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Determination of some coagulation parameters according to age and sex in Sivas Kangal dogs

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Abstract: Hemostasis is stopping bleeding in a controlled manner whereas coagulation is a series criterion and to diagnose disorders caused by coagulation in an early period is to determine the coagulation time. These phases are controlled with various tests. In this study, the aim is to determine some coagulation parameters in Sivas Kangal dogs by taking the age and sex into consideration. Coagulation is vitally important in many physiological and pathological cases. The most important tests, complete blood count with peripheral blood smear, prothrombin time (PT), activated partial thromboplastin time (aPTT), and D-dimer fibrin degradation product assay were applied to all subjects, respectively. It was found that thrombocyte counts in Sivas Kangal dogs were affected by the age factor in automatic and manual counting, whereas they were not affected by sex. In the same way, while PT times showed a meaningful change among groups depending on age, aPTT times were not affected by sex or age. The D-dimer assay results, which show the existence of fibrin degradation product, change depending on age and sex.

Key words: Coagulation, coagulation tests, Sivas Kangal dogs

1. Introduction

Hemostasis starts with the formation of a blood clot for the repair of the damaged area without forming a pathological clot (thrombosis) after the disintegration of vascular integration. After that, blood flows in the vessels by fibrinolysis in a controlled manner [1,2]. Coagulation, which is a part of hemostasis and has vital importance, is a series of events that the body performs to prevent excessive blood loss from damaged tissue, blood vessels, and organs as a result of disintegration of the blood vessels [3]. Blood clotting starts with thrombocyte plugs formed by active thrombocytes as a result of adhesion and aggregation of active thrombocytes, which are found in blood circulation and are one of the elements of the cellular system. This process continues with clotting factors in protein using intrinsic, extrinsic, and common paths to form thrombin, which is the most important enzyme in the thrombosis process. Coagulation stops with the formation of fibrin plaque shaped by thrombin activation. However, the process ends after the degradation of the fibrin plaque with fibrinolytic enzymes and the blood flows in the vessel properly [2,3]. Coagulation times vary among individuals depending on the functions of clotting factors playing roles in all processes of coagulation. These phases are controlled with various tests just as they are applied to humans [4,5]. Tests that are properly applied to determine the phase of the coagulation in which the problem exists can be categorized as tests related to thrombocyte activation, coagulation cascade, and fibrin formation and fibrinolysis [6–9].

In a literature review, sufficient numbers of studies regarding the coagulation system peculiar to Sivas Kangal dogs [10–12] which are indigenous to Turkey today and important for the world due to their genetic features, have not been encountered. For that reason, the aim of this study is to observe some coagulation parameters and to present possible differences depending on Sivas Kangal dogs' age and sex. Additionally, another target in this study is to ensure that alternative patient approaches are determined by veterinarians correctly in situations requiring surgical operations and to prevent animal deaths caused by coagulation problems secondarily with the diagnosis of diseases caused by coagulation. Thus, findings from the obtained data that can benefit both basic sciences and clinical sciences are sought.

2. Materials and methods

The study was carried out with dogs in the Cumhuriyet University Faculty of Veterinary Medicine's Sivas Kangal Dogs Research and Breeding Center. Dogs that were healthy in clinical examination and had normal values with respect to body temperature and hematologic tests

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were included in the study. The sampling size of the study was determined with effect size (f) = 0.47, α (type 1), error = 0.05, and test power (1 – β) = 0.85, and accordingly the minimum sampling size was estimated as 42 as n = 7/number of groups. These dogs were categorized into 3 subcategories based on their age and into 2 subcategories based on sex. Accordingly, female puppies of 0–6 months were in the 1st group, male puppies of 0–6 months in the 2nd group, female dogs of 10–14 months in puberty in the 3rd group, male dogs of 10-14 months in puberty in the 4th group, older adult female dogs of 16–24 months in the 5th group, and older adult male dogs of 16–24 months in the 6th group.

