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RESEARCH ARTICLE

Investigation of cTn I, CK-MB, Myoglobin and D-Dimer Levels at Anemic Dogs Infected with *Ehrlichiosis*

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ABSTRACT

Ehrlichiosis is a vector-borne disease that affects humans and animals. Multiple tissues and organs are fascinating, and *Ehrlichiosis* can cause multiple organ failure. In one's way the known clinical findings and pathogenesis, it is not clear how *Ehrlichia* is effective and important on myocardial damage and disseminate intravascular coagulation (DIC) profile. Anemia is seen as one of the common clinical conditions in *Ehrlichiosis*. In this study, it was determined to state the existence of myocardial damage and thromboembolic condition at *Ehrlichiosis* with the severity of anemia. The animal material of this study consisted of 24 mono-infected, 9 co-infected, and 10 healthy animals. Additionally, animals were separated into mono, co-infected; with anemia profile non-anemic, mild, moderate, and severe anemic, and according to serologic and molecular results; acute infected, ınfected, and active ınfected. CK-MB, cTnI, and myoglobin levels were calculated in all groups to identify myocardial damage. Accordingly, D-Dimer concentrations were determined to set down the potential of the DIC profile. As a result of the data, D-Dimer levels significantly increased in mono, co-infected, mild, moderate, severe, and active infected animals ($p<0.05$). Significant statistical difference was seen in cTnI levels in mono, mild, moderate, and active infected groups ($p<0.05$). There were no significant statistical differences between CK-MB and myoglobin levels between study groups. As a result; It was observed that ischemia caused damage to the myocardium in the long view and composed DIC profile.

Key Words: CK-MB, cTn I, D-Dimer, *Ehrlichia*, Myoglobin

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Ehrlichiosis ile Enfekte Anemili Köpeklerde cTn I, CK-MB, Miyoglobin ve D-Dimer Seviyelerinin Belirlenmesi

ÖZ

Ehrlichiosis birçok doku ve organı etkileyen, çoklu organ yetmezliklerine neden olabilen bir hastalıklır. *Ehrlichia*nın yaygın damar içi pıhtılaşma (YDP) bozukluğu ve miyokardiyal hasar yönünden ne derece etkili ve önemli olduğu hala netlik kazanmamıştır. Anemi *Ehrlichiosis*'te yaygın görülen klinik tablolardan biridir. Yapılan bu çalışmada *Ehrlichiosis*'li hayvanlarda gelişen miyokardiyal hasar ve potansiyel YDP tablolarının anemi ile birlikte değerlendirilmesi amaçlandı. Çalışmanın hayvan materyali 10 sağlıklı, 24 mono-enfekte ve 9 ko-enfekte hayvandan oluşturuldu. Bunun yanında enfekte hayvanlar serolojik ve moleküler sonuçlarına göre aktif enfekte, enfekte ve akut enfekte; hastalık etkenlerine göre mono ve co enfekte, anemi durumlarına göre non anemik, hafif, orta ve şiddetli anemik olarak gruplandırıldı. Bu gruplarda cTnI, CK-MB ve miyoglobin seviyeleri, myokardiyal hasarın tespit edilmesi amacı ile ölçüldü. YDB profiline yatkınlığın belirlenmesi için D-Dimer konsantrasyonları saptandı. Elde edilen sonuçlar doğrultusunda D-Dimer konsantrasyonlarında anlamlı yükselmeler mono enfekte, co enfekte, hafif, orta ve şiddetli ile aktif enfekte gruplarında tespit edildi ($p<0.05$). Çıkan bu sonuçlar doğrultusunda, cTnI seviyelerinde mono enfekte, orta ve şiddetli anemili hayvanlar ile aktif enfekte gruplarında anlamlı istatistiksel farklar belirlendi ($p<0.05$). Diğer parametrelerde herhangi bir farklılığa rastlanmadı. Sonuç olarak *Ehrlichia* sonucu gelişen anemi doku ve organlarda iskemiye neden olarak, aneminin şiddeti ile uyumlu bir şekilde uzun süreli olgularda myokardiyal hasara sebep olduğu ve YDB profili geliştirdiği görüldü.

