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Comparison of selected clinical pathology analytes in two outbred strains of Rat at different ages

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ABSTRACT

Outbred rats are laboratory animals widely used in preclinical research and investigation of clinical pathology parameters is an important part of the preclinical evaluation of drug safety. The objective of the present study was to determine the influence of strain and age on selected hematological and biochemical analytes in Sprague Dawley (Crl: CD[SD]) and Wistar (Crl: WI[Han]) rats. Selected analytes were studied in 390 rats per strain with an equal representation of both sexes at 6-8 weeks, 10-14 weeks, and 6-10 months of age. The data were statistically compared to assess the changes with age and strain. The distribution of results by strain showed that SD rats of both sexes had significantly higher values of MCHC, PLT, WBC, LYMPH, and AST, ALT concentration. In comparison, male Wistar rats had significantly higher values of RBC, E% and female Wistar rats had N% and UREA concentration. The distribution of the results according to age showed that the increase in the age of the rats significantly reduced the MCV, MCH, PLT, L%, and the concentration of AST, and ALP. The concentration of GLU, TP, CREA, and percentage of neutrophils and eosinophils significantly increased with the increasing age of rats. In conclusion, this research demonstrated that age and strain significantly influence most outbred rats' hematological and biochemical parameters.

Keywords: Hematology, Biochemistry, Rat, SD, Wistar, Age

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INTRODUCTION

Outbred albino strains of rats are popular rats used in laboratory research (Koolhaas, 2010), owing to their anatomical, genetic, and physiological similarity to humans, and other advantages like small size, low space, and resource requirement, ability to produce a good number of offspring with short gestation period, rapid development, and short life span (Bryda, 2013). These rats share about 95% of the human DNA, which makes it easier for the induction of diseases and testing since they respond to treatments like humans. (Van Zutphen, 2001). Hence, these rats are used in toxicology, safety, and efficacy studies, apart from the research conducted in reproduction and development, behavior, and nutrition. As a result, a large volume of historical data generated with these rats makes them a useful tool for testing drug effect and safety, which is a must for regulatory studies leading to drug approvals in all parts of the world.

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Wistar and Sprague-Dawley (SD) rats are maintained as outbred rat models. A population's maximum heterogeneity is guaranteed by outbred breeding systems. Population size, generational order, future breeder selection, and breeding strategy can all have an impact on the retention of genetic variability within an outbred colony. (Krinke, 2000) It is crucial to control these production factors to preserve the stock's genetic integrity. Although a minimum breeding unit of 25 pairs is advised, it is suggested to begin the colony with as many breeder pairs as feasible to create genetic heterogeneity within a population. (Sukow, 2006) Following the establishment of an outbred colony, effective colony management requires making use of each generation's full breeding life span to reduce allele loss within the gene pool. Outbred rats are primarily preferred over mice models because their larger body size allows serial blood draws. Surgical manipulations and blood pressure measurement by telemetry are easier to perform and they share a similar pathway with humans for eradicating toxins, so common choice for toxicology and pharmacology studies. Hematological and biochemical tests are important for humans and animals since the blood is the body's main transport system and contains the input and output materials for nearly all metabolic activities. Additionally, blood profiles can be used to identify any abnormalities from normal. Evaluation of the hematological profile provides clinically meaningful and vital information on the response of the body to injury, deprivation, and/or stress. Such evaluation is indispensably important for diagnosis, determining prognosis, assessment of the efficacy of therapy, and toxicity of drugs and chemical substances. Standard reference values for hematology and biochemistry analytes have been reported by researchers (SD Rat: He Q, 2017; Delwatta, 2018; Aleman, 1998; Zhong, 2010; Wolford, 1987) (Wistar Rat: Boehm, 2007; Öztürk, 2021; de Kort, 2020; Jacob, 2018, Hayakawa, 2013; Kampfmann,2012; Patel, 2024). These values can be influenced by age, sex, physiological activity status, nutrition, climate factors, stress, etc. However, there are few reports comparing strain differences or age differences in terms of hematology and biochemistry analytes. To evaluate the influence of the age and rat strain used as the experimental animal model, we compared hematology and biochemistry analysis differences between SD and Wistar rats at three different ages.

MATERIALS AND METHODS METHODS

Animals and Diets

Wistar (Crl: WI[Han]) and Sprague Dawley (Crl: CD[SD]) rats used for the data collection were bred and maintained at the Animal Research Facility of Zydus Research Centre, accredited by AAALAC. Breeders from the Charles River Laboratory were used to establish a colony at Zydus Research Centre and rats were bred using an outbreed breeding strategy. A total of 780 rats (390 rats/strain) in the age range of 6-8 weeks, 10-14 weeks, and 6-10 months were included in this comparison. All rats were reared in individually ventilated cage (IVC) (2-4 rats/cage) filled with sterile corncob bedding, which was changed, and cages were washed once a week. The rats were housed at controlled room temperature (23 ± 2 °C) and relative humidity (50 ± 15 %) with a 12/12 h light/dark cycle. Each IVC cage had a ventilation rate set at 40-50 air changes per hour, and the housing room had a ventilation rate set at 10-15 air changes per hour. The animals had free access to a standard chow diet (2018 Teklad global 18% protein rodent diets, Inotiv) and reverse osmosis-treated water. All rats were housed socially and enrichment items, such as rat tunnels and huts, were provided in cages for the well-being of animals. All health monitoring studies were performed in compliance with standard operating procedures (SOPs) of the facility and were approved by the Institutional Animal Ethics Committee (IAEC). Additionally, growth curves for both rat strains (64 rats/strain: 32 male and 32 female) were generated up to 12 weeks of age by weighing them every week. Body weight results were expressed as mean ± standard deviation.

Specimen Collection

A randomly selected group of rats were screened for hematology and biochemistry analytes as a part of the routine health monitoring program. In each health monitoring study, 30 rats (15 males and 15 females) randomly selected from three age ranges: 6–8 weeks, 10–14 weeks, and 6-10 months, were used. Selected rats were fasted overnight (water ad libitum). Under isoflurane anesthesia, animals were bled by retro-orbital plexus puncture. Blood samples for hematology (approximately 450 µL/rat) were collected in an anticoagulant tube (50 µl/vial, 2% EDTA) and approximately 700 µL/rat were collected in a centrifuge tube for biochemistry. Blood samples from each age range were collected by following the same procedure and rats were euthanized after completion of specimen collection by carbon dioxide (CO2) using a gradual fill method in a transparent euthanasia chamber (30 L/min flow rate). Data compiled from thirteen health monitoring studies per strain carried out over the years (2016-2024) were used in this comparison.

Hematology and Biochemistry analyte measurements

All samples were transported promptly to the National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited Clinical Pathology Laboratory at Zydus Research Centre. Samples collected

for clinical chemistry analysis were centrifuged at 4000 rpm for 10 min at 24 °C within two hours of collection to harvest serum. The samples were analyzed within five hours of collection. Whole blood was used for the determination of hematology analytes, namely red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), white blood cell (WBC) and differential leucocyte count: neutrophil (NEU, N%), lymphocyte (LYMPH, L%), eosinophil (EOS, E%), monocyte (MONO, M%), basophil (BASO, B%). The analyses were performed on the automated blood cell analyzer ADVIA 2120i (Siemens Healthineers, USA) using commercially available test methods (flow cytometry). Two-level quality controls were run before analysis and after successful analyses of quality controls, hematology samples were analyzed. Serum samples were processed for biochemistry analytes by method/ techniques described as follows: glucose (GLU) by hexokinase method; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) by IFCC (International Federation of Clinical Chemistry) method; total protein (TP) by colorimetric Biuret method; albumin (ALB) by bromocresol green method; urea (UREA) by kinetic method and creatinine (CREA) by Jaffe method. The analyses were performed using a Cobas C311 analyzer (Roche Diagnostics, Switzerland). Details of calibrators, lot number, and standard values were manually installed in the analyzer, and calibration was performed as per standard operating procedure.

