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RESEARCH ARTICLE

Biochemical and hematological profiles of wistar rats at the Selcuk University experimental medicine research and application center

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Selçuk Üniversitesi deneysel tıp araştırma ve uygulama merkezindeki wistar ratların biyokimyasal ve hematolojik profili

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Öz

Amaç: Yetiştiriciler, belirli popülasyonlar için bir dizi referans değeri sağlar, ancak yerel deneysel hayvan araştırma merkezlerinde üretilen sıçanlar fizyolojik ve genetik değişikliklere uğramış olabilir. Bu çalışmadaki amacımız, Konya, Türkiye, Selçuk Üniversitesi Deneysel Tıp Araştırma ve Uygulama Merkezi'nde (SUDAM) standart yerel koşullarda yetiştirilen Wistar ratların biyokimyasal ve hematolojik profillerini araştırarak gelecekteki çalışmalara katkıda bulunmaktır.

Gereç ve Yöntem: Çalışmaya SUDAM Hayvan Evinde normal hayatlarını süren 1 (erkek = 15, dişi = 15), 3 (erkek = 15, dişi = 15), 6 (erkek = 15, dişi = 15) ve 12 (erkek = 15, dişi = 15) aylık Wistar ratlar dahil edildi. Seçilen biyokimyasal ve hematolojik parametreler sırasıyla Beckman AU 5800 ve Beckman Coulter LH-780 analizörleriyle ölçüldü.

Bulgular: Üre, kreatinin, ALT, albümin, globulin, RBC, hemoglobin, hematokrit ve MCH düzeyleri arasında aylara göre istatiksel olarak anlamlı bir farklılık vardı ($p<0.05$). Seçilen biyokimyasal ve hematolojik parametreler cinsiyete göre karşılaştırıldığında glikoz, üre, ALP, amilaz, albümin, total protein, CK, kolesterol, trigliserit, sodyum, potasyum, RBC, hematokrit, MCH, trombosit, WBC, nötrofil, monosit, eozinofil, bazofil düzeyleri arasında istatistiksel olarak anlamlı bir farklılık vardı ($p<0.05$).

Öneri: Bu çalışma, Wistar ratların biyokimyasal ve hematolojik parametrelerinin yaş, cinsiyet, çevre ve beslenme gibi faktörlerle değişebileceğini kanıtlamıştır.

Anahtar kelimeler: Referans değerler, hematolojik parametreler, biyokimyasal parametreler, sıçan, wistar

Abstract

Aim: Breeders provide a set of reference values for the specific populations, however, rats produced in local experimental animal research centers may have undergone physiological and genetic changes. Our aim in this study was to contribute to further studies by investigating the biochemical and hematological profile of Wistar rats raised under standard local conditions at the Selcuk University Experimental Medicine Research and Application Center (SUDAM), Konya, Turkey.

Materials and Methods: The study included 1 (male=15, female=15), 3 (male=15, female=15), 6 (male=15, female=15), and 12-month-old (male=15, female=15) Wistar rats living their normal lives in the Animal House of SUDAM. Selected biochemical and hematological parameters were measured with the Beckman AU 5800 and Beckman Coulter LH-780 analyzers, respectively.

Results: Urea, creatinine, ALT, albumin, globulin, RBC, hemoglobin, hematocrit, and MCH levels differed significantly between rats according to months ($p<0.05$). When the selected biochemical and hematological parameters were compared according to gender, it was found that glucose, urea, ALP, amylase, albumin, total protein, CK, cholesterol, triglyceride, sodium, potassium, RBC, hematocrit, MCH, platelet, WBC, neutrophil, monocyte, eosinophil, basophil levels showed a statistically significant difference ($p<0.05$).

Conclusion: This study proved that biochemical and hematological parameters of Wistar rats may alter with factors such as age, gender, environment, and nutrition.