Anahtar kelimeler: CK-MB, cTn I, D-Dimer, *Ehrlichia*, Miyoglobin

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INTRODUCTION

Ehrlichiosis; is a zoonotic disease that can be transmitted by vectors. It affects both humans and animals. It has multiple clinical signs and when it is not treated, it could be severe and fatal (Paşa et al. 2017). Studies show that the disease can be seen at rates between 2.2% and 70% in different countries all over the World (Harrus et al. 2016). Fever, depression, lethargy, anorexia, loss of weight, secretion on eyes and nose, dyspnoea, lymphadenopathy, splenomegaly, edema at extremities and scrotum, disposition at bleeding, petechia on skin and mucosa, ecchymose and rarely epistaxis are most common clinical findings in Ehrlichiosis (Yağcı et al. 2010). As a laboratory result, thrombocytopenia, mild to moderate anemia (normocytic, normochromic, non-regenerative), and mild leukopenia can be monitored (Vismaya et al. 2020).

Several diseases can cause cardiac damage directly or indirectly. Cardiac myocardial damage can produce pro-inflammatory cytokines; like tumor necrosing factor alfa and interleukin 1, 6, 18. After damage to the myocardium, cardiac output can be formed and will end up with hypoperfusion at skeletal muscles. Anemia and vasculitis contrubite the heart damage with hypoperfusion (Gonzalez et al. 2022). This causes the activation of monocytes and produces the same cytokines. These cytokines affect myocardial functions negatively and spark off an increase in myocardial damage (Krejci et al. 2016). As a result of this damage to the myocardium; cardiac troponin I (cTn I), CK-MB, and Myoglobin can get into blood circulation from different tissues. By this purpose; cardiac troponin I, CK-MB, and Myoglobin can be used as diagnostic and prognostic marker of cardiac damage (Slack et al. 2005).

Hemostatic disorders are used as important markers for detecting the prognosis of diseases. Additionally, these disorders can cause bleeding problems (Zoia et al. 2022). disseminated intravascular coagulation profile (DIC) is defined as a syndrome in which thrombosis can be seen at capillary vessels and can progresses to secondary fibrinolysis (Stokol et al. 2022). This condition is not a primer defect. It develops because of reflection of the underlying primary factors (Bruchim et al. 2017). DIC is reported with Ehrlichiosis but the pathogenesis is not clearly explained (Dalugama and Gavarammana 2018). In the determination of DIC; It is reported that D-Dimer value, which is a cross-linked pure fibrin degradation product, can be used for diagnostic purposes (Machida et al. 2010; Sadosty et al. 2011).

Ehrlichiosis has different agents that can cause disease in dogs and mammalians. *Ehrlichia canis* is the causative agent of canine monocytic ehrlichiosis (CME). CME has different effects on several organs and makes multıple clinical signs. Similarly to other infectious diseases such as Babesiosis, Parvovirus, Chagas Disease, Leishmaniosis and Leptospirosis, Ehrlichiosis can cause myocarditis (Vitt et al. 2016). In addition to the reported effects of ehrlichiosis, its vital damage to the heart is not fully known. Evaluation of CME in terms of disseminated intravascular coagulation disorder (DIC) and thromboembolism risk, is considered important for both human and animal health (Evermann et al. 2012). In this study, we aimed to find if there is a relationship between the infected dogs with CME and the myocardial damage markers (cTn I, Myoglobin, CK-MB) and D-dimer levels, which are the markers of coagulation tendency.

MATERIAL METHODS

Animal Material and Study Groups

The study was ethically approved by the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (Decision Number: 64583101/2016/60). The animal material of the study was organized with dogs that applied to Aydın Adnan Menderes University Veterinary Faculty Animal Hospital. 43 dogs of different ages, breeds and genders, without any previous heart disease, constituted the animal material of the study. The control group was created according to clinical, hematological, biochemical, serological and moleculer results. As a serologic method, immunochromatographic Rapid Diagnostic Test Kits (IDEXX, SNAP 4DX® plus, Ehrlichia Canis, A. phagocytophilum, Borrelia burgdorferi, Dirofilaria immitis; IDEXX, SNAP® Leishmania, Westbrook USA) were used and for molecular method we implemented real-time polymerase chain reaction (PCR). Presence of CME was searched with animals that had clinical findings suitable with Ehrlichiosis. The animals that had fever, depression, lethargy, anorexia, loss of weight, lymphadenopathy, splenomegaly, secretion on eyes and nose, dyspnoea, edema at extremities and scrotum, disposition at bleeding, petechia on skin and mucosa, ecchymose and epistaxis were subjected diagnostic methods for detecting Ehrlichia. The animals that had no clinical findings, normal hematologic and biochemical parameters, and negative results with serologic and molecular examinations constituted the control group. The Ehrlichia positive group was composed had positive results from one of the serological or molecular diagnostic methods.

