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## Ischemia-modified albumin levels as a biomarker of ischemic injury in dogs with parvoviral enteritis

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**ABSTRACT:** The aim of this study was to determine ischemia-modified albumin levels in dogs with parvoviral enteritis. The study was carried out on a total of 24 dogs, 16 with parvoviral enteritis (sick group) and 8 healthy (control group). The diagnosis of parvoviral enteritis was made by clinical findings, immunochromatographic rapid diagnostic kit, and PCR analysis of stool samples. For blood analysis, 3 ml of blood was collected from the cephalic vein of dogs into tubes without anticoagulant prior to treatment. The tubes were maintained at room temperature for 30 minutes, before which they were centrifuged at 5000 rpm for 5 minutes. Serum samples were then extracted and stored at -20°C until further analysis. While IMA, IMAR, and cTn I levels and capillary refill time were significantly higher in dogs with parvoviral enteritis than in healthy dogs ( $P<0.05$ ), albumin levels were significantly lower ( $P<0.05$ ). As a result, it was observed that cardiac damage developed in dogs with parvoviral enteritis, and it would be useful to evaluate IMA and IMAR levels in the evaluation of ischemic damage.

**Keyword:** Canine parvovirus; Ischemia modified albumin; PCR, cTnI

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## INTRODUCTION

Canine parvoviral enteritis is a disease caused by canine parvovirus type 2 and is characterized by acute, contagious, and hemorrhagic gastroenteritis. There are two forms of the disease: enteritis and myocarditis. While dogs younger than six months of age usually exhibit symptoms of the severe disease, adults with compromised immune systems may also be susceptible. Parvoviral enteritis begins clinically with nonspecific findings such as anorexia, depression, and fever. Symptoms of vomiting and diarrhea follow within 24-48 hours. Intense fluid and protein loss from the gastrointestinal tract can cause severe dehydration and hypovolemic shock (Judge 2015; Mazzaferro 2020).

Cardiac damage may occur in dogs with parvoviral enteritis due to myocarditis, hypovolemia, septicemia, and endotoxemia. Biomarkers are used to determine cardiac damage. For this purpose, cardiac troponins are widely used (Gulersoy et al., 2020; Kocaturk et al., 2012). In a study, it was determined that myocardial damage developed in canine parvoviral enteritis, and the increase in cardiac troponin I (cTnI) levels was important in determining the damage (Kocaturk et al., 2012). In recent years, ischemia-modified albumin has been used as a new biomarker in the determination of cardiac damage in human medicine (Can and Yosunkaya 2017). The most prevalent protein in blood is albumin, which makes up 35–50% of plasma proteins in healthy dogs. Albumin is responsible for approximately 75% of the plasma osmotic pressure. Moreover, albumin is an essential source of amino acids that the animal's body can use when needed (Cerón et al., 2005). The amino-terminal end (N terminal) of the albumin molecule is the primary attachment point of transition metal ions such as cobalt, nickel and copper. For various reasons (free radical damage, acidosis and hypoxia, etc.), the N-terminal end of albumin is modified and its capacity to bind transition metals such as cobalt, nickel and copper decreases. This modified form of albumin is called ischemia modified albumin (IMA) and is determined spectrophotometrically using the albumin cobalt binding test. The stability of ischemia-modified albumin is 2 hours at 4-20°C. Values are stated to be stable when stored at -20°C. Although classical cardiac biochemical markers (e.g., cTnI) have high sensitivity and specificity, the rise of these markers occurs hours after acute coronary syndrome in humans. As a newly studied marker for the early diagnosis of the

acute coronary syndrome, ischemia-modified albumin stands out as a licensed, sensitive, inexpensive method to detect ischemia early for routine use. It is reported that ischemia-modified albumin rises within minutes after the onset of ischemia, remains elevated for 6-12 hours, and returns to normal levels within 24 hours (Can and Yosunkaya 2017).

