



TOTAL ANTIOXIDANT CAPACITY, TOTAL OXIDANT STATUS, D-DIMER AND NITRIC OXIDE LEVELS IN DOGS WITH PARVOVIRAL ENTERITIS

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Summary

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Canine parvoviral enteritis remains one of the commonest causes of death in young dogs. This study aimed to determine serum total antioxidant capacity (TAC) and total oxidant status (TOS) levels in dogs with canine parvoviral enteritis (CPV) and reveal the risk factors of DIC formation. Thirty dogs were included in the study. The dogs were divided into two groups; the first group consisted of 20 dogs diagnosed with CPV (study group) and the other group consisted of 10 healthy dogs aged 0–12 months (control group). The mean TAC value was 0.354 ± 0.018 mmol Trolox equiv./L in the control group and 0.295 ± 0.007 mmol Trolox equiv./L in the study group. The mean TOS level was determined as 6.539 ± 0.154 $\mu\text{mol H}_2\text{O}_2/\text{L}$ in the control group and 7.934 ± 0.364 $\mu\text{mol H}_2\text{O}_2/\text{L}$ in the study group. The mean nitric oxide (NO) concentration was 18.613 ± 0.856 $\mu\text{mol/L}$ in the control group and 21.781 ± 0.913 $\mu\text{mol/L}$ in the study group. D-dimer levels were found to be 0.170 ± 0.070 mg/dL in the control group and 2.585 ± 0.584 mg/dL in the study group. As a result, it was demonstrated that it is essential to consider that oxidative stress increases and antioxidant capacity decreases in dogs with CPV and that disseminated intravascular coagulation (DIC) may develop in dogs with CPV. It is thought that considering oxidative stress and DIC during the treatment may contribute positively to prognosis and survival.

Key words: canine parvoviral enteritis, coagulation, DIC, TAC, TOS

INTRODUCTION

Canine parvovirus infection (CPV) is characterised by acute, highly contagious, and haemorrhagic enteritis in dogs. It has been widely seen worldwide for many years. On the other hand, CPV is one of

the commonest causes of death in young dogs (Mazzaferro, 2020). Haemorrhagic gastroenteritis, acute myocarditis, and neonatal mortality were identified in affected dogs (Killian *et al.*, 2018). The

acute onset of haemorrhagic enteritis is characterised by excessive blood in the stool, vomiting, and depression in puppies younger than 12 months. Myocarditis is mainly seen in puppies younger than eight weeks of age and progresses with death (Goddard & Leisewitz, 2010).

D-dimer is a fibrin formation and degradation biomarker that can be measured in whole blood and plasma. Circulating D-dimer levels in healthy animals are very low but can be rapidly elevated in thrombosis-related conditions. Nowadays, D-dimer levels estimation is mainly used to detect venous thromboembolism (VTE). Also, they are frequently used to determine the optimum anticoagulation time and monitor disseminated intravascular coagulation (DIC) in patients with VTE (Weitz *et al.*, 2017).

Total antioxidant capacity (TAC) is mainly used to determine the antioxidant level in biological samples (Fraga *et al.*, 2014). Typically, antioxidants block the harmful effects of oxidants and protect cells from oxidative damage caused by reactive oxygen species. Diminished levels of antioxidants in the blood indicate overproduction of oxidants (Prior & Cao, 1999; Halliwell, 2011). Oxidants cause DNA, lipid, and protein damage and induce oxidative stress, which plays an important role in the etiology or progression of many diseases (Prior & Cao, 1999; Halliwell, 2011; Fraga *et al.*, 2014; Diez-Espinosa *et al.*, 2015).

NO is a signalling molecule produced in mammalian cells by NO synthase. It is involved in a wide range of physiological processes, including inflammatory response, bronchodilation, vasodilation, intraocular pressure modulation, neuronal function regulation, and signal transmission. It has been well established that NO has a role in the pathogenesis of many

human viral infections (Fang, 2004; Gartwaite, 2014).

This study aimed to determine serum total antioxidant capacity, total oxidant status and D-dimer levels in dogs with CPV.

MATERIALS AND METHODS

Ethical statement

The study was approved by Afyon Kocatepe University Animal Experiments Local Ethics Committee (Approval No: 131-16).

Animals

In this study, the data were taken from 30 dogs with different breeds and ages brought to Afyon Kocatepe University Animal Hospital. The dogs were divided into two groups. The diagnosis of CPV was made in the light of clinical findings and faecal antigen test. Parasitological examination was performed to all dogs to exclude parasitic infestation. The first group consisted of 20 dogs diagnosed with CPV (study group). The other group consisted of 10 healthy dogs aged between 0–12 months (control group). Blood samples were taken from both groups via *vena cephalica antebrachii* in tubes with clot activator for serum and tubes containing EDTA for plasma and kept at -20°C till measurement.