Keywords: Reference values, hematological parameters, biochemical parameters, rat, wistar

Introduction

Animals have been used in scientific research throughout human history (Andersen and Winter 2019). Animal experiments have made important contributions to modern medicine, including vaccine and drug development, understanding of pathological pathways in human disease (Franco 2013). Currently, approximately 20 million animal subjects are used in biomedical studies (Robinson et al 2019). Laboratory mice and rats are the most researched animal group with a rate of 80-90% (Sengupta 2013). The reason is the anatomical, genetic (95%) and physiological similarity of laboratory rats and mice to humans, small size, low space, and resource requirements, their ability to give a large number of offspring despite short gestation period, rapid development, and short life span (Bryda 2013). Wistar rats were developed in 1906 at the Wistar Institute to be used in biomedical research. It is an outbred albino rat and is one of the most popular rats used in laboratory research today (Koolhaas 2010). Biochemical and hematological laboratory tests have an important role in evaluating specific changes in the physiological and functional profile of a laboratory animal. In experiments where pathological mechanisms are induced, it is important to know normal reference values to determine the degree of disease or treatment efficacy, as some pathologies affect metabolism and cause changes in laboratory results (Pessini et al 2020). Breeders provide a set of reference values under certain conditions for the different parameters of their specific populations, however, rats produced under standard conditions in local experimental animal research centers may have undergone physiological and genetic changes over time. Therefore, all experimental research centers must determine the reference values for the animals they breed under local conditions (Leineweber et al 2018). Our aim in this study was to contribute to further studies by investigating the biochemical and hematological profile of Wistar rats raised under standard local conditions at the Selcuk University Experimental Medicine Research and Application Center (SUDAM).

Material and Methods

Animals

The study included 1 (male=15, female=15), 3 (male=15, female=15), 6 (male=15, female=15), and 12-month-old (male=15, female=15) Wistar rats living their normal lives in the Animal House of SUDAM, Konya, Turkey. In this study, no statistical method was adopted to determine the sample size and the number of subjects was kept at a minimum as stated in the ethical application. The Wistar rats were fed ad libitum with a pelleted diet (crude protein more than 23%, crude fiber less than 7%, crude ash less than 8%, moisture less than 12%, and metabolic energy at least 2600 Kcal/kg) (Bil Yem, Turkey) and tap water. During the study, rats were housed in polycarbonate thermoregulated cages with a length of 595 mm, a width of 380 mm, a height of 200 mm, and a floor area of 1800 cm^2, with 5 rats per cage based on sex. Necessary environmental conditions (temperature 24 ± 2 °C, humidity 60%, 12/12 hours light - dark cycles, ventilation set at 10-15 changes per hour) were provided for the rats. The bedding was altered and the cages were cleaned daily. This study was approved by the local ethics committee of SUDAM (Number: 2019/3, Date: 29.01.2021).

Sample collection

In December 2019, the animals were anesthetized with 75 mg/kg ketamine plus 8 mg/kg xylazine, and 0.5 and 1 mL of blood samples were taken into tubes with EDTA and serum seperator gel respectively, between 8.00 and 10.00 AM from animals that were at rest for at least 2 hours. The blood samples in serum separator gel tubes were centrifuged at 2000 x g for 10 min and the serum samples were separated. Selected biochemical parameters in serum samples were measured with the Beckman AU 5800 (Beckman Coulter, Brea, USA) autoanalyzer, and hematological parameters from whole blood samples were measured with the Beckman Coulter LH-780 (Beckman Coulter, Miami, FL, USA). Selected biochemical parameters included glucose, sodium, potassium, calcium, phosphorus, urea, creatinine, total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), amylase, creatine kinase (CK), total protein, albumin, globulin, cholesterol, triglyceride, glucose and selected hematological parameters included red blood cell (RBC), hemoglobin, hematocrit, white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophil, lymphocyte, neutrophil/lymphocyte ratio, monocyte, eosinophil, basophil, platelet.