This study aimed to evaluate ischemia-modified albumin levels in dogs with parvoviral enteritis and to determine their relationship with myocardial damage.

## MATERIALS AND METHODS

### Animals

The study was carried out on a total of 24 dogs, 16 with parvoviral enteritis (sick group) and 8 healthy (control group), brought to veterinary clinics in Balıkesir province and Animal Hospital Faculty of Veterinary Medicine, Balıkesir University for diagnosis and treatment. The diagnosis of canine parvoviral enteritis was made by clinical findings, an immunochromatographic rapid diagnostic kit (Asan Easy, South Korea) and PCR analysis of stool samples. For blood analysis, 3 ml of blood was taken from vena cephalica antebrachii of dogs into tubes without anticoagulant before treatment, and the tubes were kept at room temperature for 30 minutes, then centrifuged at 5000 rpm for 5 minutes, serum samples were removed, and the samples were stored at -20°C until analysis.

### Blood Analyzes

The cTnI levels in the serum samples were determined using the chemiluminescent immunoassay (Hamacher et al., 2015) method (Beckman Coulter DXI 800, USA), and the ischemia-modified albumin levels (Değirmençay et al., 2021) were determined as colorimetric (Rel Assay Diagnostics, Mega Medicine, Gaziantep, Türkiye). IMA levels were expressed as absorbance units (absu). Optical density was determined using a microplate reader (SPECTROstar Nano, BMG Labtech, Ortenberg, Germany).

### Determination of Ischemia Modified Albumin/Albumin Ratio (IMAR)

IMAR levels were calculated by dividing IMA levels by serum albumin (g/dL) levels.

### Polymerase Chain Reaction

Extraction of viral DNA was performed according to the kit's procedure using the viral nucleic acid isolation kit (Jena Bioscience, Viral RNA+DNA Prepa-

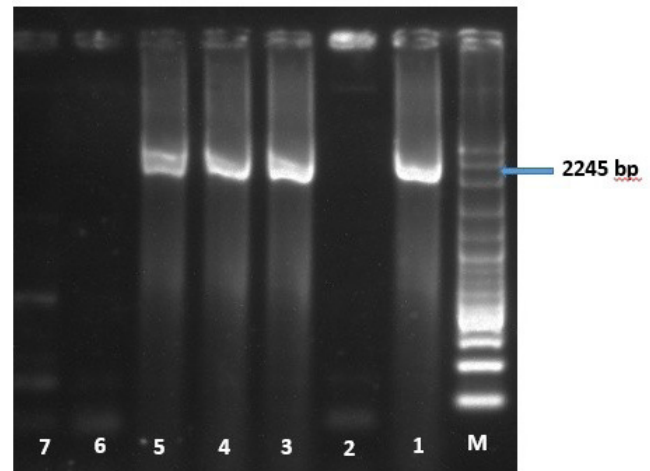
ration Kit, Germany) from stool samples. CPV-P1-forward ATggCACCTCCggCAAAGa, CPV-P2-reverse-TTTCTAggTgCTAgTTgAg primers specific to the CPV VP1-VP2 gene region were used for nucleic acid amplification (Özkul et al., 2002). For the CPV amplification process, following the first denaturation phase for 6 minutes at 94 °C, the heat programs applied in the thermal cycler (Himedia, Prima-Trio, India) were applied for 60 seconds at 94 °C, 60 seconds at 52 °C, 150 seconds at 72 °C and this heat programs were repeated for 40 cycles. After the final extension at 70°C for 10 minutes, 2245 bp of DNA product was obtained (Figure 1). Positive control in our Virology Department was used as positive control in PCR reactions. The 100 bp standard (Vivantis VC 100 bp Plus DNA Ladder NL 1407/100-3000 bp) was used for the evaluation of the obtained PCR products and the amplified DNA products were stained (GelRed Nucleic Acid Gel Stain) on a 1, 5 % agarose gel and detected under UV light in a gel imaging device.

