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Araştırma Makalesi

THE EFFECT OF BLOOD WITHDRAWAL SITE ON HEMATOLOGICAL VALUES IN MICE

Hümeyra ÜNSAL*[1], Muharrem BALKAYA*, Cengiz ÜNSAL*

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Farelerde Kan Alma Yerinin Hematolojik Değerler Üzerine Etkisi

Özet: Bu çalışmada kan alma yerinin bazı hematolojik değişkenler üzerine etkilerinin araştırılması amaçlanmıştır. Bu amaçla toplam 21 Swiss albino dişi fare kullanılmıştır. Fareler üç gruba ayrılmışlar ve her grup farklı bir kan alma yeri için kullanılmıştır. Kan her hayvandan kalp, kuyruk veya retro-orbital pleksustan bir kez alınmıştır. PCV değerlerinin retro-orbital pleksusdan alınan kanda diğer iki yöntemle alınanlardakinden daha yüksek olduğu belirlenmiştir ($P<0.01$). Retro-orbital pleksustan alınan kanda lökosit konsantrasyonunun da yüksek olduğu ve bunun lenfosit konsantrasyonunun daha yüksek olmasının bir sonucu olduğu saptanmıştır (ikisi de $P<0.05$). Buna karşın eozinofil oranının kuyruk kanında retro-orbital pleksusdan alınan kana göre daha yüksek olduğu saptanmıştır ($P<0.01$). Monosit oranı kalp kanında en yüksektir ve farklılık kuyruk ve retro-orbital pleksus kanına göre istatistiksel olarak onaylanmıştır ($P<0.05$). Kanın kompozisyonunun kan alma yerine bağlı olarak değişebildiği, bu nedenle hematolojik ve muhtemelen serum biyokimyasal değerlerin karşılaştırılmasında kan alma yerinin göz önünde bulundurulması gerektiği vurgulanmıştır.

Anahtar Kelimeler: kan alma, örnek yeri, hematoloji, fare

Summary: In this study, the investigation of the effect of the blood withdrawal site on certain hematological values was aimed. For this purpose a total of 21 female Swiss albino mice were used. The mice were divided into three equal groups. Each group served for a different blood collection technique. Blood was withdrawn from the heart, the tail or from the retro-orbital plexus only for one time from each animal. The mean PCV value in blood gathered from retro-orbital plexus was higher than in blood collected from other two blood sampling sites ($P<0.01$). It was seen that the blood obtained from retro-orbital plexus had also

* Adress: Adnan Menderes University, School of Veterinary Medicine, Department of Physiology, West Campus 09016 Işıklı- Aydın.
[1] Corresponding author e-mail: hceler@yahoo.com

higher mean leukocyte concentrations as a result of higher lymphocyte concentrations (both $P<0.05$). However, the percentage of eosinophils was highest in the tail blood, and the differences between blood samples obtained from tail and retro-orbital plexus were statistically significant ($P<0.01$). Similarly, the percentage of monocytes was highest in the hearth blood, and the differences with tail and retro-orbital plexus sites were statistically confirmed ($P<0.05$). It was concluded that blood composition may change depending on sampling site and the blood sampling site have to be taken into consideration when hematological and possibly serum biochemical values are evaluated.

Key words: blood withdrawal, sampling site, hematology, mice

Introduction

It is obvious that in men and animals, the physiological variation of hematological values are in wide ranges **(3,8)**. Furthermore, the differences related to the gender or genetic origin as well as the method and site of the blood withdrawal have great effect on certain hematological variables **(1,2,8,11,16)**. However, recently the effects of blood withdrawal techniques on hematological and biochemical variables have been investigated in many species and races of animal because of there are controversial aspects about this matter **(5,10,14,18)**. Schermer **(15)** reviewed the early literature to explain this problem and suggested that the blood obtained from tail might have higher concentrations of leukocytes than withdrawn by decapitation or by *Arteria femoralis* whereas blood from orbital plexus could have lower hemoglobin and erythrocyte concentrations. Nichols and Miller **(11)** could not find out any difference in leukocyte concentrations in blood withdrawn from the hearth and the tail vein of rats. Scheufler **(16)** suggested that the concentrations of erythrocytes, leukocytes and hemoglobin in blood obtained from the tails of mice were higher than in blood samples taken by decapitation. Schnell et al. **(14)** reported that blood obtained from retro-orbital plexus had higher erythrocyte, hemoglobin and hematocrit concentrations than the cardiac blood, whereas the cardiac blood had lower WBC and lymphocyte concentrations compared with blood samples taken from caudal vena cava and retro-orbital plexus in mice. Similarly, blood samples collected by cardiac puncture had less leukocyte counts than those of obtained from tail and saphenous vein in male and female C57BL/6 mice **(5)**. A comparison of hematological values from the peripheral blood of the ear and venous blood of infant baboons showed that leukocyte and erythrocyte concentrations, packed cell volume and hemoglobin values in peripheral blood of the ear were higher than the venous blood, while total leukocyte concentrations had no consistent pattern **(2)**. A comparison of the venous and skin puncture blood of children and adults revealed that erythrocyte, hemoglobin, hematocrit and neutrophil values in the blood samples collected by skin puncture were higher than in venous blood whereas the platelet values in venous blood were higher than taken by skin puncture **(1)**. But the results of a similar study in adult volunteers were suggested that leukocyte concentration was higher in blood from skin puncture than the venous blood but the other hematological variables did not differ **(18)**.