First of all, body temperatures of all the subjects in the groups were measured. After that, blood specimens from the vena cephalica antebrachii in their forelegs were collected for hematologic and hemostatic tests to be performed. In order to prepare the peripheral blood smear, a drop of blood obtained from the area was dripped on a clean microscope slide and it was dried by applying smear. After that, surfaces of the smear were dyed with Wright's stain in accordance with the procedures. Thrombocytes were counted with a microscope for the evaluation of smear count in 1 µL of blood and the number of thrombocytes was calculated roughly. Additionally, for the thrombocyte and complete blood count approximately 2 mL of blood was drawn in tubes including K_2EDTA. The samples collected in EDTA tubes were tested on a fully automatic blood counting device (Mindray BC-2800Vet, China) at the Cumhuriyet University Veterinary Faculty's Diagnostic Laboratory [4,8]. On the other hand, for coagulation tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and D-dimer, blood was collected into tubes including 0.2 mL of 3.2% trisodium citrate with mixing ratio of blood with anticoagulant of 1:9 (9 volumes of blood for 1 volume of citrate). Eluted blood plasma was processed by using appropriate reactive substances for PT and aPTT to be determined. Finally, the D-dimer test was carried out to measure the amount of fibrin degradation product as a result of degradation of fibrin polymers by plasmin enzyme and to show the D-dimer assay results quantitatively [4]. For that purpose, an ELISA test was applied using a 96-well canine D-dimer test kit (Elabscience Products, Houston, TX, USA) in accordance with the kit's protocol. Based on the flash occurring in the wells, D-dimer levels of the subjects (r^2 = 0.99) for which the optical density (OD) values were specified were evaluated in ng/mL. Statistical evaluation of the data obtained in the research was conducted with SPSS 14.01 (SPSS Inc., Chicago, IL, USA; license number: 9869264). Compatibility of data with normal distribution was checked with the Shapiro–Wilk test. The effect of Sivas Kangal dogs' age and sex on the studied blood parameters was analyzed with two-way analysis of variance. The statistical significance limit was accepted as $P < 0.05$. The Bonferroni test was used as an advanced stage test to analyze the significant effects found in this study.

3. Results

When age- and sex-related changes in body temperature were examined, age-related changes ($P < 0.001$) were found to be significant. The mean body temperature of the dogs in the puberty period, which was 38.8 ± 0.26 °C, was higher than that of puppies of 37.9 ± 0.50 °C and adult dogs of 38.2 ± 0.36 °C, regardless of sex. In this study, hematological test values were examined in order to get an idea about the general health status of animals and to interpret the hemostatic test values in order to eliminate the problems related to hematological disorders and to evaluate the hemostatic test results correctly. When hematological data were evaluated according to age and sex, no results were found to be associated with hemostatic test parameters since the study was conducted with healthy dogs. The hematological tests were examined between the groups and the findings are presented in detail in Table 1. When the platelet count results of the groups obtained from the automatic counting device were compared with the results of manual platelet counting from peripheral smear preparations, the lowest platelet count was recorded in dogs in puberty from both counting methods. When changes by sex were examined, it was found that the platelet count in males was lower than that in females. All values are presented in detail in Table 2. When PT and aPTT data obtained from all groups were examined, the female puppies' PT (8.93 ± 0.34 s) and aPTT (16.09 ± 1.25 s) were the lowest values among all groups. Among all groups, the longest PT time was determined as 10.26 ± 0.27 s in female dogs in puberty. As for aPTT time, among all groups, the longest aPTT time was found in male dogs in puberty as 20.84 ± 2.04 s. In this study, age-related change in PT time, regardless of sex, was found to be significant at $P = 0.036$ (Table 2). However, it was determined that aPTT time was not affected by age or sex (Tables 2). When the study was evaluated in terms of the D-dimer test, the lowest D-dimer value was observed in male puppies as 94.28 ± 33.12 ng/mL. The highest value was recorded in adult female dogs as 466.10 ± 98.62 ng/mL (Table 2). When the obtained data were evaluated, the changes in D-dimer values depending on age ($P = 0.004$) and sex ($P = 0.024$) were found to be statistically significant (Tables 2).