Animals were separated into two groups; control (n=10) and CME positive (n=33). The infected group was dissociated from the mono (n=24) and co-infected (n=9) group. Co-infection status was evaluated according to the presence of A. phagocytophilum, Anaplasma platys, Borrelia burgdorferi, Dirofilaria immitis and Leishmaniosis.

These agents were searched with rapid tests. Ehrlichia mono-infected animals were divided into two groups according to their anemia status anemic (n=18) and non-anemic (n=6). Anemic animals were also grouped as mild (n=6), moderate (n=6) and severe (n=6). The severity of anemia was determined by the hematocrit value; mild: 30-37%, moderate: 20-29% and severe 13-20% (Turgut 2000). Both mono- and co-infected animals with CME were classified by serological and molecular testing results (Tanikawa et al. 2013; Barrantes-Gonzales et al. 2016). Serologically positive, PCR positive animals were classified as active infected, serologically positive, PCR negative animals were called infected, serologically negative, PCR positive animals were staged acute infected.

Sample Collection

For the collection of hematological data, 2.5 mL blood samples were taken into tubes with Ethylenediamine Tetraacetic Acid (EDTA), from vena cephalica antebrachium of animals. This sample was used for detecting whole blood, rapid test kits and PCR results. To determine cTn I, CK-MB, and myoglobin levels, 5 mL blood samples were collected in serum tubes (with silicone) from the same vena. Also, 2 mL blood samples were taken in tubes containing 3.2% sodium citrate for detecting D-Dimer levels.

Laboratory Analyses

Whole blood parameters were determined with an automatic hematology analyzer cell counter machine (Coulter-Abacus Junıor Vet, Hungary). Serum samples and plasma that was acquired from containing 3.2% sodium citrate tubes, were handled at fluorescence immunoassay rapid quantitative test machine (Finecare, Wondfo Biotech, China) to determine cTn I, CK-MB, Myoglobin and D-Dimer levels. Whole blood parameters, cTn I, CK-MB and D-Dimer levels were obtained immediately after blood samples were collected.

Serological Analyses

For serological diagnosis, blood samples, that taken into EDTA tubes, were processed with rapid diagnostic test kits. ELISA-based immunochromotographic rapid diagnostic test kits were used to detect CME mono and co-infection situation (IDEXX, SNAP 4DX® plus, Ehrlichia Canis, A. phagocytophilum, Borrelia burgdorferi, Dirofilaria immitis; IDEXX, SNAP® Leishmania, Westbrook USA). Test kits were applied within three hours after blood samples were taken.

Moleculer Analyses

Real-time PCR was used for molecular analysis. In PCR analysis DNA was prepared suitably to the protocol with 200 µL QiaGen® Genomic DNA Purification Kits (Qiagen Company, Germany). In this study, two Ehrlichia-specific PCR primers, that amplify 455 bp of the gene and are prepared based on the 16S rRNA gene sequence, were selected and used (Inokuma et al. 2004). The EDTA tube samples were used and stored at -20 °C until PCR applications were applied.

Statistical Analyses

In this study, in the analysis of values obtained for each group, the arithmetic mean ($\bar{x}$), standard deviation (ss), minimal-maximal values (Xmin-Xmax) and median values of the parameters were calculated, and whether the values normality distrubution condition was determined by Shapiro-Wilk test. For this purpose, the Kruskall Wallis and Mann Whitney U tests methods were applied to determine the difference between groups. Probability (p-value) < 0.05 was considered significant. SPSS 23 Statistics Packet Programme (IBM, Armonk, NY, USA) was used for statistical analyses.