### Statistical Analysis

Data were presented as mean and standard error of mean (Mean±SEM). Whether the data showed normal distribution or not was analyzed with the Kolmogorov Smirnov test. The difference between the groups was determined using the independent T-test for normally distributed data (IMA, albumin, IMAR, body temperature, CRT, and age) and the Mann Whitney U test for data that did not show normal distribution (cTnI).  $P<0.05$  was considered statistically significant. Statistical analyzes were determined using the SPSS software program (Version 22).

### RESULTS

The dogs that were found to have parvoviral enteritis were nine Kangals, two Pointers, one Toy Poodle, and four crossbreeds. In addition, 6 of the dogs with parvoviral enteritis were male and 10 were female. There were six Kangals, one German Shepherd, and one Golden Retriever in the control group. In ad-



**Figure 1.** Agarose gel image of PCR products amplified with specific CPV primers [Lane M: DNA ladder marker (100-bp), 1: positive control, 2: negative control (distilled water); 3,4,5: positive products; 6,7: negative products].

dition, 4 of the control group dogs were male and 4 were female.

Changes in age, body temperature and capillary refill time of dogs with parvoviral enteritis and healthy are given in Table 1. The mean body temperature of dogs with parvoviral enteritis was lower than the healthy ones, but no statistical difference was found. The body temperature was above 40 °C in 2 of the dogs with parvoviral enteritis and below 38 °C in 7 of them. Capillary refill time of dogs with parvoviral enteritis was significantly ( $P<0.05$ ) higher compared to healthy dogs.

### DISCUSSION

Despite vaccinations, canine parvoviral enteritis continues to be a common disease, especially in puppies, which can cause death. Therefore, numerous studies on canine parvoviral enteritis are still being conduct-

**Table 1.** Changes in clinical findings in dogs with parvoviral enteritis and healthy

Parameters	Control	Sick	P
Temperature (°C)	38.41±0.09	37.18±0.80	0.150
CRT (sec)	2.25±0.16	4.85±0.27	<0.001
Age (day)	86.25±4.70	81.87±9.20	0.750

CRT: Capillary refill time

IMA, albumin, IMAR and cTnI levels of dogs with parvoviral enteritis and healthy are presented in Table 2. IMA, IMAR and cTnI levels of dogs with parvoviral enteritis were significantly higher ( $P<0.05$ ) and albumin levels were significantly lower ( $P<0.05$ ) compared to healthy dogs.

**Table 2.** IMA, albumin, IMAR and cTnI levels of dogs with parvoviral enteritis and healthy

Parameters	Control	Sick	P
IMA (absu)	0.34±0.01	0.37±0.005	0.032
Albumin (g/dL)	2.86±0.08	2.27±0.07	<0.001
IMAR	0.12±0.005	0.17±0.004	<0.001
cTnI (pg/mL)	7.84±1.29	112.67±44.01	0.003

IMA: ischemia-modified albumin, IMAR: ischemia-modified albumin/albumin ratio, cTnI: cardiac troponin I

ed around the world. A significant portion of deaths in this disease are associated with ischemic damage due to sepsis/endotoxemia. In the present study, it was determined that IMA levels, which is a biomarker of ischemic damage, increased and ischemic damage occurred in dogs with parvoviral enteritis.