In most of these studies the same subjects were used for the collection of the blood from different sites simultaneously. It is well known that the repeated blood

sampling could affect the blood composition **(4)**. Thus, the aim of this study was to compare the values of certain hematological variables from blood samples withdrawn from tail, retro-orbital plexus and hearth of Swiss albino mice by using each animal for only one sampling technique. For blood withdrawal, these sampling sites were chosen because blood in hearth was a mix of venous blood and lymph, blood in retro-orbital plexus was the representative of the venous blood and blood obtained by tail clipping was a mix of the arterial, venous and capillary blood. Furthermore, they are most often preferred sites for blood sampling in small experimental animals.

Materials and Methods

A total of 21 Swiss albino female mice served as blood donors. The mean body weight of animals was ca. 28 g, with a range of 23 and 37 g. Animals were divided randomly into three equal groups, each served for a blood sampling technique.

The body weights of animals were also recorded immediately before blood sampling. Blood was withdrawn with three different techniques; by tail clipping method, retro-orbital plexus punctuation and the heart puncture, under ether anesthesia by using EDTA as anticoagulant. For packed cell volume (PCV) determination blood was obtained directly in heparinized capillary tubes. Blood sampling procedures were carried out within 3 days for which ca. 1/3 of animals in each group used for each day. All sample collections were carried out in the mornings between $8^{.30}$-$9^{.30}$ hours following a 15 hour food and water withdrawal period. The total leukocyte concentration (TWBC), concentration of erythrocytes (RBC) and hemoglobin (Hb), and values of PCV were determined in all blood samples. TWBC and RBC counts were made by standard methods in improved Neubauer hemocytometry. PCV were determined by microhematocrit method. Hemoglobin values were determined by cyanmethemoglobin method via spectrophotometer. Differential leukocyte count was determined by counting 400 leukocytes *per* animal on two separate blood films prepared by panoptic method *ad modum* Pappenheim.

Statistical Analyses

The data were analyzed by analyses of variance (ANOVA) and expressed as mean ± standard deviation, with their minima and maxima. *Post hoc* tests were made by Least Significant Difference test. Differences were considered significant at $P<0.05$.

Results

The results and the statistical evaluation of the hematological values summarized in Table 1.

Erythrocytes

Blood obtained from retro-orbital plexus had higher PCV values than the blood withdrawn from hearth and tail ($P<0.01$). There was not any significant difference in erythrocyte concentrations and hemoglobin values between blood sampling sites.

Table 1. The body weights and the values of certain hematological variables of mice.
Tablo 1. Farelerin vücut ağırlıkları ve bazı hematolojik değişkenleri.

Variables	The Blood Sampling Site		
	Hearth X ± SD (Min-Max)	Tail X ± SD (Min-Max)	Retro-Orbital Plexus X ± SD (Min-Max)
BW [g]	27.64 ± 3.26 (23.45 - 32.78)	28.76 ± 3.39 (25.12 - 32.60)	28.90 ± 4.37 (24.07 - 37.55)
RBC [x10^6/µl]	10.65 ± 1.12 (8.87 - 11.99)	10.44 ± 2.11 (8.15 - 13.57)	11.43 ± 2.51 (7.13 - 15.24)
Hb [g/dl]	14.03 ± 0.93 (13.10 - 15.18)	14.71 ± 0.66 (14.10 - 15.70)	14.93 ± 2.59 (10.40 - 18.36)
PCV [%]	46.14 ± 3.13^{B} (42.00 - 50.00)	45.40 ± 4.04^{B} (40.00 - 51.00)	51.43 ± 1.72^{A} (49.00 - 54.00)
WBC [x10^3/µl]	3.77 ± 1.01^{b} (2.60 - 5.60)	3.72 ± 0.91^{b} (2.89 - 5.28)	6.11 ± 2.68^{a} (2.98 - 10.13)
MATURE NEUTROPHIL [x10^3/µl]	0.99 ± 0.35 (0.49 - 1.39)	1.16 ± 0.76 (0.63 - 2.49)	1.26 ± 0.61 (0.45 - 2.25)
YOUNG NEUTROPHIL [x10^3/µl]	0.09 ± 0.05 (0.03 - 0.17)	0.10 ± 0.02 (0.07 - 0.12)	0.13 ± 0.10 (0.03 - 0.32)
LYMPHOCYTE [x10^3/µl]	2.30 ± 0.48^{b} (1.75 - 3.18)	2.11 ± 0.40^{b} (1.43 - 2.43)	4.42 ± 2.16^{a} (2.28 - 7.92)
MONOCYTE [x10^3/µl]	0.22 ± 0.19 (0.09 - 0.64)	0.08 ± 0.03 (0.04 - 0.12)	0.17 ± 0.17 (0.08 - 0.55)
EOSINOPHIL [x10^3/µl]	0.19 ± 0.15) (0.05 - 0.40)	0.28 ± 0.17 (0.10 - 0.55)	0.14 ± 0.10 (0.05 - 0.29)
MATURE NEUTROPHIL [%]	25.89 ± 5.65 (18.75 – 35.25)	29.50 ± 11.78 (19.00 – 47.25)	21.29 ± 8.45 (13.75 – 37.00)
YOUNG NEUTROPHIL [%]	2.54 ± 1.33 (1.00 - 4.50)	2.85 ± 0.86 (2.00 - 4.00)	2.04 ± 0.89 (0.75 - 3.50)
LYMPHOCYTES [%]	62.18 ± 5.66^{ab} (55.50 - 70.00)	58.10 ± 12.91^{b} (39.50 - 67.75)	71.75 ± 8.68^{a} (56.50 - 82.25)
MONOCYTES [%]	5.43 ± 3.00^{a} (2.50 - 11.50)	2.40 ± 1.02^{b} (0.75 - 3.50)	2.64 ± 1.55^{b} (1.50 - 6.00)
EOSINOPHILS [%]	4.96 ± 3.38^{AB} (1.75 - 11.00)	7.35 ± 2.87^{A} (2.75 - 10.50)	2.64 ± 1.60^{B} (0.50 - 4.75)