RESULTS

Control group was constituted with normal hematologic parameters and negative serologic and molecular results (n=10). CME-positive animals had clinical complaints compatible with Ehrlichiosis. All infected dogs showed fever, edema at extremities, ecchymose, and epistaxis. The dogs were between two and eight years old. 13 dog was mixed breed. 5 dog's breed was Anatolian Shepherd Dog, 12 dog was Golden Retriever, and 3 dog was Pointer. All control group animals were constituted from mixed breed dogs. Statistical evaluation of the animals used in the study according to breed and age was not carried out because there were not enough numbers to form groups. CME positive animals (n=33) were classified according to the presence of *A. phagocytophilum, Borrelia burgdorferi, Dirofilaria immitis and Leishmaniosis,* as mono and co infected groups. All co-infected animals (n=9) had anaplasmosis and four had leishmaniosis. These agents' diagnoses were made with immunochromatographic rapid diagnostic test kits. Mono-infected CME positive animals were classified according to anemia situation. They were separated into 4 groups; non anemic (n=6), mild anemic (n=6), moderate anemic (n=6) and severe anemic (n=6). Mono-infected animals RBC, Hgb and HCT values and anemia classification status was shown in Table 1. Serological and molecular classification condition was shown in Table 2 and animals were staged as active infected (n=12), infected (n=15) and acute infected (n=6).

Table 1. Anemia classification results of CME mono-infected animals

No (Protocol No)	RBC (10^12/L)	Hgb (g/dL)	HCT (%)	Group
1 (10)	5.52	15.64	40.13	Non Anemic (n=6)
2 (15)	6.3	14.65	38.42	
3 (16)	5.62	18.22	37.12	
4 (17)	6.21	15.15	42.12	
5 (2)	6.01	12.11	35.12	
6 (8)	5.64	13.24	30.12	
7 (Fiona)	5.49	10.12	36.70	Mild Anemic (n=6)
8 (403)	5.26	10.12	35.50	
9 (14)	5.22	9.87	36.79	
10 (9)	5.4	8.65	36.5	
11 (3)	5.48	10.15	36.17	
12 (1)	5.46	8.64	30.41	
13 (SG 5)	3.49	10.98	22.66	Moderate Anemic (n=6)
14 (SG 1)	3.84	10.24	26.64	
15 (167)	4.71	10.25	28.33	
16 (12)	3.70	10.12	23.94	
17 (13)	3.61	6.24	24.90	
18 (7)	3.42	5.12	24.09	
19 (387)	3.46	10.12	19.90	Severe Anemic (n=6)
20 (508)	2.03	8.24	12.70	
21 (535)	2.53	6.12	17.22	
22 (SG 13)	4.70	3.15	5.56	
23 (SG 16)	1.76	2.24	12.62	
24 (SG 20)	2.94	2.25	19.41	

Table 2. Serological and molecular classification result

Group	Serological Result (Immunochramotographic Rapid Test)	Moleculer Result (Polymerase Chain Reaction)
Active Infected (n=12)	+	+
Infected (n=15)	+	-
Acute Infected (n=6)	-	+

In this study, control (n=10) and CME positive animals' (n=33); D-Dimer, cTn I, CK-MB, myoglobin levels were shown in Table 3. In the control groups, the plasma D-Dimer concentration was 0.49±0.51 mg-L and control group cTn I level 0.04±0.01 ng-mL. For D-Dimer levels, both co ($p=0.021$) and mono ($p=0.017$) infected groups had significantly different statistical value ($p<0.05$). Also, apart from non-anemic animals (n=6), D-Dimer levels of anemic animals in the study group were found to be significantly different ($p<0.05$) when compared to the control group. The p-values for mild (n=6), moderate (n=6), and severe (n=6) anemia groups were 0.012, 0.011, and 0.011, respectively. In serological and molecular classification; only active infected group ($p=0.01$) had significantly different statistical result ($p<0.05$). Mono infected groups cTn I value ($p=0.01$) were found meaningful statistical difference with control groups ($p<0.05$) but co-infected group had no statistical difference ($p>0.05$). Concurrently, moderate ($p=0.004$) and severe anemic ($p=0.042$) groups had significant statistical differences. In this study, it was determined that active infected group cTn I value ($p=0.042$) were statistically significantly different when compared with the control group ($p<0.05$). It was determined that there was no statistically significant ($p>0.05$) difference between CK-MB and myoglobin levels between the groups subjected to the classification groups.