Statistical analysis

The data was grouped by age and strain for both sexes. A boxplot for each hematology and biochemistry analyte was visually checked for outliers, and significant outliers were eliminated by the Tukey method. (Horn PS, 2003) Outliers were eliminated if biologically implausible or incredible and were excluded from further analysis. To determine the normality of the data distribution, the Shapiro-Wilk test was applied after the elimination of significant outliers. In each rat strain, hematology and biochemical values were expressed as mean ± standard deviation (mean ± SD). The differences between strains for each analyte were compared using an independent-sample t-test (parametric) when conditions of normality were met and when it failed, the Mann-Whitney U test (nonparametric) was used. The differences linked to age for each rat strain were performed by one-way ANOVA (post-hoc analysis by Tukey HSD and Bonferroni test). The possible effects of age and sex were tested together by factorial ANOVA using a statistical software program (SPSS 21.0). P-value <0.05 was considered statistically significant.

RESULTS

Body Weight

The weight curves (mean ± SD) of both rat strains up to 12 weeks of age are shown in Figure 1. All rats appeared to be healthy during weight curve, and SD rats were noticeably heavier than Wistar rats in both sexes. Female rats presented lower values of body weight compared to males of the same age in both strains.

Strain differences

The results of strain differences for hematology and biochemistry analytes at three different age ranges are summarized in Tables 1 and 2. In addition, Box and Whisker plots of each analyte for both sexes are depicted in Figures 2, 3, and 4. In comparison between strains statistical differences were observed in the majority of analytes. In male SD rats (Table 1), MCH, MCHC, PLT, WBC, NEU, LYMPH, AST and ALT were significantly higher, whereas RBC and E% were significantly lower than Wistar rats in all age ranges. HGB concentrations were similar between strains in all age ranges. 6-8 weeks male SD rats had significantly higher MCV, MONO, BASO, GLU, ALP and

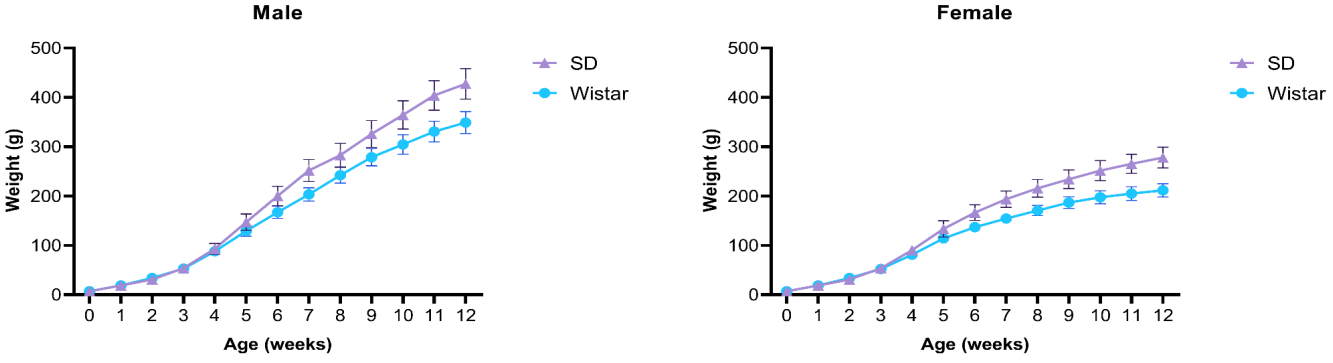

Figure 1. Mean weekly body weight (mean ± standard deviation) of SD (n=32) and Wistar (n=32) rats up to 12 weeks of age.

Table 1: Hematology and Biochemistry values of SD Rats and Wistar Rats – Male

Analytes	6-8 Weeks				10-14 Weeks				6-10 Months			
	SD		Wistar		SD		Wistar		SD		Wistar	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
RBC (106/µL)	61	6.48 ± 0.44	63	6.81 ± 0.41^{***a}	62	7.83 ± 0.48	55	8.01 ± 0.42^{*a}	63	8.10 ± 0.54	65	8.36 ± 0.51^{**a}
HGB (g/dL)	60	13.2 ± 0.75	62	13.4 ± 0.65^{a}	63	14.6 ± 0.76^{a}	59	14.4 ± 0.68	64	14.3 ± 0.90	65	14.6 ± 0.66
HCT (%)	61	41.4 ± 2.76	64	42.8 ± 3.20^{*a}	62	45.3 ± 2.90^{a}	60	45.2 ± 2.95	62	44.5 ± 2.63	64	45.7 ± 2.72^{*a}
MCV (fL)	56	64.8 ± 2.55^{**a}	64	62.6 ± 3.93	63	58.1 ± 4.54	60	56.9 ± 4.54	64	54.8 ± 4.32^{a}	64	54.7 ± 3.33
MCH (pg)	61	20.4 ± 0.84^{***a}	62	19.5 ± 0.84	63	18.8 ± 1.08***	58	18.0 ± 0.72	63	17.8 ± 1.08^{**a}	62	17.3 ± 0.65
MCHC (g/dL)	57	31.8 ± 0.95^{**a}	64	31.2 ± 1.33	57	32.4 ± 0.98^{**a}	60	31.9 ± 1.44	63	32.5 ± 1.35^{**a}	64	31.8 ± 1.50
PLT (103/µl)	59	998 ± 235.02^{***a}	59	763 ± 151.34	59	880 ± 198.22***	59	694 ± 118.93	63	829 ± 157.25^{***a}	62	631 ± 115.05
WBC (103/µL)	60	7.90 ± 2.44^{***a}	64	5.39 ± 1.00	62	10.20 ± 2.79^{***a}	58	6.77 ± 1.37	64	9.00 ± 2.68^{***a}	65	5.03 ± 1.25
NEU (103/µl)	61	0.95 ± 0.59***	63	0.53 ± 0.18	63	1.31 ± 0.55***	57	1.00 ± 0.32	62	2.06 ± 1.09***	63	1.31 ± 0.49
N %	60	11.1 ± 4.91^{a}	63	10.1 ± 3.67	63	12.7 ± 4.42	59	14.9 ± 3.86^{**a}	64	23.8 ± 10.48	65	27.3 ± 9.07^{*a}
LYMPH (103/µl)	61	6.69 ± 2.09^{***a}	64	4.56 ± 0.93	62	8.30 ± 2.42^{***a}	57	5.35 ± 1.09	64	6.12 ± 2.25***	61	3.12 ± 0.78
L %	61	83.8 ± 5.44	64	84.4 ± 4.78a	61	82.1 ± 4.84^{***a}	59	78.4 ± 4.56	64	67.6 ± 13.09^{a}	63	64.4 ± 10.38
MONO (103/µl)	60	0.21 ± 0.11^{***a}	62	0.13 ± 0.07	59	0.26 ± 0.15a	60	0.24 ± 0.12	59	0.34 ± 0.17***	64	0.23 ± 0.15
M %	61	2.68 ± 1.25^{a}	62	2.53 ± 1.28	56	2.30 ± 1.02	59	3.33 ± 1.60^{***a}	59	3.93 ± 2.16	64	4.48 ± 2.40
EOS (103/µl)	61	0.04 ± 0.02	56	0.04 ± 0.01	62	0.08 ± 0.05	54	0.08 ± 0.04	61	0.12 ± 0.06*	61	0.10 ± 0.05
E %	59	0.42 ± 0.23	56	0.67 ± 0.26***	60	0.70 ± 0.34	55	1.26 ± 0.73***	63	1.36 ± 0.69	58	1.81 ± 0.83**
BASO (103/µl)	61	0.09 ± 0.09*	64	0.07 ± 0.07	57	0.10 ± 0.10	60	0.11 ± 0.11	62	0.14 ± 0.17*	63	0.08 ± 0.10
B %	61	1.34 ± 1.32	64	1.23 ± 1.17	60	1.25 ± 1.46	60	1.56 ± 1.44	61	1.70 ± 2.11	60	1.31 ± 1.53
GLU (mg/dL)	55	61.1 ± 13.08^{*a}	61	55.7 ± 11.81	62	95.3 ± 21.25^{a}	63	93.5 ± 24.88	65	118.2 ± 20.28^{a}	64	111.1 ± 23.12
AST (U/L)	63	187.1 ± 45.65^{***a}	63	130.0 ± 23.66	65	166.5 ± 42.99***	61	118.3 ± 26.25	64	155.1 ± 32.99***	59	105.1 ± 24.45
ALT (U/L)	65	49.1 ± 10.33***	65	30.5 ± 7.59	65	44.7 ± 8.85^{***a}	63	31.5 ± 5.77	64	45.5 ± 11.33***	59	36.3 ± 8.16
ALP (U/L)	65	295.9 ± 88.68**	63	245.6 ± 73.96	61	129.2 ± 27.77	63	125.0 ± 48.71	59	81.5 ± 18.89*	63	74.2 ± 19.27
TP (g/dL)	65	5.86 ± 0.35	65	6.03 ± 0.27**	64	6.60 ± 0.32^{*a}	63	6.47 ± 0.28	65	7.03 ± 0.34***	64	6.82 ± 0.32
ALB (g/dL)	65	3.99 ± 0.48	65	4.04 ± 0.40	65	4.16 ± 0.44	63	4.16 ± 0.44	65	4.15 ± 0.42	65	4.21 ± 0.51
UREA (mg/dL)	65	22.8 ± 5.98	65	26.9 ± 6.65^{***a}	65	30.8 ± 5.60	63	33.1 ± 5.16^{*a}	64	31.8 ± 5.33^{a}	64	31.5 ± 5.64
CREA (mg/dL)	65	0.35 ± 0.17	65	0.36 ± 0.17	65	0.44 ± 0.15	63	0.46 ± 0.13	65	0.46 ± 0.14	65	0.51 ± 0.15

