and Argentino, 2006; Mazzaccara *et al.,* 2008). The leucocyte variations found in present study might be related to development of immuno-competence and surveillance system and were affected by age (Wolford *et al.,* 1987).

In a study, it was found that concentrations of renal markers creatinine and urea are related to glomerular filtration rate. Renal organic anion transporter 1mRNA expression was higher in male than in female mice, reflecting significantly higher fraction of creatinine secretion in males, which most likely contributed to the low plasma creatinine levels generally found in male mice (Eisner *et al.,* 2010). In our study, triglyceride levels are significantly higher in female rats as compared to male rats. This finding is in agreement with findings of He *et al.* (2017), who reported in their study that total cholesterol in female rats was higher than male rats. This might be explained by physiological events developed at early stage of life that has sex-related cholesterol metabolism (Choi *et al.,* 1988), such as absorption synthesis and catabolism. Another study showed that testosterone suppresses plasma cholesterol levels in animals when fed a hypercholesterolemic diet (Lee *et al.,* 2008). The ALP value was significantly higher in male rats as compared to female rats. This finding is similar to findings of Delwatta *et al.,* 2018, who reported that liver enzymes ALP and AST showed statistically significant gender difference.

In present study, findings of significantly higher values of RBC and HGB in female rats as compared to male rats, are in contrast to findings of He *et al.,* 2017, who reported that sex differences were observed in RBC parameters. The RBCs were significantly higher in males as compared to female rats, the same trends were reported in previous studies (Kane *et al.,* 2012). It might be due to effect of testosterone which activates erythropoiesis by stimulating erythropoietin production (Mirand *et al.,* 1965). One hypothesis for the difference between male and female erythrocyte indices is that male has greater muscle mass and therefore requires greater hemoglobin-carrying capacity. However, other factors affecting erythrocyte indices cannot be excluded. For, example, in rats, the difference between male and female RBC count and hemoglobin varies with age and in some cases, reverse; for example, female rats have greater RBC values at 3-7 weeks than do males and at 88-150 week old, hemoglobin in female rats is greater than that of males of similar age (Kane *et al.,* 2012). These findings of Kane *et al.* (2012) are somewhat in agreement with findings of the present study.

Conclusion

The current study is the first to be conducted to establish a reference database for selected hematological and biochemical parameters of Wistar rats and to check any significant difference between male and female rats, for benefit of future researchers. This report shows significant difference in parameters of male and female Wistar rats in some hematologic and biochemical analytes. Therefore, it is necessary to partition the reference intervals.

Table 1: Hematological and biochemical profile of male and female Wistar rats

Hemato-biochemical parameters	Normal Range[1,2,3]	Wistar rat (Male) Group A	Range (Male)	Wistar rat (Female) Group B	Range (Female)	Combined range (Male & Female)	F-value	P-value
WBC ($X10^3/\mu l$)	5-13	13.01±0.78*	8.7-19.1	10.58±0.87*	4.13-16.2	4.13-19.1	0.149	0.047
RBC ($X10^6/\mu l$)	6-10	7.17±0.18*	5.39-8.14	7.71±0.17*	6.92-8.94	5.39-8.94	0.00	0.038
HGB (g/dl)	11-17	12.87±0.38*	8.5-15.3	14.26±0.23*	12.9-15.4	12.9-15.4	0.99	0.004
HCT (%)	37.6-50.6	44.47±1.44	31.2-52.1	47.45±0.98	41.9-51.7	31.2-52.1	0.814	0.098
MCV (fl)	48.9-58.3	61.93±0.80	57.9-69.1	61.62±0.63	57.6-65.9	57.6-69.1	0.438	0.097
MCH (pg)	17.1-20.9	61.93±0.80	15.8-20.6	18.55±0.32	16.5-20.6	15.8-20.6	0.437	0.159
MCHC (g/dl)	32.9-37.9	29.04±0.56	26.1-31.8	30.12±0.44	27.3-31.6	26.1-31.8	3.658	0.139
PLT ($X10^3/\mu l$)	638-1200	746.93±42.06	350-1010	634.40±75.29	292-1184	292-1184	9.297	0.205
Neutrophil (%)	6.2-33.2	20.80 ±1.66	11-34	22.05±3.12	08-34.4	08-34.4	1.215	0.726
Lymphocyte (%)	62.2-90.3	77.87±1.88	63-88	76.14±3.17	42-91	42-91	1.088	0.643
Eosinophil (%)	0.2-4.5	1.13±0.13	0-2.0	1.51±0.31	0-5.1	0-5.1	4.068	0.264
Monocyte (%)	0.8-3.9	0.73±0.15*	0-2.0	0.31±0.10*	0-0.6	0-2.0	2.505	0.031
Basophil (%)	0-0.8	0.00±0.00	00	0.47±0.03	0-0.4	0-0.4	11.299	0.110
RBS (mg/dl)	70-208	95.53±4.86	65-122	89.13±5.42	55-116	55-122	0.253	0.550
Urea (mg/dl)	12.3-27.1	47.12±1.84	33.6-59	45.55±2.70	22-62.5	22-62.5	1.194	0.636
Creatinine (mg/dl)	0.2-0.6	0.50±0.03*	0.32-0.8	0.68±0.04*	0.5-1.18	0.5-1.18	0.694	0.003
Total Cholesterol (mg/dl)	24-85	66.67±4.51	40-105	65.60±4.57	42-100	40-105	0.217	0.869
Triglycerides (mg/dl)	40-130	78.20±6.06*	30-120	103.73±9.33*	60-170	30-170	3.382	0.029
HDL (mg/dl)	38.33-63.33	28.67±3.04	05-46	27.00±3.59	04-50	04-50	0.447	0.726
T. Bilirubin (mg/dl)	0.05-0.18	0.24±0.02	0.11-0.4	0.29±0.05	0.13-0.96	0.11-0.96	4.497	0.399
Direct Bilirubin (mg/dl)	0.03-0.06	0.13±0.02	0.04-0.4	0.13±0.03	0.01-0.41	0.01-0.41	1.082	0.904
Total Protein (g/dl)	5.2-7.7	5.88±0.34	3.9-7.98	6.39±0.34	4.4-8.8	3.9-8.8	0.101	0.291
Albumin (g/dl)	3.4-5.5	3.70±0.20	2.0-4.8	3.92±0.21	2.9-5.45	2.0-5.45	0.042	0.439
Globulin (g/dl)	1.5-2.5	2.18±0.20	1.2-4.06	2.38±0.22	1.23-4.24	1.2-4.24	0.899	0.514
SAG Ratio	1.58-3.0	1.82±0.13	0.97-2.56	1.79±0.15	0.77-3.0	0.77-3.0	0.082	0.886
AST (IU/L)	65-203	240.33±13.42	160-360	241.93±19.69	145-390	145-390	2.115	0.947
ALT (IU/L)	16-48	101.80±12.03	54-220	73.87±9.74	30-170	30-220	0.019	0.082
ALP (IU/L)	26-230	555.47±71.47*	242-1240	265.13±31.52*	135-590	135-1240	5.443	0.001

All values are expressed as Mean±SE. Starmark (*) shows significant difference between means of both groups when compared at a 95% confidence interval (when $P<0.05$).

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Understanding the significant discrepancies in hematological and biochemical analytes between male and female Wistar rats provides important insight into physiological effects in test rats. The establishment of locally sex-specific reference intervals allows more precise evaluation of animal quality and experimental results of Wistar rats in our research studies. These reference values may help to verify results using Wistar rats as a model and also to reduce to some extent the number of rats in the control groups of future research projects.

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