

Some Hematological and Biochemical Characteristics of Male and Female Sprague-Dawley Rats

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Erkek ve Diři Sprague-Dawley Ratların Bazı Hematolojik ve Biyokimyasal Parametreleri

Abstract: This study was carried out to determine the biological norms of some hematological and serum biochemical variables of the male and female Sprague-Dawley rats, which have been bred since ca. two decades in Türkiye, and also since two years in our Laboratory Animal Unit as a relatively closed stock colony.

Erythrocyte, leukocyte and eosinophil counts were made by standard methods using Turk's solution in improved Neubauer hemocytometry. Packed cell volume (PCV) was determined with microhematocrit method, and leukocyte differentials were carried out on two slides for which a total of 500 leukocytes were considered (2x250). Serum biochemical variables were determined by commercial kits according to the proposal of the producer. In serum; total protein level, alkaline phosphatase activity and, calcium, phosphorus, magnesium and cholesterol levels were determined. The results were statistically analysed for which Man Whitney-U test and t-Test for independent groups were used to compare the means. That is because there was no difference in results of these two tests, t-Test results were considered.

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Except the packed cell volume (PCV) value and relative lymphocyte rate, there was no difference between male and female animals for all blood constituents studied. The males had a higher mean PCV value than the females ($p < 0.05$), whereas the mean relative percentage of lymphocytes of females was higher than the of males ($p < 0.05$).

Key Words: Rats, Sprague-Dawley, hematology, serum, normal values.

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Özet: Bu çalışmada yaklaşık 20 yıldır Türkiye'de ve iki senedir de laboratuvar hayvanları ünitemizde relatif olarak kapalı bir koloni beklende yetiştirilen erkek ve dişi Sprague-Dawley ratlarında bazı hematolojik ve serum biyokimyasal parametrelerin biyolojik normlarının belirlenmesi amacıyla gerçekleştirildi.

Eritrosit, lökosit ve eozinofil sayımlarında standard yöntemler kullanıldı. Hematokrit, mikrohematokrit yöntemle saptandı. Diferensiyal kan tablosu, taze hazırlanmış ve Pappenheim'in panoptik yöntemine göre boyanmış olan iki frotiden toplam 500 hücrenin değerlendirilmesi ile yapıldı. Serum alkalen fosfataz aktivitesi, total protein, kalsiyum, fosfor, magnezyum ve kolesterol değerlerinin belirlenmesinde ticari kitler kullanıldı. Ortalamaların karşılaştırılmasında bağımsız gruplar için t-Testi ve Man Whitney-U testi kullanıldı.

Hematokrit değer ve relatif lenfosit oranı dâhilinde, araştırılan diğer parametreler açısından erkek ve dişi ratlar arasında önemli bir fark bulunmadı. Hematokrit değer erkeklerde, relatif lenfosit oranı ise dişilerde daha yüksek bulundu ($p < 0.05$).

Anahtar Sözcükler: Rat, Sprague-Dawley, kan, serum, normal değerler.

Introduction

Over 3 century laboratory animals have been used in different research activities (15,17,18,26). In general; laboratory animals are used to get information about their biological nature, to study as experimental models of human and agricultural animal diseases or conditions, to develop and/or improve techniques, protocols, test materials and drugs etc., as well as effective therapeutic approaches (2). The exact interpretation of the results of such experimental works require a preceded thoroughly examination of the research subjects and the existence of a detailed information about their nature. This necessity to establish some basis for comparison has led to the numerous hematological and biochemical investigations using control and apparently healthy animals, and will do so also in the future.

The results of these investigations showed that concentrations of blood constituents were influenced by several factors like analytical, methodological, biological factors and the interactions between them (16). In addition, environment has also been known as a very important aspect affecting the concentration of blood constituents directly or indirectly. In other words, there are different intrinsic and extrinsic factors controlling and affecting the circulating concentrations of blood cells and other constituents of the blood through stimulation of their production and release into blood stream as well as eliminating them from the blood in different manner. Biological factors include genetic constituents like species, strain and line of subjects (6,20,25,32). Demographic characteristics include sex (34), age (5,7,13,21,24,36) and some other properties characterizing an individual. Also, physiological conditions consist of some properties like gestation, parturition or

lactation, and different physiopathological situations (12,25,30). Under environmental factors affecting different biological values, especially nutrition and housing conditions of animals could be highlighted (8,19,27,40). Earlier studies related to hematological and clinical biochemical values of different laboratory animal species as well as different strains of the same species were summarized by Jain (25), Mitruka and Rawnsley (29) and Schmidt and von Forstner (33).