*, **, *** Statistically significant different values: $p<0.05$, $p<0.01$ and $p< 0.001$ (t-test).

^{a}Statistical comparison based on parametric test.

Table 2: Hematology and Biochemistry values of SD Rats and Wistar Rats – Female

Analytes	6-8 Weeks				10-14 Weeks				6-10 Months			
	SD		Wistar		SD		Wistar		SD		Wistar	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
RBC (10^6/µL)	65	6.76 ± 0.52	62	7.03 ± 0.32^{**a}	62	7.50 ± 0.38	61	7.64 ± 0.45	60	7.80 ± 0.55^{a}	61	7.71 ± 0.47
HGB (g/dL)	65	13.6 ± 1.13^{a}	63	13.6 ± 0.49	63	14.2 ± 0.92^{**a}	61	13.8 ± 0.57	59	14.5 ± 0.94^{a}	62	14.2 ± 0.85
HCT (%)	65	42.2 ± 3.82	65	43.3 ± 2.81^{a}	63	43.3 ± 3.93	62	43.4 ± 2.69^{a}	59	44.3 ± 3.01	62	44.6 ± 2.96^{a}
MCV (fL)	65	62.5 ± 3.55	65	61.3 ± 3.80	63	58.0 ± 4.45	62	57.1 ± 3.71	60	57.5 ± 4.55	62	58.1 ± 3.27
MCH (pg)	65	20.2 ± 0.81^{***a}	62	19.3 ± 0.58	62	19.0 ± 0.81^{***a}	62	18.2 ± 0.62	58	18.7 ± 1.00	59	18.5 ± 0.55
MCHC (g/dL)	64	32.4 ± 1.12^{***a}	65	31.5 ± 1.51	63	32.9 ± 1.72*	62	32.0 ± 1.60	59	32.9 ± 1.45^{***a}	62	31.9 ± 1.56
PLT (10^3/µl)	63	973 ± 205.84^{***a}	59	773 ± 137.33	58	894 ± 137.06^{***a}	61	694 ± 138.60	57	809 ± 160.07^{***a}	61	659 ± 152.53
WBC (10^3/µL)	63	7.26 ± 1.93^{***a}	65	5.10 ± 1.33	62	7.6 ± 2.5^{***a}	59	4.32 ± 1.06	60	5.10 ± 1.85^{***a}	58	3.01 ± 0.85
NEU (10^3/µl)	65	0.63 ± 0.31*	61	0.48 ± 0.15	63	0.84 ± 0.34*	61	0.71 ± 0.23	56	0.80 ± 0.40	56	0.72 ± 0.26
N %	65	8.41 ± 3.65	64	9.90 ± 2.75**	62	11.1 ± 4.29	61	16.6 ± 4.70^{***a}	58	17.1 ± 7.17	58	25.5 ± 7.93***
LYMPH (10^3/µl)	65	6.44 ± 1.91^{***a}	65	4.31 ± 1.17	62	6.39 ± 2.29^{***a}	59	3.31 ± 0.86	57	3.44 ± 1.33***	61	1.97 ± 0.62
L %	63	87.1 ± 4.24***	62	84.6 ± 3.22	60	83.7 ± 4.42^{***a}	61	76.6 ± 5.39	56	73.6 ± 9.92***	62	63.4 ± 11.04
MONO (10^3/µl)	63	0.16 ± 0.09	64	0.14 ± 0.07	61	0.21 ± 0.09***	60	0.14 ± 0.09	55	0.21 ± 0.15**	61	0.14 ± 0.08
M %	62	2.10 ± 0.95	62	2.61 ± 1.21^{*a}	60	2.75 ± 1.19	61	3.38 ± 1.98	54	4.05 ± 2.4	57	3.91 ± 1.86
EOS (10^3/µl)	59	0.06 ± 0.03	62	0.05 ± 0.02	61	0.08 ± 0.04*	54	0.06 ± 0.04	55	0.09 ± 0.05*	59	0.07 ± 0.04
E %	58	0.77 ± 0.36	60	1.04 ± 0.41***	59	1.0 ± 0.44	56	1.50 ± 0.78**	56	1.94 ± 1.22	60	2.36 ± 1.18
BASO (10^3/µl)	63	0.06 ± 0.05	64	0.05 ± 0.05	63	0.08 ± 0.07**	61	0.04 ± 0.04	54	0.05 ± 0.07	60	0.05 ± 0.06
B %	64	0.96 ± 0.85	65	1.14 ± 1.03	62	1.12 ± 1.09	62	1.11 ± 1.09	54	1.31 ± 1.80	58	1.56 ± 1.89
GLU (mg/dL)	56	79.2 ± 16.22*	59	72.9 ± 15.56	60	93.4 ± 14.85***	63	84.3 ± 18.67	63	102.5 ± 21.47^{**a}	65	91.1 ± 17.39
AST (U/L)	60	161.4 ± 24.36^{***a}	62	117.4 ± 21.88	62	154.2 ± 38.37***	62	105.9 ± 22.53	63	131.9 ± 31.58***	63	102.8 ± 28.73
ALT (U/L)	65	39.3 ± 9.68***	64	26.6 ± 5.77	63	36.0 ± 7.68^{***a}	63	25.5 ± 5.15	63	35.5 ± 9.67^{***a}	56	27.1 ± 6.82
ALP (U/L)	61	152.7 ± 41.41^{a}	61	140.9 ± 43.48	61	77.8 ± 19.26***	64	66.4 ± 34.94	63	63.4 ± 33.93***	57	30.8 ± 13.66
TP (g/dL)	63	6.25 ± 0.32^{a}	65	6.20 ± 0.26	63	6.87 ± 0.39	65	6.89 ± 0.39^{a}	65	7.25 ± 0.53*	63	7.03 ± 0.38
ALB (g/dL)	65	4.33 ± 0.61	65	4.28 ± 0.47	65	4.64 ± 0.74	65	4.71 ± 0.60	65	4.66 ± 0.75	65	4.57 ± 0.61
UREA (mg/dL)	64	33.6 ± 7.36	65	37.7 ± 8.50^{**a}	64	33.9 ± 5.72	63	39.2 ± 7.77^{***a}	64	36.1 ± 6.27	65	42.69 ± 8.48^{***a}
CREA (mg/dL)	65	0.39 ± 0.15	65	0.41 ± 0.16	65	0.50 ± 0.14	65	0.53 ± 0.16	65	0.53 ± 0.15	65	0.58 ± 0.13*

