Original article

Assessment of intestinal and cardiac-related biomarkers in dogs with parvoviral enteritis

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Abstract

The aim of this study was to evaluate the intestinal and cardiac biomarkers in the determination of intestinal and cardiac damage in dogs with parvoviral enteritis. The material of this study consisted of 10 healthy dogs (control group) and 30 dogs with parvoviral enteritis (experimental group) admitted to the Department of Internal Medicine, Faculty of Veterinary Medicine, Selcuk University.

Serum samples were extracted from the collected blood samples taken from *vena cephalica* venipuncture for analysis of blood gases, haemogram and to measure the levels of intestinal-fatty acid-binding protein (I-FABP), trefoil factor 3 (TFF-3), claudin-3 (CLDN-3), heart-type fatty acid-binding protein (H-FABP), cardiac troponin I (cTnI), and creatine kinase-myocardial band (CK-MB) by enzyme linked immunosorbent assay (ELISA) test kits.

Statistically significant decreases in the blood gas hydrogen ion concentration (pH), partial pressure of oxygen (pO_2), sodium (Na), bicarbonate (HCO_3), and oxygen saturation ($SatO_2$) levels and significant increase in the levels of I-FABP, TFF-3, CK-MB, cTnI and also in the haemogram, a decrease in leukocyte (WBC) level and an increase in platelet (THR) level were detected in parvoviral dogs compared to the control group ($p<0.05$). Also ROC analysis revealed on 0th hour for the utility of I-FABP and on 48th hour for TFF-3 in differentiating in the experimental group between the survivor and non-survivor dogs. Other intestinal-related biomarker (CLDN-3) and none of the cardiac-related biomarkers (H-FABP, CK-MB and cTnI) are not high enough for prediction of mortality.

In conclusion, it was determined that I-FABP and TFF-3 for the intestinal injury and mortality prediction, and CK-MB and cTnI for the cardiac injury were useful and reliable biomarkers to determine the damage caused by parvovirus in dogs.

Key words: biomarkers, cardiac biomarkers, dog, intestinal biomarkers, parvoviral enteritis

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Introduction

Canine Parvovirus infection is an acute, very contagious, and lethal viral disease of dogs (Goddard and Leisewitz 2010, Er and Ok 2015). The agent responsible for parvoviral infection is parvovirus Type-2 (CPV-2) (Lamm and Rezabek 2008, Goddard and Leisewitz 2010). In dogs, CPV-2a, 2b, and 2c strains cause acute haemorrhagic gastroenteritis and myocarditis (Elia et al. 2007, Hong et al. 2007, Goddard and Leisewitz 2010). Although the severe clinical disease occurs mostly in dogs less than 6 months of age, adult dogs with immune deficiency are also potentially at risk (Mylokanis et al. 2016). Acute haemorrhagic enteritis is the most common form. The myocarditis form is less common than the enteritis form. This form causes peracute death due to heart failure in puppies which are infected in fetal life or as infants younger than 8 weeks of age whose dams are not vaccinated (Hoskins 1997). In dogs which are not treated for parvoviral enteritis, the mortality rate can be as high as 90%, whereas in treated dogs the mortality rate may vary between 4-7% depending on the therapeutic approach (Sarpong et al. 2017).

Some biomarkers, I-FABP for example, are used to determine the intestinal damage due to acute intestinal ischemia and necrotic enterocolitis (NEC) (Kanda et al. 1996, Guthmann et al. 2002, Benkoe et al. 2014, Yildiz et al. 2018). Ng et al. (2013) reported that the mortality rate in infants with NEC was 45% and the levels of liver-type fatty acid binding protein (L-FABP), intestinal-type fatty acid binding protein (I-FABP), and trefoil factor 3 (TFF-3) were significantly higher in the infants who died than those who survived. It was reported that I-FABP and L-FABP intestinal biomarkers could be used to evaluate the prognosis of preterm infants with NEC (Benkoe et al. 2014).

In patients with NEC, after an inflammatory response due to intestinal mucosal damage, it was reported that the concentration of TFF-3 increases along with L-FABP, I-FABP (Ng et al. 2013, Srivastava et al. 2015). Yildiz et al. (2018) found that serum TFF-3 levels in calves with atresia coli were significantly higher than the healthy calves. Thuijls et al. (2010) reported that claudin-3 (CLDN-3) was significantly reduced in infectious bowel disease and disruption of intestinal wall integrity, and this biomarker may be a reliable marker for the detection of intestinal damage.