It is well known that any population derived from a definite strain stock could differ from its origin in some extent when strain stock is bred over 22 generations as a closed colony (14). This is why there are sometimes great differences between different populations of the same strain or line as well, although they are also held under relatively similar conditions. Because of this, it is withstanding to check out the biological norms of every population in a laboratory animal unit in certain intervals, regularly or if there is a necessity.

Therefore, the aim of this study was to determine the levels of some hematological and serum biochemical parameters of the Sprague-Dawley rats bred since ca. two years in our laboratory animal unit as a closed colony and to compare them with accepted biological norms for rats of the same strain, given in present literature.

Materials and Methods

In this study, a total of 16, 105 days old, conventionally maintained Sprague-Dawley rats of both sexes were used, which made up ca. 18% of our population of this strain used for breeding. The mean body weight of the female rats ($n = 8$) was 108 g, and the of male rats ($n = 8$) 138 g. These animals have been bred since ca. two decades in Türkiye and also since two years in our institution as a relatively closed colony. They were held in polypropylene cages on wood shavings in groups of

3 to 5 animals in a semi-climate room, where the temperatures ranged between 22 and 27 °C, relative humidity between 60% and 75% and a light-dark cycle of 14:10 hours until the study conducted. A commercial food for mouse and rats (Best Yem, Ýzmit) and water was given *ad libitum*.

After a preceding of 18 h fasting period, the rats were weighed and blood samples were taken between 09.00 and 12.00 h by tail cutting under light ether anesthesia into two different Eppendorf tubes. The blood used for hematologic examinations anticoagulated with a small amount of EDTA-K₃ (1 mg / 1 ml blood). The blood samples without anticoagulant were left at room temperature for 45 min for clotting, then centrifugated at about 3000 rpm for 15 minutes and the serum was separated for biochemical analyses.

The blood cell counts were made within 4 hours following blood withdrawal. A hemocytometer with improved Neubauer rule was used to determine erythrocyte (RBC) and total leukocyte (TWBC) counts by standard methods (10,25). That is because of the very rarity of eosinophils in the peripheral blood of the rats, the eosinophil count was also carried out directly in hemocytometer; for this reason a hemocytometer with a rule of Fuchs-Rosenthal with a volume of 3.2 µl and a method *ad modum* Pilot (31) was employed. The packed cell volume (PCV) was determined with microhematocrit method by centrifugation of blood at 12800 rpm for 10 minutes. Two blood films per animal were made with fresh blood and stained *ad modum* Pappenheim, and a total of 500 leukocytes were differentiated. The counts were calculated as absolute concentrations per µl of blood and as percentages. Serum alkaline phosphatase (AP) activity and the levels of total protein (TP), cholesterol, calcium (Ca), phosphorus (P) and magnesium (Mg) were determined by Microlab

2000 (Merck®) with standard commercial kits (DiaSys®, Germany). The biochemical analyses were carried out according to the manufacturer's prescriptions.

Man Whithney-U test and two-tailed t-Test for independent groups were used for statistical analyses of the results (23).

Results

The results are summarized in Tables 1 to 3 as the mean values and standard deviations ($\bar{X} \pm S.D.$) with minimum and maximum.

Table 1. The body weights and some hematological variables.

Groups	Male rats (n = 8)	Female rats (n = 8)	
Variables	$\bar{X} \pm S. D.$ Minima - Maxima	$\bar{X} \pm S. D.$ Minima - Maxima	Significant Level
BW (g)	138 \pm 2.80 (120-154)	108 \pm 9.65 (93-126)	n. s.
RBC ($\times 10^6 / \mu\text{L}$)	7.86 \pm 1.32 (6.22-9.71)	6.54 \pm 1.75 (3.11-8.65)	n. s.
PCV (%)	46.00 \pm 2.88 (43.00-50.00)	43.88 \pm 1.48 (42.00-46.00)	p < 0.05
TWBC (/ μL)	14784 \pm 4536 (8462-22400)	11047 \pm 3158 (6800-15488)	n. s.
LYM (/ μL)	9446 \pm 3540 (4959 - 15232)	7094 \pm 2097 (4284-9580)	n. s.
MO (/ μL)	1203 \pm 616 (686 - 2330)	637 \pm 293 (266-1011)	n. s.
NEUT (/ μL)	1155 \pm 616 (686 - 2330)	1002 \pm 415 (519 - 1580)	n. s.
EOS-D (/ μL)	38 \pm 28 (11 - 98)	43 \pm 20 (11 - 70)	n. s.
EOS-ID (/ μL)	20 \pm 24 (0 - 65)	32 \pm 17 (0 - 54)	n. s.
MNL (/ μL)	10649 \pm 3982 (5991 - 17562)	7731 \pm 2311 (4624 - 10780)	n. s.

n. s. = not significant.