*, **, *** Statistically significant different values: $p<0.05$, $p<0.01$ and $p< 0.001$ (t-test).

^{a}Statistical comparison based on parametric test.

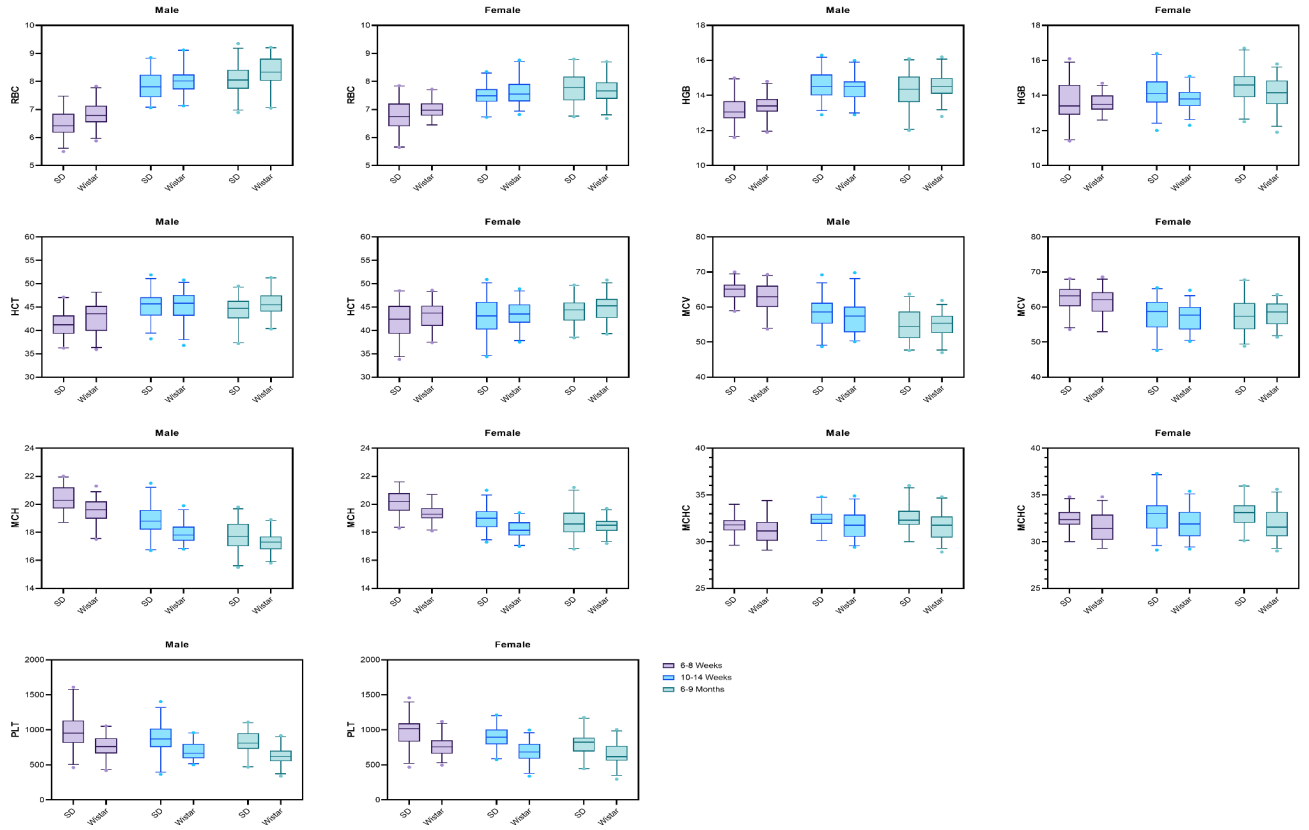

Figure 2. Comparison of RBC analytes and PLT counts of SD and Wistar Rats at three different ages. The box-and-whisker-plots show median values, 25% and 75% quartiles (box range), adjacent values (whiskers), and extreme values (dots).