4. Discussion

Studies emphasize the existence of many diseases caused by bleeding, such as thrombocytopenia, thrombocytopathy, hemophilia, von Willebrand disease, and disseminated intravascular coagulopathy (DIC) that have been identified

Table 1. The effect of age, sex, and interaction of age × sex on the hematological test results in Sivas Kangal dogs.

	n	WBC, $\times 10^9$/L	RBC, $\times 10^{12}$/L	Hb, g/dL	HCT, %	MCV, fL	MCH, pg	MCHC, g/dL
Age	(Mean ± SEM)							
Puppies	14	12.4±3.8	6.09 ± 1.6^{a}	10.90 ± 3.7^{a}	44.56 ±11.3^{a}	73.5 ± 5.5	21.7 ± 1.8	29.57 ± 0.7^{a}
Puberty	14	14.5±7.5	7.24 ± 0.9^{b}	15.67 ± 1.5^{b}	52.95 ± 5.3^{b}	73.5 ± 4.3	21.6 ± 1.1	29.54 ± 0.8^{ac}
Adult	14	13.1±4.9	7.58 ± 0.9^{cb}	16.01 ± 2.2^{bc}	55.82 ±7.0^{bc}	73.7 ± 2.4	21.4 ± 1.4	27.91 ± 2.4^{b}
P-value		-	0.008	0.001	0.003	-	-	0.006
Sex	(Mean ± SEM)							
Male	21	11.2 ± 1.1^{b}	7.1 ± 1.53	13.7 ± 4.4	52.3±10.9	74.7 ± 4.4	21.8 ± 1.4	29.2 ± 0.6^{b}
Female	21	15.4 ± 1.1^{a}	6.8 ± 1.14	14.5 ± 2.2	49.8 ±7.4	72.4 ± 3.7	21.1 ± 1.3	28.7 ± 2.3^{a}
P-value		0.014	-	-	-	-	-	0.032
Age × sex	(Mean ± SEM)							
Male puppies	7	11.3 ± 3.5	6.2 ± 2.1	8.8 ± 3.9^{b}	45.4±14.6	74.5 ± 7.1	22.0 ± 2.5	29.5 ± 0.6
Male puberty	7	11.2 ± 2.4	7.3 ± 0.4	15.8 ± 0.6	54.4±2.9	74.6 ± 3.1	21.7 ± 0.5	29.1 ± 0.7
Male adult	7	11.2 ± 3.8	7.6 ± 1.3	16.6 ± 2.8	57.3±9.4	75.1 ± 1.7^{b}	21.7 ± 0.2^{b}	29.0 ± 0.6
Female puppies	7	13.6 ± 4.0	5.9 ± 0.9	12.9 ± 2.2^{a}	43.7±7.7	72.4 ± 3.5	21.3 ± 0.9	29.5 ± 0.9
Female puberty	7	17.8 ± 9.6	7.2 ± 1.3	15.4 ± 2.2	51.5±6.8	72.4 ± 5.2	21.6 ± 1.5	29.9 ± 0.8
Female adult	7	15.0 ± 5.4	7.5 ± 0.4	15.3 ± 1.2	54.2±3.2	72.3 ± 2.2^{a}	20.4 ± 1.4^{a}	26.8 ± 3.1
P-value		-	-	0.034	-	0.028	0.05	-

$^{a, b}$ Values with different superscript letters in the same column are statistically significant; otherwise, P ≥ 0.05.

Table 2. The effect of age, sex and interaction of age × sex on the hemostatic test results in Sivas Kangal dogs.