The mean body weights of males and females were 138 g and 108 g, respectively and did not differ significantly. As shown in Table 1, only the differences obtained from mean PCV values were statistically confirmed. Exactly, the males had higher PCV values than the females ($p < 0.05$). Differences in the concentrations of all other blood variables were not significant.

Table 2. The relative leukocyte counts (%).

	LYM	MON	NEUT	EOS	MNL
Male rats (n = 8)					
\bar{X}	62.65*	8.15	9.02	0.15	70.80
S. D.	6.20	2.83	6.13	0.18	6.20
X_{Min}	54.20	4.20	1.60	0.00	38.40
X_{Max}	69.80	12.20	12.20	0.40	61.00
Female rats (n = 8)					
\bar{X}	64.03*	5.68	9.18	0.33	69.70
S. D.	2.78	1.91	3.12	0.18	3.01
X_{Min}	59.60	3.20	4.80	0.00	65.20
X_{Max}	68.40	8.60	14.40	0.60	75.00

* $p < 0.05$

In differential blood picture (Table 2), there was only one statistically confirmed difference in relative percentages of lymphocytes, where mean relative values of females were higher than the of males ($p < 0.05$). Such differences in blood picture could be partly due to the sex of animals; for example because the sexual hormones. On the other site, the body weight difference between male and female rats could

also be the cause of differences in the concentrations of blood cells or their relative percentages (3).

Table 3. The values of some serum biochemical parameters of rats studied.

	TP (g/dL)	AP (IU/L)	Ca (mg/dL)	P (mg/ dL)	Mg (mg/ dL)	Cholesterol (mg/dL)
Male rats (n = 8)						
\bar{X}	7.33	580	11.26	11.66	2.54	52.16
S. D.	0.44	142	1.58	3.27	0.23	4.97
X_{Min}	6.70	364	9.70	6.70	2.20	44.30
X_{Max}	7.90	751	13.50	16.50	2.90	60.30
Female rats (n = 8)						
\bar{X}	7.30	513	11.38	9.73	2.34	53.48
S. D.	0.35	108	0.97	1.90	0.17	7.62
X_{Min}	6.80	390	9.60	6.40	2.00	44.50
X_{Max}	7.80	707	12.50	12.40	2.60	64.90

The results of some serum constituents studied are presented in Table 3. A t-Test for independent groups showed that there was no significant difference between males and females in relation to all serum constituents studied. Only the mean calcium value of the female animals showed a small tendency to be higher than of the males ($p = 0.063$, $DF = 4,068$).

Discussion

There are many evidences about sex dependent differences of blood constituents between adult animals, although some discrepancies and discussions in

this aspect are also present. It is generally accepted that in adult animals the mean erythrocyte concentrations and hematocrit values of the males are higher than of the females (1,10,25). Furthermore, some investigators reported that the female rats had more leukocytes than the males from the 8th week of life (9,25,37), while others failed to find any difference in the concentrations of leukocytes as well as in differential white cell pictures (11,22,28). In contrary, in a study on adult Wistar albino rats the concentrations of total leukocytes, mononuclear leukocytes and eosinophils of males have been found to be significantly higher than the of females, while in differential blood picture only the relative higher percentage of eosinophils in males could be statistically confirmed (3). The great variations in the eosinophil counts could be partially due to the small numbers of animals used as well as due to the method chosen for counting of these cells (39).

The results of the present study have been fully in agreement with the discrepant nature of the hematological picture and could be seen as a contribution to the discussions about its sex dependent behavior. Shortly, the higher PCV value of males was statistically confirmed ($p < 0.05$), but not the higher concentrations of erythrocytes of these animals. The TWBC concentration was higher in males than in females, but this difference could not be confirmed statistically. In differential blood picture, the lymphocytes were the dominating cells, but only the higher mean relative percentage value of the lymphocytes of female rats was statistically significant ($p < 0.05$). Absolute and relative mononuclear leukocyte (MNL) counts also did not show any difference. Neither the concentrations nor the percentages of the other cell types showed any important difference between both sexes. In addition, eosinophil values gathered by direct and indirect methods were not different, although it was well accepted that with direct method always higher numbers of cells could be

enumerated (3,25). No basophil could be observed during the differentiation of the blood cells. In addition, the relatively small percentages of neutrophils in both sexes were obvious.

The mean values of RBC, PCV and leukocytes of Sprague-Dawley rats, summarized by Mitruka and Rawnsley (29), are shown in Table 4.

Table 4: Reference values for studied hematological parameters of the Sprague-Dawley rats (29).