Statistical analysis

Statistical analysis was performed with SPSS statistical software package version 21.0. One-Sample Kolmogorov-Smirnov test was performed to find out the distribution. Student's t and Mann-Whitney U tests were used to compare the mean and median values between two groups, respectively. One-way ANOVA analysis (post-hoc analysis with LSD or Tamhane's T2 tests) and Kruskal – Wallis test (post-hoc analysis Mann-Whitney U) were also performed. $p<0.05$ was considered as statistically significant.

Results

Hematological and biochemical parameters of Wistar rats are given in Table 1 and Table 2, respectively. Results are expressed as Mean ± SD (standard deviation).

As a result of the study, it was found that biochemical and hematological parameters, especially urea, creatinine, ALT,

Table 1. Hematological profiles for Wistar rats in SUDAM

	1		3		6		12	
	Female	Male	Female	Male	Female	Male	Female	Male
RBC (10e6/Ul)	6.6±0.4 a,b,c	6.1±0.3	7.9±1.1 d,e	7.0±1.1	8.6±0.3	8.3±0.4	8.6±0.3 g	8.3±0.3
Hemoglobin (g/dL)	13.4±0.6 a,b,c	12.5±0.5	14.6±1.2 d,e	14.4±1.1	15.4±0.6	15.1±1.6	15.3±0.6	15.1±0.6
Hematocrit (%)	40.8±2.5 a,b,c	37.2±1.8	43.6±6.2 d,e	39.4±6.0	47.1±2.4	46.7±2.2	46.9±1.9 g	45.8±2.2
MCV (fL)	61.8±2.5 a,b,c	60.3±1.1	54.6±1.8	55.9±1.9	54.4±1.4	56.1±1.5	54.4±2.1	55.2±1.7
MCH (pg)	20.2±0.9 a,b,c	21.3±3.6	18.8±3.9 d,e	20.8±2.8	17.7±0.3	18.1±1.9	17.8±0.7 g	18.2±0.6
Platelet (K/uL)	1171±324 c	1206±196	681±274	1302±352	1179±254	1090±371	926±233 g	939±199
WBC (K/Ul)	7.0±2.9	5.7±1.1	10.6±5.3	3.6±1.7	7.65±2.43	5.86±2.34	7.4±2.3 g	5.0±2.0
Neutrophil (K/uL)	1.0±0.9	0.7±0.3	1.6±0.6	0.5±0.2	1.6±1.1	0.63±0.51	2.2±2.8 g	1.1±0.7
Lymphocyte(K/uL)	5.8±2.0 c	4.8±0.8	7.7±2.6	3.0±1.5	5.7±2.1	4.92±2.04	4.9±2.5	3.5±1.5
Monocyte (K/uL)	0.09±0.08	0.06±0.02	0.23±0.05	0.09±0.02	0.09±0.01	0.66±0.11	0.09±0.04 g	0.10±0.02
Eosinophil (K/uL)	0.09±0.05	0.08±0.02	0.10±0.06	0.06±0.03	0.11±0.03	0.06±0.01	0.12±0.05 g	0.09±0.02
Basophil (K/uL)	0.11±0.05	0.09±0.01	1.14±0.89	0.07±0.05	0.17±0.19	0.27±05	0.26±0.41 g	0.29±0.73

a: 1 vs 3 month, b: 1vs 6 month, c: 1 vs 12 month, d: 3 vs 6 month, e: 3 vs 12 month, f: 6 vs 12 month comparison. g: female vs male comparison. p<0.05 was considered as statistically significant. a, b, c, d, e, f, g: means that there was a statistically significant difference between the defined groups.