Parvoviruses initially infect the lymphoid tissue in the pharynx before entering the bloodstream, and then come to the intestines, bone marrow, and heart cells at very young ages, infecting these cells. Canine parvovirus replicates in dividing cells and thus typically causes disease symptoms in tissues with rapidly dividing cells, such as the gastrointestinal tract and bone marrow. In young dogs born to unvaccinated parents and infected during the first two weeks of life, the heart muscle is affected as cardiac muscle cells replicate during the first two weeks of life and can cause myocarditis. However, the myocarditis form of canine parvoviral enteritis is much less common today, as most dogs have a vaccination history. Because maternal antibodies usually protect newborns against parvovirus infection in the first two weeks (Judge 2015). Apart from myocarditis directly caused by viruses, it has been reported that myocardial damage develops due to sepsis in parvoviral enteritis (Kocaturk et al., 2012; Judge 2015). Kocaturk et al. (2012) reported that the presence of clinical and hematological findings indicative of sepsis and SIRS was detected in dogs with PVE. Sepsis has been shown to be one of the major causes of myocardial damage in both humans and dogs. Excessive cytokine production and release during the course of parvoviral enteritis causes the development or exacerbation of myocardial cell damage (Otto et al., 1997; Yilmaz and Senturk 2007). In dogs with parvoviral enteritis, anaerobic bacteria present in the intestines under normal conditions can enter the bloodstream from the damaged intestinal mucosa and cause SIRS and sepsis (Yilmaz and Senturk 2007; Kocaturk et al., 2012). Biomarkers are used to detect cardiac damage. Cardiac troponins provide reliable detection of car-

diac damage, and even small increases are important to indicate myocardial damage. Cardiac troponin I is considered a reliable serum biomarker for myocardial ischemia and necrosis in humans and animals (Burgener et al., 2006; Er and Ok 2015; Mair et al 2018; Gulersoy et al., 2020). Burgener et al. (2006) found that in dogs with acute myocardial injury, the level of cTnI increased significantly within 24 to 48 hours and began to return to normal after 48 hours. Kocaturk et al. (2012) reported that myocardial damage was observed in dogs with parvoviral enteritis and that increased cTnI levels could be considered as a poor prognostic indicator. Gülersoy et al. (2020) stated that serum cTnI levels were significantly increased in dogs with parvoviral enteritis compared to healthy dogs, and dogs with parvoviral enteritis may have developed mild or moderate myocarditis with hemorrhagic enteritis. Similarly, in the present study, cTnI levels were found to be significantly higher ( $P<0.05$ ) in dogs with parvoviral enteritis compared to healthy dogs. These results showed that myocardial damage developed in canine parvoviral enteritis, as in previous studies. Myocardial damage in canine parvoviral enteritis may be directly related to myocarditis caused by parvovirus, or it may be a result of sepsis observed during the course of the disease.

Albumin is a negative acute phase protein, and its concentration decreases by more than 25% during the inflammatory response (Schmidt and Eckersall 2015). Studies in canine parvoviral enteritis have found a decrease in albumin levels (Kocaturk et al., 2012; Başbuğ et al., 2020). It has been reported that the reason for this decrease is loss of proteins due to enteritis and/or hemorrhage, decreased food intake/anorexia and malabsorption (Wingfield and Raffe 2002). In addition, hypoalbuminemia can be observed as a result of the increase in vascular permeability associated with SIRS (Kalli et al., 2010; Alves et al., 2020). Although albumin measurement is easy and inexpensive, it has low clinical value in the diagnosis and monitoring of inflammation. In

cases of inflammation or infection in dogs and cats, the reduction in albumin levels provides an estimate of the acute phase reaction. However, the sensitivity and specificity rates are not high for detecting clinical or subclinical diseases (Cerón et al., 2005). In this study, serum albumin levels were found to be significantly lower ( $P<0.05$ ) in dogs with parvoviral enteritis compared to the control group. This reduction may possibly be related to intestinal loss of albumin due to enteropathy, decreased food intake or anorexia and being a negative acute-phase protein.