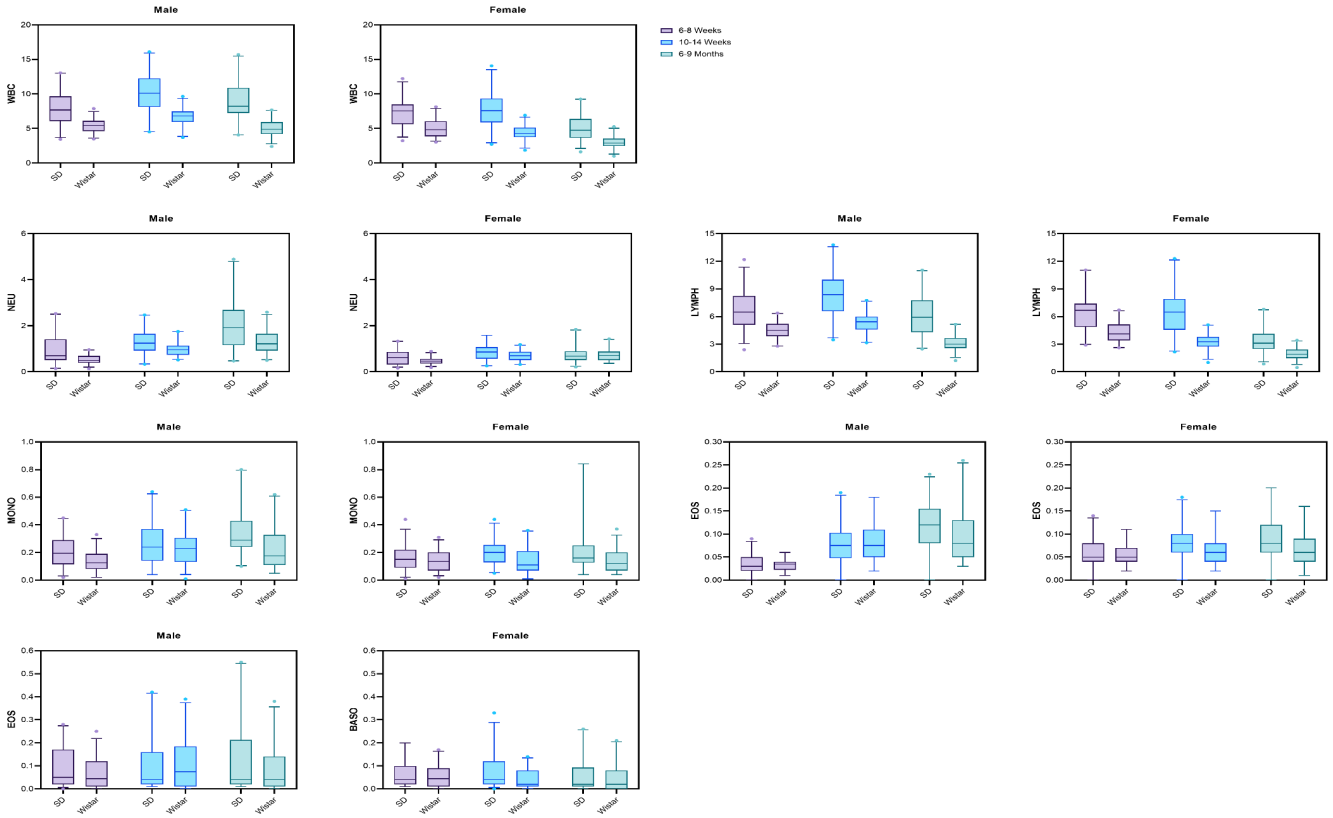

Figure 3. Comparison of differential white blood cell (WBC) counts of SD and Wistar Rats at three different ages. The box-and-whisker-plots show median values, 25% and 75% quartiles (box range), adjacent values (whiskers), and extreme values (dots).

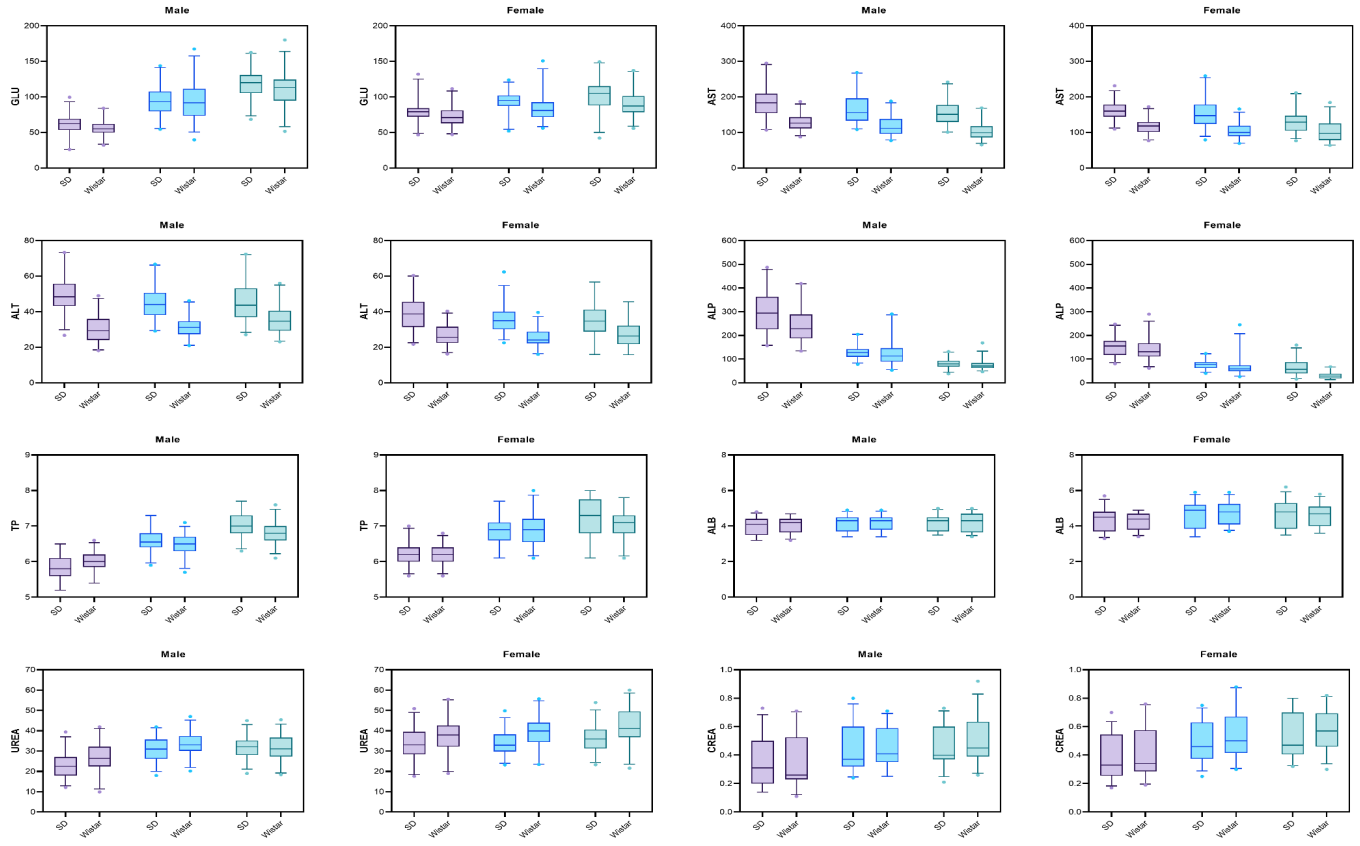

Figure 4. Comparison of biochemical analytes of SD and Wistar Rats at three different ages. The box-and-whisker-plots show median values, 25% and 75% quartiles (box range), adjacent values (whiskers), and extreme values (dots).

lower HCT, TP, and UREA than Wistar rats. N%, M%, and UREA of male Wistar rats (10-14 weeks) were significantly higher and L% and TP were lower in contrast to SD rats. 6-10 months male SD rats had significantly higher MONO, EOS, BASO, ALP, TP, and lower HCT and N% compared to Wistar rats.

In female SD rats (Table 2), MCHC, PLT, WBC, LYMPH, L%, GLU, AST, and ALT were significantly higher, whereas N% and UREA were significantly lower than Wistar rats in all age ranges. HCT, MCV, B%, and ALB were similar between strains in all age ranges. RBC, M%, and E% of male Wistar rats (6-8 weeks) were significantly higher and MCH and NEU were lower in contrast to SD rats. 10-14 weeks female SD rats had significantly higher HGB, MCH, NEU, MONO, EOS, BASO, and ALP, whereas lower E% compared to Wistar rats. In 6–10-month-old rats, male SD rats had significantly higher MONO, EOS, ALP, and TP and lower CREA than Wistar rats.