Table 2. Biochemical profiles of Wistar rats in SUDAM

	1		3		6		12	
	Female	Male	Female	Male	Female	Male	Female	Male
Glucose (mg/dL)	251±57 c	260±36	247±53	290±48	230±37 f	287±49	270±43 g	304±56
Urea (mg/dL)	48.8±6.5 a,b,c	42.8±4.9	65.0±10.7 d,e	55.8± 5.6	44.0±5.6 f	36.8±4.4	39.5±3.7 g	34.2±5.0
Creatinine (mg/dL)	0.2±0.03 a,b,c	0.22±0.02	0.33±0.05 e	0.33±0.05	0.31±0.06 f	0.33±0.04	0.38±0.09	0.34±0.02
AST (U/L)	120±17 a	96±20	84±13 d,e	101±30	120±31	96±18	112±23	111±35
ALT (U/L)	44±10.1 a,b,c	44.7±5.9	59.3±12.6 d,e	56.6±13.2	69.0±11.7 f	59.1±13.9	81.3±22.8	77.4±28.8
ALP (U/L)	378±69 a,c	393±71	461±145	226±69	511±127	229±125	415±192 g	210±100
Amylase (U/L)	2145±411 b	1772±192	2457±333 d	1649±183	2290±288 f	2461±413	1881±351 g	1990±455
Albumin (mg/dL)	3.0±0.19 a,b	3.0±0.09	3.2±0.2 d,e	3.4±0.1	3.0±0.1 f	3.2±0.1	2.8±0.2 g	3.2±0.2
Total protein (mg/dL)	5.2±0.2 a,b,c	5.4±0.1	6.0±0.3	6.7±0.2	6.3±0.3	6.5±0.3	6.2±0.3 g	6.8±0.3
Globulin (mg/dL)	2.2±0.1 a,b,c	2.3±0.1	2.6±0.2 d,e	3.2±0.2	3.2±0.2 f	3.3±0.1	3.4±0.3	3.5±0.2
Phosphorus (mg/dL)	7.7±0.5 a,b,c	8.2±0.4	6.3±1.4	5.6±0.9	6.3±2.0	5.8±0.7	6.8±2.2	4.7±0.6
Total bilirubin (mg/dL)	0.14±0.02 a,b	0.14±0.02	0.15±0.03	0.15±0.01	0.10±0.04 f	0.12±0.02	0.14±0.03	0.14±0.03
Creatine kinase (U/L)	604±173 a	359±161	349±136 d, e	334±120	598±224	401±73	609±225 g	505±351
Cholesterol (mg/dL)	76.3±14.6 b,c	91.0±13.5	58.6±8.8	69.5±10.6	60.3±11.7 f	62.1±11.3	60.2±9.9 g	77.8±15.6
Triglyceride (mg/dL)	54±24.6 a,b,c	87.2±36.3	78.6±20.8	102±28.4	49.6±19.3	102±28.1	81.1±27.6 g	97.4±31.8
Sodium (mEq/L)	145.5±2.0 a,b	145.8±1.6	143.2±2.2 d,e	139.9±1.7	145.8±2.6	143.2±1.5	146.4±1.6 g	142.8±2.8
Potassium (mEq/L)	4.7±0.3	4.7±0.3	4.8±0.7 e	4.3±0.3	5.0±0.7	4.3±0.4	5.7±1.0 g	4.4±0.5

a: 1 vs 3 month, b: 1vs 6 month, c: 1 vs 12 month, d: 3 vs 6 month, e: 3 vs 12 month, f: 6 vs 12 month comparison. g: female vs male comparison. p<0.05 was considered as statistically significant. a, b,c, d, e, f,g: means that there was a statistically significant difference between the defined

albumin, globulin, RBC, hemoglobin, hematocrit, and MCH levels differed significantly between rats according to months ($p<0.05$). When the selected biochemical and hematological parameters were compared according to gender, it was found that glucose, urea, ALP, amylase, albumin, total protein, CK, cholesterol, triglyceride, sodium, potassium, RBC, hematocrit, MCH, platelet, WBC, neutrophil, monocyte, eosinophil, basophil levels showed a statistically significant difference ($p<0.05$).