RBC ($\times 10^6/\mu\text{L}$)	PCV (%)	TWBC ($\times 10^3/\mu\text{L}$)	LYMPH ($\times 10^3/\mu\text{L}$)	MON ($\times 10^3/\mu\text{L}$)	NEUT ($\times 10^3/\mu\text{L}$)	EOS ($\times 10^3/\mu\text{L}$)	BASO ($\times 10^3/\mu\text{L}$)
6.26 -8.96	40.0 - 49.0	9.40 -14.9	6.80 -10.72	0.05 -0.53	0.06-3.45	0.03 -0.06	0.00-0.02
Relative percentages			72.0-94.5	0.50-3.50	4.50-23.5	0.35-0.60	0.00-0.20

If it is compared with the reference values in the Table 4, it can be seen that TWBC values found in this study lie on the higher limits of the norms. The concentrations of monocytes of both sexes are also higher than normal values given. But by interpretation of the values from present study in comparison with these references, it must be considered that the reference values have been arisen from SPF animals. Furthermore, it must also be highlighted that, by interpretation of all these results the specificity and sensitivity of the methods used for enumeration of the blood cells on the one side, and the relatively small size of experimental animals on the other side should also be considered.

Table 5 shows a general view from serum biochemical values of normal rats. A circular of the Schmidt and von Forstner (33) gave also some detailed information

about the norms and biological variations of different blood constituents in relation to characteristics of animals like age, sex, strain and species as well as with their hygienic conditions.

Table 5. Reference values for studied biochemical parameters of the Sprague-Dawley rats, with maxima and minima (29).

	TP (g/dL)	AP (IU/L)	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	Cholesterol (mg/dL)
Male animals						
\bar{X}	7.61	466	12.20	7.56	3.12	48.30
S. D.	0.50	-	0.75	1.51	0.41	10.20
X_{Min}	4.70	300	7.20	3.11	1.60	10.00
X_{Max}	8.15	640	13.90	11.00	4.44	54.00
Female animals						
\bar{X}	7.52		10.60	8.26	2.60	44.70
s_x	0.32		0.89	1.14	0.21	9.62
X_{Min}	6.80		9.60	3.11	1.60	10.00
X_{Max}	7.80		12.50	11.00	4.44	54.00

The total protein values in the present literature varied between 5.19 and 7.43 g/dL (4,6,8,33). Different factors like the strain and age of animals as well as nutrition and restraining techniques seemed to be an important factor influencing the mean protein values of rats significantly. The mean protein values found in the present

study were 7.33 ± 0.44 g/dL for males and 7.30 ± 0.35 g/dL for females, and so were consonant with the normal protein values given for rats (29). Thompson et al. (35) reported the range of alkaline phosphatase values of the rats between 300 and 640 IU/L. But a detailed study of reference values for this parameter showed that the mean alkaline phosphatase values of rats could vary widely and lie between 65.8 and 713 IU/L (29). In this study we have found that the mean alkaline phosphatase values were 580 ± 142 IU/L and 513 ± 108 IU/L in male and female rats, respectively. The mean Ca values in this study were found to be 11.26 ± 1.58 mg/dL for males and 11.38 ± 0.97 mg/dL for females, and good in accordance with the values given in literature in which the mean Ca values for rats were varying in mean between 10.66 and 11.46 mg/dL (4). Normal serum P values of albino rats varied between 6.72 and 10.30 mg/dL (29). In this study, the mean serum P values were found as 11.66 ± 3.27 mg/dL for males and 9.73 ± 1.90 mg/dL for females. Thus, these values were sometimes higher than normal limits which could lye on the food given. Normal serum Mg values of albino rats were varying between 2.16 and 3.12 mg/dL (29,37). Our results, 2.54 ± 0.23 mg/dL for male rats and 2.34 ± 0.17 mg/dL for female rats were good in agreement with this norms. Normal serum cholesterol values of albino rats varied between 44.7 and 110 mg/dL (29). Our results were 52.16 ± 4.97 mg/dL for males and 53.48 ± 7.62 mg/dL for females, and thus seemed to be in a lower portion of normal limits.

All serum biochemical constituents studied were failed to show any sex dependent difference. Only Ca had a tendency to be higher in females than in males ($p = 0.063$, $DF = 4,068$).

Conclusions

The question of the fragility of hematological and biochemical results and their dependency on several different factors rise the necessity of using the most sensitive methods like fully automated cell counters produced or adapted specifically for veterinary use as well as biochemical analyzers. Furthermore, it is also very essential to monitor the normal values of a given population in certain intervals by using a representative number of animals through periodical systematically analyses of the cellular and biochemical blood constituents in respect to intrinsic and extrinsic confounding factors in a strain related basis.

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