Table 3. D-Dimer, cTnI, CK-MB and Myoglobin levels of control and study groups.

Group	Parameters											
	D-Dimer (mg-L)			cTnI (ng-mL)			CK-MB (ng-mL)			Myoglobin (ng-mL)		
	$\bar{x}\pm ss$ (min-max)	Median	p-value (Controlx Group)	$\bar{x}\pm ss$ (min-max)	Median	p-value (Controlx Group)	$\bar{x}\pm ss$ (min-max)	Median	p-value (Controlx Group)	$\bar{x}\pm ss$ (min-max)	Median	p-value (Controlx Group)
Control	0.49±0.51 (0.10-1.20)	0.10	-	0.04±0.01 (0.03-0.07)	0.05	-	0.65±0.94 (0.00-3.12)	0.43	-	10.90±5.39 (2.24-18.45)	10.72	-
Mono Infected	4.09±4.12 (0.00-10.00)	2.35	0.017 p<0.05*	0.63±0.82 (0.10-2.86)	0.18	0.000 p<0.05*	3.78±6.80 (0.30-33.36)	1.36	0.060 p>0.05	10.74±8.67 (2.00-41.85)	7.71	0.664 p>0.05
Co Infected	5.17±3.93 (0.00-10.00)	4.00	0.021 p<0.05*	0.56±0.83 (0.10-1.93)	0.10	0.077 p>0.05	2.14±2.48 (0.00-7.34)	2.29	0.060 p>0.05	8.22±3.83 (3.14-13.73)	7.57	0.664 p>0.05
Non Anemic	0.80±0.95 (0.10-2.60)	0.45	1.00 p>0.05	0.70±1.07 (0.10-2.81)	0.18	0.108 p>0.05	1.34±1.23 (0.30-3.45)	0.77	1.000 p>0.05	9.42±7.43 (2.00-19.70)	8.37	0.562 p>0.05
Mild Anemic	7.08±4.55 (0.30-10.00)	10.00	0.012 p<0.05*	0.60±1.11 (0.00-2.86)	0.17	0.144 p>0.05	3.01±3.36 (0.00-9.11)	2.25	0.929 p>0.05	12.26±6.81 (4.82-19.02)	12.57	0.562 p>0.05
Moderate Anemic	6.11±4.25 (2.00-10.00)	6.20	0.011 p<0.05*	0.83±0.60 (0.00-1.65)	0.76	0.004 p<0.05*	8.73±12.30 (0.98-33.36)	4.76	0.026 p<0.05*	14.35±13.51 (7.45-41.85)	9.20	0.562 p>0.05
Severe Anemic	5.71±3.70 (1.60-10.00)	4.95	0.011 p<0.05*	0.46±0.57 (0.10-1.38)	0.76	0.042 p<0.05*	1.90±2.91 (0.00-7.36)	0.30	1.000 p>0.05	6.92±4.86 (2.29-15.68)	6.63	0.562 p>0.05
Active Infected	4.99 ±4.31 (0.10-10.00)	5.30	0.011 p<0.05*	0.63±1.04 (0.10-1.10)	0.10	0.042 p<0.05*	2.19±2.45 (0.30-9.11)	1.84	1.000 p>0.05	10.65±6.88 (2.00-19.70)	7.06	0.562 p>0.05
Infected	4.43 ±4.09 (0.10-10.00)	2.30	0.762 p>0.05	0.68±0.72 (0.00-1.93)	0.27	0.681 p>0.05	2.53±2.61 (0.00-7.34)	1.25	0.753 p>0.05	8.26±4.41 (2.00-15.78	9.54	0.707 p>0.05
Acute Infected	3.08 ±3.67 (0.10-10.00)	2.60	0.762 p>0.05	0.43±0.48 (0.10-1.38)	0.27	0.681 p>0.05	7.71±12.85 (0.30-33.36)	2.53	0.753 p>0.05	13.35±13.98 (6.71-41.85)	7.71	0.707 p>0.05

***:** Statistically significant difference between control and group (p<0.05).

DISCUSSION

The World Health Organization (WHO) defines Ehrlichiosis as an important disease with zoonotic potential, which can be severe and fatal if left untreated. It has been reported that 50% of Ehrlichia infections can be seen in Asia, Africa, Europe and America continents throughout the world (Tsachev et al. 2006), and the prevalence of the disease can reach 67%, especially in regions with tropical climate characteristics (Karagenç et al. 2005).