The amino-terminal end (N terminal end) of the albumin molecule is the primary binding site of transition metal ions such as cobalt, nickel, and copper (Can and Yosunkaya 2017). Due to various reasons (free radical damage, inflammation, energy-induced membrane damage, exposure to free iron and copper, acidosis and hypoxia), the amino-terminal end of albumin is modified and its capacity to bind transition metals such as cobalt, nickel and copper decreases (Worster et al., 2005; Can and Yosunkaya 2017; Park et al., 2021). In some studies, it has been shown that ischemia-modified albumin can be a potential marker of tissue ischemia (Roy et al., 2004; Sinha et al., 2004; Peacock et al., 2006). Ischemia-modified albumin is therefore considered a candidate marker of transient myocardial (Sinha et al., 2004; Peacock et al., 2006) or non-myocardial ischemia (Roy et al., 2004). While IMA is at the level of 1-2% of the total albumin in non-pathological conditions, this rate can increase up to 6-8% in ischemic conditions (Sbarouni et al., 2011; Can and Yosunkaya 2017). Today, biomarkers (such as cTnI) that are used extensively for the detection of myocardial damage are secreted from myocytes when cell necrosis develops. Although classical cardiac biomarkers such as cardiac troponin I have high sensitivity and specificity, their increase occurs hours after myocardial injury (Burgener et al., 2006; Mair et al., 2018). This suggests that IMA may serve as an early biomarker of ischemia. In a study, it was stated that ischemia-modified albumin is better in monitoring ischemia than CK-MB in patients with and without ischemia (Chawla et al., 2006). Ischemia-modified albumin may occur due to increased oxidative stress in different ischemia models that affect not only the myocardium but also other organs (Sbarouni et al., 2011; Can and Yosunkaya 2017). Serum IMA level is also increased in diseases that cause non-cardiac ischemia such as pulmonary embolism (Turedi et al., 2008), end-stage renal diseases, cerebrovascular ischemia, post-exercise skeletal muscle ischemia (Roy et al., 2004), diabetes mellitus (Balamir et al., 2018),

metabolic syndrome (Valle et al., 2010), some cancers (Stachowicz-Stencel et al., 2011; Eryilmaz et al., 2020), infection, sepsis (Park et al., 2021), peripheral vascular diseases (Roy et al., 2004) and liver diseases (Kim et al., 2010; Can and Yosunkaya 2017). It has been reported that oxidative stress is also observed in dogs with parvoviral enteritis, in addition to sepsis and SIRS (Panda et al., 2009; Kocarturk et al., 2010; Kocaturk et al., 2012; Aydoğdu et al., 2018; Gaykwad et al., 2018). In the present study, it was observed that IMA and IMAR levels significantly ( $P<0.05$ ) increased compared to the control group. This increase may occur directly as a result of myocardial damage caused by the virus or sepsis, or as a result of non-cardiac ischemia independent of myocardial damage (oxidative damage, sepsis, etc.).

### Limitations

The fact that the Sequential Organ Failure Assessment (SOFA) score in dogs with parvoviral enteritis, mentioned in some recent studies (Kalogianni et al., 2022), was not performed in the sick dogs in this study is a limitation of the study. In addition, the inability to perform other cardiac function tests (such as electrocardiography, echocardiography and histopathological evaluations) other than cTnI and therefore the inability to determine whether myocardial damage is due to myocarditis or other secondary causes (dehydration, hypotension, sepsis, etc.) was considered as another limitation. In addition to this, a more comprehensive evaluation by performing the analysis of biomarkers associated with oxidative stress that can be triggered by ischemia may allow the results to be supported. We believe that the results of the study can lead to current studies on this subject.

### Conclusions

In conclusion, increased cTnI levels in dogs with parvoviral enteritis showed that myocardial cell damage developed. High levels of IMA and IMAR in dogs with parvoviral enteritis also showed that cardiac or non-cardiac ischemia is present, and these parameters may be useful in determining ischemic damage. In addition, it was concluded that new studies are needed to investigate the relationship of IMA with ischemia in cardiac and other non-cardiac diseases.

### FINANCIAL SUPPORT

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## ETHICAL STATEMENT

This study was approved by the Balıkesir University Animal Experiments Local Ethics Committee (Approval no: 2019/3-6).

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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