Age differences

Age-dependent effects in hematological and biochemical analytes of both strains are summarized in Table 3. In both sexes of SD and Wistar rats, RBC, HGB, and HCT levels were significantly higher in older rats (6-10 months) compared to younger rats (6-8 weeks). In addition, concentrations of RBC tend to increase with age (Table 3) in both strains. There were significant age differences in MCV and MCH levels in both sexes of SD and Wistar rats; however, both analytes were significantly higher in 6-8 weeks old rats compared to 6-10 months old rats. There was also an interaction between the effects of sex and age for RBC, HGB, HCT, MCV, and MCH levels in both strains. MCHC concentrations were found to be similar in female rats of both strains; however, male rats had age differences. PLT counts had significant age differences in both strains (male and female) and were found significantly higher in younger rats than older rats.

In male rats of both strains (Table 3), there were age differences in WBC counts, and found significantly higher in 10-14 weeks old rats when age ranges were compared (Table 3). Furthermore, WBC counts tend to decrease with age in female Wistar rats, which was not observed in female SD rats. When comparing age differences for absolute leukocyte counts, there were age differences for NEU, N%, LYMPH, L%, M%, EOS, and E% in both sexes of SD and Wistar rats; however, BASO and B% were found to be similar in both sexes of SD and Wistar rats. Furthermore, NEU, N%, EOS, and E% tend to increase with age in both

Table 3: Age-related Hematology and Biochemistry mean values of SD and Wistar Rats

Analytes	Sex	SD			Interactive effects of Sex and Age	Wistar			Interactive effects of Sex and Age
		6-8 weeks	10-14 weeks	6-10 months		6-8 weeks	10-14 weeks	6-10 months	
RBC (106/µL)	Male	6.48	7.83^{A}	8.10b^{C}	p<0.001	6.81	8.01^{A}	8.36^{BC}	p<0.001
	Female	6.76	7.50^{A}	7.80b^{C}		7.03	7.64^{A}	7.71^{C}	
HGB (g/dL)	Male	13.2	14.6^{A}	14.3^{C}	p<0.05	13.4	14.4^{A}	14.6^{C}	p<0.001
	Female	13.6	14.2^{a}	14.5^{C}		13.6	13.8	14.2^{bC}	
HCT (%)	Male	41.4	45.3^{A}	44.5^{C}	p<0.05	42.8	45.2^{A}	45.7^{C}	p<0.05
	Female	42.2	43.3	44.3^{c}		43.3	43.4	44.6^{c}	
MCV (fL)	Male	64.8^{AC}	58.1^{B}	54.8	p<0.05	62.6^{AC}	56.9^{b}	54.7	p<0.001
	Female	62.5^{AC}	58.0	57.5		61.3^{AC}	57.1	58.1	
MCH (pg)	Male	20.4^{AC}	18.8^{B}	17.8	p<0.001	19.5^{AC}	18.0^{B}	17.3	p < 0.001
	Female	20.2^{AC}	19.0	18.7		19.3^{AC}	18.2	18.5^{b}	
MCHC (g/dL)	Male	31.8	32.4^{a}	32.5^{c}		31.2	31.9^{a}	31.8	
	Female	32.4	32.9	32.9		31.5	32.0	31.9	
PLT (103/µl)	Male	998a^{C}	880	829		763^{aC}	694^{b}	631	
	Female	973a^{C}	894^{b}	809		773^{aC}	694	659	
WBC (103/µL)	Male	7.90	10.20^{A}	9.00^{b}	p<0.001	5.39	6.77^{AB}	5.03	p<0.001
	Female	7.26^{C}	7.60^{B}	5.10		5.10^{AC}	4.32^{B}	3.01	
NEU (103/µl)	Male	0.95	1.31^{a}	2.06^{BC}	p<0.001	0.53	1.00^{A}	1.31^{BC}	p<0.001
	Female	0.63	0.84^{a}	0.80^{c}		0.48	0.71^{A}	0.72^{C}	
N %	Male	11.1	12.7	23.8^{BC}		10.1	14.9^{A}	27.3^{BC}	p<0.05
	Female	8.41	11.1^{a}	17.1^{BC}		9.90	16.6^{A}	25.5^{BC}	
LYMPH (103/µl)	Male	6.69	8.30^{aB}	6.12	p<0.001	4.56^{C}	5.35^{AB}	3.12	
	Female	6.44^{C}	6.39^{B}	3.44		4.31^{AC}	3.31^{B}	1.97	
L %	Male	83.8^{C}	82.1^{B}	67.6		84.4^{AC}	78.4^{B}	64.4	p<0.001
	Female	87.1a^{C}	83.7^{B}	73.6		84.6^{AC}	76.6^{B}	63.4	
MONO (103/µl)	Male	0.21	0.26	0.34^{bC}	p<0.05	0.13	0.24^{A}	0.23^{C}	p<0.05
	Female	0.16	0.21	0.21		0.14	0.14	0.14	
M %	Male	2.68	2.30	3.93^{BC}		2.53	3.33^{a}	4.48^{bC}	
	Female	2.10	2.75	4.05^{BC}		2.61	3.38^{a}	3.91^{C}	
EOS (103/µl)	Male	0.04	0.08^{A}	0.12^{BC}	p<0.05	0.04	0.08^{A}	0.10^{C}	p<0.001
	Female	0.06	0.08^{a}	0.09^{C}		0.05	0.06	0.07^{c}	
E %	Male	0.42	0.70^{a}	1.36^{BC}		0.67	1.26^{A}	1.81^{BC}	
	Female	0.77	1.04	1.94^{BC}		1.04	1.50^{a}	2.36^{BC}	
BASO (103/µl)	Male	0.09	0.10	0.14	p<0.05	0.07	0.11	0.08	p<0.05
	Female	0.06	0.08	0.05		0.05	0.04	0.05	
B %	Male	1.34	1.25	1.70	p<0.05	1.23	1.56	1.31	
	Female	0.96	1.12	1.31		1.14	1.11	1.56	
GLU (mg/dL)	Male	61.1	95.3^{A}	118.2^{BC}	p<0.001	55.7	93.5^{A}	111.1^{BC}	p<0.001
	Female	79.2	93.4^{A}	102.5^{bC}		72.9	84.3^{a}	91.1^{C}	
AST (U/L)	Male	187.1a^{C}	166.5	155.1		130.0^{aC}	118.3^{b}	105.1	
	Female	161.4^{C}	154.2^{B}	131.9		117.4^{ac}	105.9	102.8	

ALT (U/L)	Male	49.1[a]	44.7	45.5		30.5	31.5	36.3[bC]	p < 0.05
	Female	39.3	36.0	35.5		26.6	25.5	27.1	
ALP (U/L)	Male	295.9[AC]	129.2[B]	81.5	p<0.001	245.6[AC]	125.0[B]	74.2	p < 0.001
	Female	152.7[AC]	77.8[b]	63.4		140.9[AC]	66.4[B]	30.8	
TP (g/dL)	Male	5.86	6.60[A]	7.03[BC]		6.03	6.47[A]	6.82[BC]	p < 0.05
	Female	6.25	6.87[A]	7.25[BC]		6.20	6.89[A]	7.03[C]	
ALB (g/dL)	Male	3.99	4.16	4.15		4.04	4.16	4.21	
	Female	4.33	4.64[a]	4.66[c]		4.28	4.71[A]	4.57[c]	
UREA (mg/dL)	Male	22.8	30.8[A]	31.8[C]	p<0.001	26.9	33.1[A]	31.5[C]	p<0.05
	Female	33.6	33.9	36.1		37.7	39.2	42.7c	
CREA (mg/dL)	Male	0.35	0.44[a]	0.46[C]		0.36	0.46[A]	0.51[C]	
	Female	0.39	0.50[A]	0.53[C]		0.41	0.53[A]	0.58[C]	

Superscript lettering A, B, C indicates statistically significant differences at p<0.001, and superscript lettering a, b, c indicates statistically significant at p<0.05.