In literature data, it has been reported that vectors can have more than one infection agent because of that co-infection is seen frequently in vectorial diseases (Solano-Gallego et al. 2011). In ehrlichiosis *Anaplasma, Leishmania, Borrelia, Bartonella, Rickettsia, Babesia,* and viral agents can be detected together. (Baneth et al. 2008; Shaw et al. 2009; Harrus et al. 2016). In this study, the presence of co-infection was detected in 9 dogs with Ehrlichiosis to the literature information mentioned above. In all of the co-ınfected animals, anaplasma was detected and leishmaniosis is found in 4 animals.

D-dimer is an important sign of fibrinolytic activation. it can increase trauma, surgery, infection, ınflammation, gestation, DIC, venous thrombosis, ischemic cardiomyopathy and thrombosis (Kobayashi et al. 2020). D-dimer is swayed from blood clots and attends blood circulation. When coagulation occurs due to many reasons such as infection; D-dimer analysis becomes a valuable marker for involuntary thrombosis (Sato et al. 2020). So D-dimer is indicated as an important marker when deep venous thrombosis and DIC occur (Han et al. 2022). In thıs study, the results of D-dimer levels are found compatible with works of literature. As a result of the analyses performed, it was determined that the D-dimer results of mono infected, co infected, mild, moderate, severe and active infected groups, were significantly different from the control group ($p<0.05$). It is recommended that more studies should be conducted on the subject of knowing how ehrlichiosis or co-infection agents predispose to DIC (Caldin et al. 2000). In the study conducted by Caldin et al. (2000), the sensitivity of D-Dimer was reported as 100% and the specificity as 97% in the development profile of DIC. It was reported by Paşa et al. (2017) that the D-Dimer level increased in infected dogs with ehrlichiosis (Paşa et al. 2017). In this study, it was seen that the findings on D-dimer were compatible with the studies of the researchers, and in line with the results obtained, it was concluded that the development potential of DIC and venous thromboembolism in Ehrlichia-infected animals were formed.

Infarctus and hypoxia consist of causing anemia Due to anemia, the increased metabolic heart requirement on the cardiovascular system and heart cannot be met (Portman et al. 1995). It has been reported that myocardial damage may develop as a result of anemia, which can occur in infections caused by Ehrlichia and other co-infected agents (Diniz 2008). One of the cardiac markers cTn I is associated with myocyte degeneration that acute heart damage (Diniz et al. 2007; Braunwald 2008). And it has been reported that myocyte degeneration usually consists of after severe ischemia (O'Brien et al. 2006; Braunwald 2008). It has been determined that myoglobin is released into the circulation due to insufficient tissue perfusion or cellular damage in trauma situations (Liu et al. 2019). Troponins and myoglobin, which are heart-derived proteins, can be used for the presence and grading of myocardial damage in the detection of possible myocardial damage (Schober et al. 2002; Oyama et al. 2004; Q'Brien et al. 2006; Langhorn and Willesen 2015). Sleeper et al. (2001) reported that healthy dogs' cTn I levels could be between <0.03 ng-ml and 0.07 ng-ml (mean and standard deviation 0.02 ± 0.01). In thıs study, it was seen that control group cTn I value was composable to works of literature. Among the study groups, cTn I levels of mono-infected animals, moderate and severe anemic group and active infected animals were found to be statistically significantly higher ($p<0.05$). This condition on cardiac troponin changes occurs due to hypoxia as described in people with anemia (Diniz et al. 2008). In humans, the half-life of cTnI is approximately 2 hours. However, a slow breakdown of contractile apparatus shows a considerably longer half-life (Langhorn and Willesen 2016). This situation is almost similar in dogs with humans. Because of this condition, cTn I is used as a sensitive biomarker in myocardial cell injuries (Burgener et al. 2006). In this study, we thouhgt that anemia in mono-infected animals can be associated with myocyte degeneration due to insufficient perfusion of the heart and the increase in cTn I levels accordingly. Similarly, the insignificance of cTn I change in animals with mild anemia supports the human studies mentioned above.