Discussion

Animals have been an important tool throughout humanity in the development of drugs, vaccines, surgical instruments, and studies on the pathogenesis of human diseases. Since humans cannot be used as subjects, animals, especially rats, are widely used in these studies (Franco 2013). Therefore, it is essential to know the biochemical and hematological parameters of the rats used in these studies to accurately evaluate the body's response to different diseases or therapies. This is the first study to create a reference database by investigating selected biochemical and hematological parameters in healthy Wistar rats grown under standard conditions in SUDAM, Konya, Turkey. Although differences were observed in various biochemical and hematological parameters according to sex and months, the living environment, food, and water contents of all animals were the same. This study demonstrates that RBC, hemoglobin, and hematocrit values progressively increased from 1 month to 6 months and remained stable at 12 months, while ALT and total protein levels progressively increased until 12 months. MCV levels were higher in 1-month-old rats compared to the other groups, while platelet, neutrophil, lymphocyte, eosinophil, and basophil levels were relatively less variable with age (Table 1). Among the selected biochemical parameters, creatinine levels were found to be lower in 1-month-old rats compared to the other groups, while glucose, amylase, cholesterol, and potassium levels showed less variability with age (Table 2). In addition, it was observed that most of the selected parameters were statistically significantly different according to gender. Among the selected hematological parameters, RBC, hematocrit, neutrophil, eosinophil, basophil, monocyte, WBC values were found to be higher in males, while MCH and platelet values were higher in females (Table 1). Among selected biochemical parameters, triglyceride, glucose, albumin, total protein, and cholesterol levels were higher in females, while ALP, amylase, CK, sodium, and potassium levels were higher in males (Table 2). When our results were compared with the values reported by international breeders and researchers, similarities and differences were found between the levels of selected parameters (Filho et al 2017, Boehm et al 2007, Liberati et al 2004, Kampfmann et al 2012, Kort et al 2020, Charles River 2018). In the study conducted by Filho et al., selected hematological parameters of 1, 2, 3, 6, 12, 18, and 24-month-old female and male Wistar rats were compared. When the results reported by Filho et al. were compared with the current study, RBC, hemoglobin, MCV, hematocrit, values were high, while monocyte% and eosinophil% levels were low in 1-month old rats in our study. MCH, lymphocyte% and neutrophil% values were similar. In 3-month-old rats, RBC, hemoglobin, and MCH levels were higher, while MCV, eosinophil%, and monocyte% values were lower in the current study. Hematocrit, lymphocyte%, and neutrophil% levels were similar to the results reported by Filho et al. RBC, hemoglobin, hematocrit, lymphocyte% levels were higher in our study, while MCV, eosinophil%, monocyte%, neutrophil% were lower. MCH levels were similar in 6-month-old rats. In 12-month-old rats, RBC, hemoglobin, MCH, hematocrit, lymphocyte% levels were higher, while neutrophil% levels were lower than the values reported by Filho. MCV and monocyte% levels were similar (Filho et al 2017). Sodium, total protein, cholesterol, glucose, ALT, AST, amylase levels were higher, potassium and creatinine levels were lower in our study when the selected biochemical parameters were reported by Boehm et al. for 5-month-old Wistar rats were compared with a similar age group in the present study. Total bilirubin, urea, triglyceride, and CK levels were similar in both studies (Boehm et al 2007). Various biochemical and hematological parameters of 70-day-old Wistar nonmated female rats were compared with pregnant rats of similar age groups in the study of Liberati et al (2004). When the values reported for nonmated rats are compared with the similar age group in the present study, RBC, hematocrit, hemoglobin, WBC, lymphocyte, total protein, albumin, creatinine, and potassium levels were lower in our study. MCV, MCH, platelet, monocyte, eosinophil, ALT, AST, ALP, CK, globulin, cholesterol, glucose, urea, phosphorus levels were higher in our study, while basophil, total bilirubin, triglyceride, sodium levels were similar (Liberati et al 2004). When the results of the present study were compared with the values reported by the Harlan Laboratory for 2-month-old female Wistar rats, WBC, RBC, hemoglobin, hematocrit, MCV, monocyte levels were lower in the current study, while the levels of MCH, basophils, lymphocyte, and neutrophils were higher. MCH and neutrophil levels were similar. Glucose, urea, and ALP levels among the selected biochemical parameters were higher in female Wistar rats, while ALT, phosphorus, sodium, and potassium levels were lower. Cholesterol, total protein, albumin, globulin, AST levels were similar. In male Wistar rats, glucose, urea, creatinine, albumin, total bilirubin, and ALP levels were higher, whereas cholesterol, total protein, globulin, AST, ALT, phosphorus, sodium, potassium levels were lower in our study. In the present study, among the hematology parameters determined for male Wistar rats, WBC, hemoglobin, hematocrit, MCV, MCH, lymphocyte, monocyte levels were lower, and eosinophil and basophil levels were higher. RBC, MCH, and neutrophil levels were similar to the values reported by the Harlan Laboratory (Kort et al 2020). When the values specified in the literature for Sprague-Dawley rats were compared with the parameters we measured in