In each line, the differences between the means with different letters are significant a,b: ($P<0.05$). A,B: ($P<0.01$)

Leukocytes

The mean leukocyte concentration in the blood obtained from retro-orbital plexus was higher than those obtained from tail and hearth ($P<0.05$). The differences between the mean values of leukocytes of this group and the others amount to ca. 50%. When the differential leukocyte count was analyzed, it was seen that the differences in WBC from different sampling sites related mainly to the higher concentrations of the lymphocytes in blood obtained from retro-orbital plexus. The mean absolute lymphocyte concentration in blood obtained from the retro-orbital plexus was higher than those of obtained from the tail and heart ($P<0.05$). But the mean percentage of lymphocytes was different only between blood obtained from the retro-orbital plexus and the tail ($P<0.05$). The percentage of eosinophils was highest in tail blood, and the differences between tail and retro-orbital plexus sites were statistically significant ($P<0.01$). However, the percentage of monocytes was highest in the hearth blood, and the differences with tail and retro-orbital plexus sites were statistically confirmed ($P<0.05$).

Discussion

When the results of earlier studies were considered, the relationship between the blood sampling sites and the hematological values are remarkable with the exception of the work of Nichols and Miller (**11**) who found no difference in hematological values related to the site of blood withdrawal. In general, the results presented here supported the earlier findings about the composition of blood obtained from different sites of the body might be different. The WBC concentration of retro-orbital blood was higher than those obtained from hearth or tail, mainly due to higher lymphocyte concentration. The PCV values were also higher in blood collected from retro-orbital plexus than the other sites. Furthermore, differences were recorded for percentages of eosinophils and monocytes. The values of the other variables did not show any significant difference in relation to the blood sampling sites. Schnell et al (**14**) suggested that blood obtained from retro-orbital plexus in mice had higher erythrocyte, hemoglobin and hematocrit values than the cardiac blood and the cardiac blood had lower WBC and lymphocyte count compared with blood samples from caudal vena cava and retro-orbital plexus. Similarly, Doeing et al (**5**) reported that blood samples collected by cardiac puncture had less leukocyte counts than those obtained by tail and saphenous vein in male and female C57BL/6 mice. Nemzek et al (**10**) also suggested that WBC counts of cardiac blood were significantly less than that of obtained from tail. Besides RBC, platelets and hematocrit were significantly higher in tail blood compared with blood from heart. In our study the blood obtained from retro-orbital plexus had also the highest PCV, WBC and lymphocyte values like the results of Schnell et al (**14**). However, in contrary to the studies of Doeing et al (**5**) and Nemzek et al (**10**) cardiac blood was not different from the tail blood. But one detail should be considered that the blood withdrawal techniques in the same site as tail clipping or puncture of tail vein as well as type of anesthetics and

counting methods are different for each study so that currently a direct comparison of the results is not always easy or meaningful.

Our study is different from most of the others because of the using three different sampling sites and usage of one animal for only one sampling method. Because the removal of blood samples excessively could affect some hematological variables **(4)**, using animals only once was important to compare the hematological values from different sampling sites. Furthermore, it is well known that blood withdrawal has profound effects on the endocrine and neural system, which in turn affects the hematopoietic activity as well as the blood composition **(6,7,9,12,13,17)**.

The controversial nature of the composition of the blood samples obtained from different sites in the body leads to the confusions in commentary of the results from different studies. It necessitates further detailed research to clear this problem. However, the results of this study as well as of other studies indicate clearly that in comparing the results of different studies in respect to the hematological values, the site of the blood sampling should be taken into consideration.

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