[A]Significant difference 6–8 weeks vs. 10–14 weeks; [B]Significant difference 10–14 weeks vs. 6–9 months; [C]Significant difference 6–8 weeks vs. 6–9 months.

strains. In male rats of both strains, LYMPH was found significantly higher in 10-14 weeks old rats and in contrast, it was found to be higher in 6-8 weeks old female rats. Age differences for MONO were found only in male rats of both strains, while no age differences were observed in female rats.

There were significant age differences for GLU, AST, ALP, TP, and CREA in both sexes of SD and Wistar rats (Table 3). The concentrations of AST and ALP were found significantly higher in younger rats (6–8 weeks) than in older rats (6-10 months). In addition, both analyte concentrations tended to decrease as age increased in both strains; however, interactive effects of sex and age were observed only for ALP. There were age differences for ALT in male rats of both strains, while female rats had similar values between age ranges. GLU, TP, and CREA were significantly higher in 6-10-month-old rats (male and female) of both strains when compared to age ranges. Furthermore, these analytes tend to increase with age in both strains. There were interactive effects of sex and age for GLU and UREA in both strains, while it was observed only in Wistar rats for ALT and TP. Male rats of both strains had significant age differences for ALB, but concentrations were found similar between age ranges in female rats of both strains. The concentrations of UREA had significant age differences in both sexes of Wistar rats and male SD rats; however, it was not observed in female SD rats.

DISCUSSION

A comparison of selected clinical pathology analytes in two outbred rat strains revealed differences in several analytes. Our results confirm previous reports, which have shown that strain and age may produce relevant differences in the mean values of hematology and biochemical analytes. The values of hematology and biochemistry analytes presented here were compared favourably to background data of Crl: CD(SD) rats (Gilkins and Cliford, 2006) Crl: WI(Han) rats (Gilkins and Cliford, 2008) and previously published articles.

This study demonstrated that RBC and HCT had strain differences; however, HGB concentrations were found to be similar between strains in all age ranges. The increase in the age of the Wistar rats was followed by a progressive increase in RBC, HGB, and HCT values in both sexes, and the findings were consistent with reports. (Jacob, 2018; Kampfmann, 2012; Özturk, 2021; Patel, 2024). In SD rats, only RBC values showed a progressive rise with age in both sexes. RBCs were found to be higher in male Wistar rats in all age ranges than in SD rats, which was not observed in female rats. The varying demands for oxygen may be the cause of variation in RBC and HB concentrations.

SD rats had higher MCH and MCHC concentrations than Wistar rats in all rage ranges; however, no strain differences were observed for MCV. Both sexes of SD and Wistar rats had age effects for MCV and MCH, whereas MCHC had no age differences in female rats of both strains. Male rats in both strains showed a decreasing trend for MCV and MCH values, which was not observed in female rats. Reported values were similar to the results reported by previous studies in SD and Wistar rats. (He Q, 2017; Jacob, 2018)

PLT counts were found higher in both sexes of SD rats than in Wistar rats and similar observations were reported in the study. (Hayakava, 2013) PLT mean values varied with age and were found significantly higher in 6-8 weeks

old rats compared to age ranges. PLT counts tended to decrease with age and similar observations were reported in the study. (Jones, 2016)

For WBC, strain differences were observed in both sexes at all age intervals (Table 1, 2) and males had higher WBC counts than females in both strains. The findings were in agreement with studies in SD and Wistar rats. (He Q, 2017; Kampfmann, 2012; Petterino, 2006, Okamura, 2011) In addition, age differences were observed in both sexes for WBC counts and were progressively decreased with age in females of both strains (Table 3). (Okamura 2011) The observed differences may be due to the effects of hormones and strain.

The absolute counts of NEU were found significantly higher in both sexes of SD rats than Wistar rats in all age ranges (except 6-10 months female rats) and in contrast, N% were found significantly higher in Wistar rats in all age ranges (except 6-8 weeks male rats). LYMPH counts were significantly higher in both sexes of SD rats in all age ranges, while L% were found significantly higher only in female SD rats. NEU and LYMPH counts were found to be higher in males than in females and findings were consistent with previous reports of SD and Wistar rats. (He Q, 2017; Kampfmann, 2012; Petterino, 2006) With age, there were age differences for NEU, N%, LYMPH, and L% in both sexes of SD and Wistar Rats. In addition, males showed an increase in NEU counts, and females showed a decrease in LYMPH counts in both strains. The increase in the age of both strains (male and female rats) was followed by a significant increase in N% and a significant reduction in L%. These findings were consistent with previous studies in Wistar rats. (Jacob, 2018; de Kort, 2020, Kampfmann, 2012)

Relatively, MONO counts and M% were found higher in SD rats than in Wistar rats (except 6-8 weeks female rats). There were no age differences for MONO in female rats of both strains; however, M% has significant age differences in both sexes of SD and Wistar rats.

Both male and female SD rats had significantly higher EOS counts in 6-10-month-old rats compared to Wistar rats. In contrast, E% was found significantly higher in both sexes of Wistar rats in all age ranges (except 6-10 months female rats). With age, there were age differences for EOS and E% in both strains, and values were found higher in older rats. BASO counts had strain differences in 6-8 weeks, 6-10 months old male rats, and 10-14 weeks old female rats; however, B% were similar between strains in both sexes. Both strains had no age effects for BASO and B%.

AST and ALT had significant strain differences and concentrations were found significantly higher in both sexes of SD rats in all age ranges. AST had significant age differences in male and female rats of both strains; however, no age differences were observed for ALT in female rats of both strains. In addition, male rats had higher AST and ALT concentrations than female rats, in agreement with previous reports. (Alemann, 1986; He Q, 2017) AST concentrations were decreased with age in male and female rats of both strains.

ALP concentrations were found similar between strains in 10-14 weeks males and 6-8 weeks females. ALP concentrations were significantly higher in 6-8 weeks old rats in both strains with irrespective of sex and a significant reduction was observed in concentrations with age. (Alemann, 1986; Wolford, 1986; Okamura, 2011) Younger rats tend to have higher ALP activities due to the predominance of isoenzymes, the activities of these isoenzymes decrease during aging.TP concentrations were found significantly higher in male SD rats than Wistar rats (except 6-8 weeks old rats), whereas it was found higher only in 6-9 months old females. TP had significant age differences and concentrations tend to increase with age in both strains irrespective of sex. ALB concentrations had no strain differences and age effects were observed only in female rats in both strains. Age-related alterations in renal functions could be the cause of differences in TP and ALB concentrations.