Rapid test kit procedure and interpretation

Canine parvovirus antigen rapid test kits (Asan Easy Test, Asan Pharmaceutial, Korea) for detecting CPV were carried out for the procedure. Stool samples or rectal smears were used for the analysis. The samples were mixed with the standard diluent, and the mixture was dropped into

the sample chamber. The reading of the test result was performed after 8–10 minutes, then evaluated according to the colour changes specified by the manufacturer.

Biochemical analysis

TAS and TOS levels were determined by a spectrophotometric method using commercial kits (Rel Assay Diagnostics, Türkiye). Nitric oxide is a very short half-life substance and is converted into stable metabolites nitrate and nitrite by oxidation. Therefore, the NO level is usually assessed by detecting these metabolites. For this reason, the amount of nitric oxide in plasma samples was determined by the vanadium chloride (III) Gries reaction method. All tests were done in Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Biochemistry.

D-dimer levels were measured by the fluorescent immunoassay method with a Finecare FIA Meter brand model device (Guangzhou Wondfo Biotech Co., Ltd., China) at Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Internal Medicine.

Statistical analysis

Analyses were performed in the PASW Statistics 18 package program. Kolmogorov-Smirnov test was used to determine the normal distribution of the data. Logarithmic transformation was applied to the data that did not show normal distribution. An independent variable *t*-test was used in the analysis of the data. Data were expressed as mean±SEM. The significance level was accepted as $P<0.05$.

RESULTS

Vomiting, haemorrhagic enteritis, inappetence, lethargy, dehydration and weakness were observed in all dogs with parvoviral enteritis.

The values of the study and control group dogs are presented in Table 1. TAC values were significantly lower in dogs in the study group when compared with the control group ($P<0.05$). When the TOS concentration of the control group was compared with the result of the study group, a significant increment was detected in the latter ($P<0.05$). Similarly, NO concentrations of the control group were statistically significantly lower than

Table 1. Changes in TAS, TOS, NO and D-Dimer levels in CPV (n=20) and healthy (n=10) dogs. Data are expressed as mean±SEM

Group	TAC (mmol Trolox equiv./L)	TOS ($\mu\text{mol H}_2\text{O}_2$ equiv./L)	NO ($\mu\text{mol/L}$)	D-dimer (mg/dL)
Control group	0.354±0.018 ^a	6.539±0.154 ^a	18.613±0.856 ^a	0.170±0.070 ^a
Study group	0.295±0.007 ^b	7.934±0.364 ^b	21.781±0.913 ^b	2.585±0.584 ^b
P	0.001	0.003	0.030	0.020

Different superscripted letters refer to significant differences between the values in the same rows ($P<0.05$).

those in the study group ($P < 0.05$). D-dimer levels of the control group were significantly increased when compared with the study group ($P < 0.05$).

DISCUSSION

CPV is quite lethal, especially in young dogs. The disease causes acute haemorrhagic enteritis in dogs younger than 12 months, and myocarditis in dogs younger than two months of age (Bloom & Kerr, 2006). During haemorrhagic enteritis, anorexia, depression, lethargy, fever, increased respiratory and heart rate, vomiting, bloody diarrhoea can be observed. Due to severe vomiting and diarrhoea, hypovolemia related to fluid-electrolyte imbalance can develop in patients (Goddard & Leisewitz, 2010; Kocatürk *et al.*, 2010). Diarrhoea, vomiting, depression, and anorexia were observed in all study group dogs.

When redox homeostasis in the cell is disrupted due to the overproduction of reactive oxygen species or the inadequacy of the antioxidant system, oxidative stress is produced. Free radical reactions take part in the defense mechanism of immune system cells such as neutrophils and macrophages. However, overproduction of free radicals can cause tissue damage and cell death (Aydoğdu *et al.*, 2018). Lots of molecules and methods have been described to determine oxidative stress. Serum/plasma levels of different oxidant species can be measured individually. However, measuring these markers individually neither gives information about the general state nor is complex and economic (Kocatürk *et al.*, 2015; Elsayed *et al.*, 2020). TAC assay is widely used in evaluating oxidative stress, being easy and inexpensive. It also reflects the antioxidant ability of the organism and the com-