In this study, there is no difference between the groups about myoglobin ($p>0.05$). Holmgren ve Valberg (1992) was reported that assessment of clinical tables with myonecrosis follow the myoglobin in plasma was a suitable method for detecting cardiac damage (Holmgren and Valberg 1992). In another study, it was reported that the first myoglobin levels were significantly higher in the gradual measurements in animals with gastric dilatation-volvulus and trauma, and the plasma MYG levels decreased in repeated measurements at the 24th and 48th hours (Burgener et al. 2006). It is thought that the decrease in myoglobin level is since the half-life is stated as 9 minutes (Klocke et al. 1982). On the other hand, Mair et al (1992, 1994) reported that there is no way to detect an increase of plasma myoglobin concentration whether it originates from skeletal muscles or heart damage (Mair et al. 1992; Mair et al. 1994). In

insufficient oxygenation in muscles Myoglobin carries essential oxygen to muscles (Karagül et al. 2000). Considering this information, it is thought that the lack of significant myoglobin levels between the groups may be due to the differences in the clinical pathogenesis of Ehrlichia in terms of anemia profile and the inability to fully clarify the origin of myoglobin from the heart.

Creatine kinase has two different bases unıt; M (Muscle) and B (Brain). As a result of the interaction of these unıts with each other, creatine kinase has three isoenzymes; CK-MB, CK-MM and CK-BB (Lang 1981). Besides the point of place that ısoenzymes came from are different, their effects are not known in veterinary medicine (Aktas et al. 1994). The most well-known information about creatine kinase belongs to dogs in veterinary medicine (Slack et al. 2005). In the last decades, there has been some suspicion of using CK-MB as a marker at cardiac damage but ıt was reported that in dogs with left ventricular hypertrophy, CK-MM levels decrease by 50%, and CK-MB levels increase %10 (Ye et al. 2001). In septic foals there is an uprising for CK-MB levels, however, it has been reported that there is no difference in CK-MB levels in living or non-viable animals, so it cannot be used as an indicator in terms of prognosis (Slack et al. 2005). And also, when compared with the cardiac-specific troponins CK-MB has lower specificity than them (Silverman et al. 1974; Adams et al. 1993).

Significant increases in CK-MB levels can occur within 2-4 hours following the development of myocardial damage. It is reported that conventional CK-MB levels decreased to normal levels 12 hours after the symptoms of acute cardiac injury were controlled and it could be detected in only 27% of the animals included in the study (Puleo et al. 1987). In the same study, it was reported that CK-MB isomers (CK-MB1, CK-MB2) expressed 98% reliability (Puleo et al. 1987). In a different study, the half-life of CK-MB concentration in plasma was defined as 2 hours (Burgener et al. 2006). The limited availability of the CK isomer, CK-MB, in the heart causes its rapid degradation. This situation limits the use of this marker, in the detection of circulating heart damage due to the inadequacy of its release in damage cases (Burgener et al. 2006). We thought that the high reversibility capacity of CK-MB could explain the absence of a significant difference in CK-MB levels between the group's capacity of CK-MB.

CONCLUSION

In conclusion, it was determined that myocardial damage was formed due to ischemia in the heart in anemic dogs with Ehrlichiosis, and in moderate and severe anemic dogs CTnI concentrations increased. D-dimer levels also increased with CME. In the fields of medicine, It is thought that these markers can be used in the clinical follow-up of the prognosis and treatment of patients with Ehrlichiosis in the follow-up of DIC status and heart damage. To gain a comprehensive understanding of all stages of CME, we suggest conducting further studies on larger populations of dogs.

Conflict of interest: The outhors declared that there is no conflict of interest.

Authors' Contributions: YP and SP contributed to the article idea, design and execution the study. YP collected datas. YP and SP analyzed data. All outhors contributed to the critical revision of the manuscript and have read and approved the final version.

Ethical Approvel: The study was ethically approved by the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (Decision Number: 64583101/2016/60).

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