Wistar rats, there were similarities and differences between the values of both species. Briefly, when the selected parameters of Wistar rats in SUDAM and other research centers were compared, it was observed that serum triglyceride and glucose values were slightly higher. However, serum triglyceride values were consistent with the levels reported in non-fasted Wistar rats (Boehm et al 2007; Liberati et al 2004). It is known that serum glucose values are affected by anesthetics as well as feeding. There are studies reporting that Ketamine/Xylazine anesthesia can increase blood glucose levels by 120 mg/dL. Therefore, considering the effect of anesthesia and feeding, our findings were consistent with these studies. For example, Saha et al. reported that basal glucose level increased from 104.8±5.7 mg/dL to 291.7±23.8 mg/dL within 120 minutes after Ketamine/Xylazine anesthesia in feded Sprague-Dawley rats. Therefore, our findings were consistent with these studies. The comparison of hematological values reported by Delwatta et al (2018). for healthy 4-5-month- old male and female rats with the findings of the current study showed that RBC, hemoglobin, values were higher than in both sexes, while WBC values were low in females and similar in males. PLT, MCV, and MCH values were low in Wistar rats in both genders according to values reported by Delwatta et al (2018). Glucose, total cholesterol, and triglyceride values were higher in Wistar rats, while ALT, and AST levels reported for Sprague-Dawley rats were higher in both sexes (Delwatta et al 2018). Similarly, when the hematological parameters reported by Aleman et al. for Sprague-Dawley rats younger than 6 months and 6-18 months old were compared with the values of Wistar rats, the hemoglobin levels of Wistar rats were low in both sexes, while the hematocrit values were similar. Serum glucose, AST and ALT levels of Wistar rats were higher than Sprague-Dawley rats, while serum urea and creatinine levels were similar (Aleman et al 1998). RBC, hemoglobin, monocyte counts reported by Ikechukwu et al. for Sprague-Dawley rats were lower than the values measured in Wistar rats in the current study, while MCV and MCH values were similar, and WBC, lymphocyte, neutrophil, monocytes, and eosinophil values were high (Ikechukwu et al 2004).

Conclusion

This study proved that biochemical and hematological parameters of Wistar rats may alter with factors such as age, gender, environment, and nutrition. Literature comparisons have shown that it is essential for each experimental application and research center to establish specific reference values for animal models grown in their local conditions. The values of biochemical and hematological determined in this study are very important in terms of guiding researchers who will use Wistar rats as an experimental model. In addition, it may contribute to reducing the number of control groups to some extent in future studies using these rat species as subjects.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Ethical Approval

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