Wistar rats had significantly higher UREA concentrations in both sexes compared to SD rats (except 6-10-month-old male rats). In addition, male rats of both strains showed an age-related increase in UREA concentrations, whereas female Wistar rats had age effects, but it was not found in female SD Rats. This may be due to increased urea reabsorption at the collecting duct resulting from low urine flow rates and to the diffusion of urea from the terminal collecting ducts into the medullary interstitium. A study reported UREA values showed age-related reduction (Alemann, 1986), but the findings were not in agreement with the report. CREA concentrations were similar between both strains and values were found significantly higher in 6-10-month-old rats of both strains. CREA concentrations also showed age-related increases in both sexes of SD and Wistar rats. (Parel, 2024) UREA and CREA concentrations were found to be higher in females than males in both strains. (He Q, 2017) This might have resulted from the reduction in glomerular filtration rate because CREA concentrations in the plasma depend largely on glomerular function or storage of CREA as a waste product in muscles. Strain differences were observed for fasting GLU level only in 6-8 weeks-old rats of both strains. Results were in agreement with the report of Ghezzi et al, 2012 who reported that mature rats showed an increase in serum glucose compared with younger rats.

CONCLUSION

In conclusion, age and strain showed a significant influence on most of the hematological and biochemical analytes of outbred rats. This study demonstrates that the concentration of MCV, MCH, PLT, AST, and ALP were markedly high in younger rats compared to older rats regardless of their sex. Finally, the levels of GLU, TP, and CREA in the serum were found to increase with age, while AST, and ALP decline with age in both strains. The limitation of this study is that animals originating from the same supplier were used for comparison, which is certainly not representative of all suppliers. Further effort should be made to more comprehensive studies with larger number of sample sizes originated from different suppliers and considering additional blood parameters (phospholipids, triglyceride, minerals, and trace elements).

AUTHOR'S CONTRIBUTIONS

Animal Research Facility team - Suresh Patel, Satish Patel, Ashvin Kotadiya, Samir Patel was involved in these studies for acquisition and interpretation of data; Tushar Patel, Harshida Trivedi analysed samples; Suresh Patel has contributed for compilation of data, literature review and wrote the manuscript. He also conducted statistical analysis and production of tables, figures. Satish Patel and Mukul Jain was involved in drafting and revising it critically for important intellectual content.

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REFERENCES

1. Alemán CL, Más RM, Rodeiro I, et al. (1998). Reference database of the main physiological parameters in Sprague-Dawley rats from 6 to 32 months. Lab Anim. 32(4):457-466. https://10.1258/002367798780599802
2. Boehm O, Zur B, Koch A, Tran N, Freyenhagen R, Hartmann M, et al. (2007). Clinical chemistry reference database for Wistar rats and C57/BL6 mice. Biol Chem. 388(5):547-54
3. Bryda EC (2013). The Mighty Mouse: the impact of rodents on advances in biomedical research. Mo Med, 110, 207-211.
4. Delwatta SL, Gunatilake M, Baumans V, et al. (2018). Reference values for selected hematological, biochemical, and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. Animal Model Exp Med. 2018;1(4):250-254. https://10.1002/ame2.12041
5. de Kort M, Weber K, Wimmer B, Wilutzky K, Neuenhahn P, Allingham P, et al. (2020). Historical control data for hematology parameters obtained from toxicity studies performed on different Wistar rat strains: Acceptable value ranges, definition of severity degrees, and vehicle effects. Toxicol Res and App. 4.
6. Ghezzi AC, Cambri LT, Botezelli JD, Ribeiro C, Dalia RA, de Mello MA (2012). Metabolic syndrome markers in Wistar rats of different ages. Diabetol Metab Syndr 4(1):16. https://10.1186/1758-5996-4-16
7. Giknis MLA, Clifford CB (2006). Clinical laboratory parameter for Crl: CD (SD). Charles River Laboratories. https://www.criver.com/sites/default/files/resources/rm_rm_r_clinical_parameters_cd_rat_06.pdf
8. Giknis MLA, Clifford CB (2008). Clinical laboratory parameter for Crl: WI(Han). Charles River Laboratories. https://www.criver.com/sites/default/files/resources/rm_rm_r_Wistar_Han_clin_lab_parameters_08.pdf
9. Hayakawa K, Mimura Y, Tachibana S, et al. (2013). Study for collecting background data on Wistar Hannover [Crl: WI(Han)] rats in general toxicity studies--comparative data to Sprague Dawley rats. J Toxicol Sci. 38(6):855-873. https://10.2131/jts.38.855
10. He Q, Su G, Liu K, et al. (2017). Sex-specific reference intervals of hematologic and biochemical analytes in Sprague-Dawley rats using the nonparametric rank percentile method. PLoS One. 12(12):e0189837. https://10.1371/journal.pone.0189837
11. Horn PS, Pesce AJ. (2003). Reference intervals: an update. Clin Chim Acta. 334(1-2):5-23. https://10.1016/s0009-8981(03)00133-5
12. Jacob Filho W, Lima CC, Paunksnis MRR, Silva AA, Perilhão MS, Caldeira M, et al. (2018). Reference database of hematological parameters for growing and aging rats. Aging Male 21(2):145-8
13. Jones CI (2016). Platelet function and aging. Mamm Genome. 2016;27(7-8):358-366. https://10.1007/s00335-016-9629-8
14. Kampfmann I, Bauer NB, Johannes S, Moritz A (2012). Differences in hematologic variables in rats of the same strain but different origin. Vet Clin Pathol. 41(2); 228-34.
15. Koolhaas JM (2010). The Laboratory Rat. In: The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, 311-326.

16. Krinke GJ ed (2000). The Laboratory Rat. San Diego, CA: Academic Press; 2000
17. Okamura T, Suzuki S, Ogawa T, et al. (2011). Background Data for General Toxicology Parameters in RccHan:WIST Rats at 8, 10, 19 and 32 Weeks of Age. J Toxicol Pathol. 2011;24(4):195-205. https://10.1293/tox.24.195
18. Özturk B, Çiftçi İ, Ecer B, Gökyaprak SM, Eryavuz Onmaz D (2021). Biochemical and hematological profiles of wistar rats at the Selcuk University experimental medicine research and application center. Eurasian J Vet Sci. 37(4):259-4.
19. Patel S, Patel S, Kotadiya A, et al. (2024). Age-related changes in hematological and biochemical profiles of Wistar rats. Lab Anim Res. 40(1):7. https://10.1186/s42826-024-00194-7
20. Petterino C, Argentino-Storino A (2006). Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. Exp Toxicol Pathol. 57(3):213±9. https://doi.org/10.1016/j.etp.2005.10.002
21. Suckow MA, Weisbroth SH, Franklin CL (2006). The Laboratory Rat. 2nd ed. Amsterdam, The Netherlands: Academic Press.
22. Van Zutphen LFM, Baumans V, Beynen AC (2001). Principles of Laboratory Animal Science, Revised Edition: a Contribution to the Humane Use and Care of Animals and to the Quality of Experimental Resu. The Netherlands: Elsevier.
23. Wolford ST, Schroer RA, Gohs FX, Gallo PP (1986). Reference range data base for serum chemistry and hematology values in laboratory animals. J Toxicol Environ Health. 18(2): 161-88.
24. Wolford ST, Schroer RA, Gallo PP, et al. (1987). Age-related changes in serum chemistry and hematology values in normal Sprague-Dawley rats. Fundam Appl Toxicol. 8(1):80-88. https://10.1016/0272-0590(87)90102-3
25. Zhong-Ze Han, Hong-De Xu and Kwang-Ho Kim et al. (2010). Reference Data of the Main Physiological Parameters in Control Sprague-Dawley Rats from Pre-clinical Toxicity Studies. Laboratory Animal Research. Vol. 26(2):153. https://10.5625/lar.2010.26.2.153