bined effects of different antioxidants on the body (Erel, 2005). In the current research studies, CPV is significantly associated with oxidative stress, reactive oxygen/nitrogen species, and lipid peroxidation (Panda *et al.*, 2009; Gaykwad, 2018). It has been reported that lipid peroxide concentrations are increased in dogs with parvoviral enteritis compared to the control group, and thus the disease causes oxidative stress (Panda *et al.*, 2009). Gaykwad *et al.* (2018) reported a significant increment in MDA and NO levels in dogs with CPV compared to the control group. Similarly, in the present study, total oxidant status and NO levels were significantly higher ($P < 0.05$) in dogs with CPV compared to the control group. It has been thought that TOS and NO levels in dogs with CPV indicate oxidative damage and that TOS and NO levels can be used safely to determine oxidative damage in dogs.

Antioxidant defense systems prevent the formation of reactive oxygen species and cell damage caused by reactive oxygen species. Antioxidants involved in the defense system against free radicals can be divided into enzymatic and non-enzymatic antioxidants. Enzyme systems are particularly effective in preventing oxidative damage. The most important enzymes are superoxide dismutase, glutathione peroxidase, and catalase. Non-enzymatic antioxidants comprise ascorbic acid, alpha-tocopherol, glutathione, beta-carotene, and other antioxidants. Under normal conditions, the current balance between the amounts and activities of antioxidants is necessary for the vitality and healthiness of the organism (Ukwueze *et al.*, 2020). The measurement of total antioxidant status, which has been used more frequently in recent years, provides more realistic information compared with the

individual measurements (Wijnberger *et al.*, 2003; Vlachos *et al.*, 2006). Gaykwad *et al.* (2018) reported that the plasma glutathione S-transferase level decreased significantly in dogs with CPV compared with the control group. On the contrary, Panda *et al.* (2009) reported that erythrocyte superoxide dismutase and catalase levels were higher in dogs with CPV when compared with the control group. Kocaturk *et al.* (2015) also affirmed no decrease in blood TAS levels in dogs with CPV. Still, there was a tendency to increase with the severity of the disease. It has been reported that the reason for this situation may be related to the synthesis of these antioxidant enzymes as a compensatory mechanism in cases of moderate and severe gastroenteritis. In addition, a study reported that serum TAS levels become elevated in critical surgery patients with septic shock which may be due to a defense mechanism against SIRS (Pascual *et al.*, 1998). There was a significant reduction in TAS level in dogs with CPV in the present study. Decreased antioxidants can indicate overproduction of oxidants resulting in DNA, lipid, and protein damage, a condition known as oxidative stress (Halliwell, 2011; Diez-Espinosa *et al.*, 2015). It is thought that the decrease in TAS level in the present study is related to the increase in TOS level.

DIC may develop due to viraemia, septicaemia, parasitic infection, severe tissue damage, poisoning, intravenous haemolysis, autoantibody formation, hepatitis, pancreatitis, and neoplasm (Caldin *et al.*, 2000; Çöl & Durgun, 2007; Googs *et al.*, 2018; Levi, 2018). On the other hand, Otto *et al.* (2000) reported that DIC could occur in dogs with CPV. In recent years, D-dimer measurement has been used in DIC cases. It has been demonstrated that for DIC diagnosis, D-dimer is more sensi-

tive and specific than many other tests (Caldin *et al.*, 2000; Stokol *et al.*, 2000; İnsal & Pişkin, 2020). Laforcade *et al.* (2003) stated that they did not detect any prolongation in prothrombin time and activated partial thromboplastin time, increase in D-dimer level, decrease in anti-thrombin-III activity, and no changes evaluated in platelet count in dogs with sepsis. Er & Ok (2015) reported that the D-dimer level was higher in dogs with CPV. Similarly, in the presented study, it was determined that the D-dimer level increased significantly ($P<0.05$) in dogs with CPV when compared to the control group. Such D-dimer levels indicate DIC situations in dogs with CPV.

It has been well established that NO has a role in the pathogenesis of many human viral infections and direct or indirect antiviral activity. Direct antiviral activity involves direct inactivation of viral particles or inhibition of their replication by NO. In contrast, indirect activity modifies the host immune response that usually produces an inflammatory response (Akaike, 2001; Gantner *et al.*, 2020; Green, 2020). In the present study, NO level has increased significantly in dogs with parvoviral enteritis.

As a result, it has been demonstrated that it is essential to consider the occurrence of increased markers of oxidative stress and decreased antioxidant defense in dogs with CPV and that DIC may develop in dogs with CPV. It is suggested that considering oxidative stress and DIC during the treatment may contribute positively to patients' prognosis and survival.

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