	n	PLT, $\times 10^9$/L, automatic	PLT, $\times 10^9$/L, manual	PT (s)	aPTT (s)	D-Dimer, ng/mL
Age	(Mean ± SEM)					
Puppies	14	406.21 ± 65.85^{ab}	423.71 ± 62.33^{ab}	9.09 ± 0.24^{a}	17.33 ± 1.3	123.17 ± 53.4^{a}
Puberty	14	250.50 ± 65.85^{a}	247.14 ± 62.33^{a}	9.98 ± 0.24^{b}	19.16 ± 1.3	230.08 ± 49.0^{ab}
Adult	14	513.00 ± 65.85^{b}	490.14± 62.33^{b}	9.75 ±0.25^{ab}	20.35 ± 1.3	372.83 ± 47.1^{b}
P-value		0.023	0.026	0.036	-	0.004
Sex	(Mean ± SEM)					
Male	21	354.6 ± 53.7	355.5 ± 50.8	9.69 ± 0.2	19.83 ± 1.0	173.894 ± 41.9^{b}
Female	21	425.1 ± 53.7	418.4 ± 50.8	9.53 ± 0.2	18.06 ± 1.0	310.163 ± 39.5^{a}
P-value		-	-	-	-	0.024
Age × sex	(Mean ± SEM)					
Male puppies	7	373.0 ± 61.5	402.8 ± 56.7	9.25 ± 0.34	18.57 ± 0.82	94.28 ± 33.12
Male puberty	7	180.8 ± 19.6^{b}	178.2 ± 19.9^{b}	9.70 ± 0.53	20.84 ± 2.04	147.84 ± 53.45
Male adult	7	510.0 ± 125.8	485.4 ± 117.9	9.63 ± 0.16	20.09 ± 1.64	279.56 ± 74.60
Female puppies	7	439.4 ± 31.3	444.5 ± 33.4	8.93 ± 0.34	16.09 ± 1.25	152.07 ± 22.61
Female puberty	7	320.1 ± 33.2^{a}	316.0 ± 36.3^{a}	10.26 ± 0.27	17.47 ± 2.81	312.32 ± 73.83
Female adult	7	516.0 ± 73.0	494.8 ± 163.2	9.88 ± 0.32	20.61 ± 1.54	466.10 ± 98.62
P-value		0.004	0.006	-	-	-

$^{a, b}$ Values with different superscript letters in the same column are statistically significant; otherwise, P ≥ 0.05.

in dogs. It was also pointed out that some of these diseases' frequency increases in some breeds depending on the breed's predisposition [13–17]. However, healthy Sivas Kangal dogs' normal body temperatures and hematological values were used in this study since sufficient numbers of sources regarding Sivas Kangal dogs are not available. Some researchers [13–19] stated that blood parameters in animals are affected by factors such as breed, age, and sex. For these reasons, in this study, while taking the age factor into consideration, groups that were created based on age were subcategorized as female and male for the categories of puppy, puberty, and adult dog. Some researchers [20,21] proposed that as hypothermia prevents coagulation factors' activity, it will affect thrombocyte activation adversely and cause coagulation disorders. They also argued that hyperthermia increases hemostatic test results. Considering this, normothermic healthy animals were chosen for the sample of this study. Hence, it is asserted that there is no possibility that any abnormality that could be encountered in hematologic and hemostatic test values is caused by hypothermia or hyperthermia. Among the groups constituting the total sample, changes in body temperature depending on age ($P < 0.001$) were found to be significant. The hematological test results of the present study were compared with those of other studies [6,19,22] including reference values of dog breeds of different ages and sexes. According to the data obtained from Sivas Kangal dogs, hematological test values of different breeds give quite similar results. Changes in white blood cell (WBC) count and mean corpuscular hemoglobin concentration (MCHC) values depending on sex are significant. The data show that the WBC count in females is higher ($P = 0.014$) than in males (Table 1). However, the MCHC value in females is lower ($P = 0.032$) than in males (Table 1). Choi et al. [6] reported that red blood cell RBC) count, hemoglobin (Hb), and hematocrit (HCT) values were not affected by sex but changed depending on age. Similarly, Harper et al. [18] stated that WBC, RBC, Hb, and HCT values were affected by changes in age. In this study, RBC, Hb, HCT, and MCHC values are affected by age changes in Sivas Kangal dogs, too. Accordingly, while RBC ($P = 0.008$), Hb ($P = 0.001$), and HCT ($P = 0.003$) values increased with age, MCHC ($P = 0.006$) values were found higher in dogs in puberty than in adults (Table 1). During the puberty period, some hormonal and metabolic events increase in animals. In this period, muscle mass increases as an effect of increasing sex hormones. This leads to an increase in physiological ATP requirement and oxygen consumption. In addition, the increased level of testosterone in the body stimulates erythropoiesis in the bone marrow. All of these physiological events explain both the sex-dependent change of WBC and MCHC and the age-related change of RBC, Hb, HCT, and MCHC [23,24].

Among the bleeding disorders of primary hemostasis, the most important role is related to thrombocytes. On the other hand, thrombocyte function disorders can occur secondarily in chronic heart, liver, and kidney diseases and failures, as well [7,25]. Automatic and manual thrombocyte counting were performed in order to detect the diseases that can be seen in primary hemostasis. It was observed that thrombocyte count in dogs in puberty is lower than in any other age group ($P < 0.05$) in both counting methods (Tables 2). However, mediators such as TXA_2 and serotonin increase with the effect of sex hormones, which are started to be secreted in this period. This leads to an increase in vasoconstriction and platelet function. Therefore, although platelet count is low in this period, clotting is not adversely affected [26,27]. When thrombocyte counts in female and male dogs in puberty are compared, the thrombocyte counts of males are lower ($P < 0.05$) than those of females in both counting methods (Table 2). It can be explained that thrombocytes of male dogs in puberty have functionally more thrombotic effect due to the effect of the increasing amount of testosterone. Nonetheless, it can function efficiently in enabling hemostasis. On the other hand, studies showed that thrombocytes in females are less thrombotic than in males [28]. In this study, PT, aPTT and D-dimer tests were conducted in Sivas Kangal dogs in order to determine whether any bleeding problems caused by sex differences and age exist for secondary hemostasis. When the data obtained from the study on PT and aPTT times were compared with other studies conducted on different animal species [3,4], it was seen that PT and aPTT times are quite close even though they are not exactly the same. PT and aPTT times show similarity among different animal species. Bruchim et al. [15] stated in their study that in dogs that were exposed to high temperatures (environmental conditions and related to exercise), PT and aPTT times were prolonged. For that reason, all the blood samples in this study were collected from animals that were not experiencing hypothermia or hyperthermia, in the same environmental conditions and while resting. Some researchers [25,29] argued that PT and aPTT times are prolonged in dogs that have acute or chronic diseases. In this study, PT and aPTT values were found in the limits of healthy animals. Neutrophil and lymphocyte ratios, which are used in the detection of infections, were also in the limits of healthy dogs. This proves that the animals used in the study did not have any infections. In this study, it was seen that PT and aPTT times and D-dimer assay results were in the reference value ranges for each age and sex group as reported for different dog breeds in the literature [4,8,30]. According to the data obtained in this study, while aPTT time was not affected by the age factor, PT ($P = 0.036$) and D-dimer assay ($P = 0.004$) were affected (Table 2). Many researchers argued that sex affects hemostatic parameters [10,13,17].

In this study, it was found that Sivas Kangal dogs' PT and aPTT values were not affected by sex in all age groups. On the contrary, as shown in Table 2, the D-dimer assay shows a change depending on sex (P = 0.024). Accordingly, the D-dimer assay results in females are higher than in males. Increased fibrin degradation product in females and lower D-dimer assay results in males are explained by the fact that high levels of estrogen increase fibrinolytic activity and a high testosterone rate inhibits fibrinolysis [28].

Some hematological and hemostatic test parameters depending on age and sex in Sivas Kangal dogs, which is an important dog indigenous to Turkey and a significant dog breed in terms of its genotypical features for the world, were determined and reference values were obtained. Thrombocyte count changes depending on age; however, it is generally not affected by sex. Similarly, PT time changes depending on age but is not affected by sex. On the contrary, it was found that aPTT time is affected by neither age nor sex, but D-dimer amount is affected by both of them.

While selecting the tests, as a result of anamnesis and clinical symptoms, physiological variables such as breed, age, and sex of the animal together with the suspected disease and practicality of the tests should be taken into consideration. While evaluating the test results that are selected, hematologic and hemostatic tests should be interpreted together. When test results are evaluated, it is recommended that studies be extended with molecular biology and genetic studies together with hormone tests. Finally, it should be considered that the D-dimer assay is high at later ages and especially in female dogs. Veterinarians should be cautious about possible hemorrhage, thrombus, and embolism in terms of the approach, diagnosis, and treatment of the patient.

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