Currently, cardiac biomarkers are also being used as reliable indicators for the diagnosis of heart disease. These biomarkers include cardiac troponins (cTnI, cTnT), creatine kinase myocardial band (CK-MB), and brain natriuretic peptide (BNP) (Apple 1999,

Ok et al. 2008, Er and Ok 2015). Cardiac troponins provide reliable information on cardiac damage and even small increases are important in terms of myocardial injury (Er and Ok 2015). Er and Ok (2015) found significant increases in the blood CK-MB and BNP levels in dogs with parvoviral enteritis and a slight increase in the cTnI levels. In recent years, heart-type fatty acid binding protein (H-FABP) has been used to determine the magnitude of heart damage. H-FABP has been reported to be a highly reliable prognostic marker in predicting mortality due to acute heart damage (Dellas et al. 2010). Serum H-FABP level has been reported to increase significantly in patients with severe heart failure, hypertrophic and dilated cardiomyopathy (Komamura et al. 2006, Arimoto et al. 2007, Renaud and Ngako 2007).

The aim of this study was to determine the extent of the intestinal injury by using the intestinal specific biomarkers and also by using the heart-specific biomarkers whether myocarditis developed in dogs with parvoviral enteritis.

Materials and Methods

Animal Material

The material of this study consisted of 30 dogs with parvoviral enteritis (experimental group) and 10 healthy dogs (control group) aged between two and six months admitted to the Department of Internal Medicine, Faculty of Veterinary Medicine, Selcuk University. The ethical approval (2018/10) was obtained from the Selcuk University Faculty of Veterinary Medicine Ethics Committee (SÜVFEK).

Clinical examinations

All dogs in the study were clinically examined (measurement of body temperature, heart and respiratory rate, heart auscultation and electrocardiographic examination). Diagnosis of parvoviral enteritis was made by laboratory tests and rapid parvovirus antigen (CPV Ag) testing in feces. Same tests were also applied to dogs in the control group. Dogs with normal clinical, laboratory findings and negative feces CPV Ag testing were considered healthy.

Taking feces samples and antigen test application

Feces samples were taken with a rectal swab and the CPV Ag test (Asan Easy Test PARVO, Asan Pharma. CO. LTD., Gyeonggi-do Korea) was applied according to the manufacturer's instructions. 30 dogs positive in CPV Ag test were the experimental group.

Feces samples of the healthy dogs were also tested for CPV Ag to ensure the absence of the virus.

Cardiologic examinations

In order to determine any heart murmur and arrhythmies heart auscultation was performed according to the point of maximal intensity (PMI). To assess type of pulse, femoral pulse was evaluated. Also electrocardiographic examination was performed using by standart bipolar lead system (I, II and III; 50 mm/sec.; 10 mm=1mV).

Taking and storing blood samples

Venous blood samples from the dogs were taken from *vena cephalica* venipuncture at the onset of treatment (0th hour) and 48 hours later (48th hour). Blood samples were taken into tubes with ethylenediaminetetraacetic acid (K_3EDTA) for haemogram, tubes with heparin for blood gases, and serum tubes for intestinal and heart biomarkers. Haemogram and blood gases were measured within 5-10 minutes. Blood samples for biochemical analysis were centrifuged and their sera were extracted and stored in a -80°C freezer until the measurement for intestinal and heart biomarkers.

Haemogram and blood gas measurements

Haemogram analyses (leukocyte (WBC), erythrocyte (RBC), haemoglobin (Hb), haematocrit (Hct) and platelet) from venous K_3EDTA blood samples were measured on MS4 device (CFE 279, Haematology Analyser). Haemogram and blood gas analysis was performed within the first 5-10 min after taking the blood samples. Blood gases (hydrogen ion concentration (pH), partial pressure of carbondioxide (pCO_2), partial pressure of oxygen (pO_2), base excess (BE), oxygen saturation ($SatO_2$), bicarbonate (HCO_3), lactate) from the venous heparin blood samples were measured on GEM Premier Plus 3000 (74351, Blood Gas/Electrolyte Analyser, Model 5700. Instrumentation Laboratories, USA).

Intestinal and cardiac biomarker measurements

Serum I-FABP, TFF-3, and CLDN-3 concentrations were measured according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanga-China) using canine specific commercial ELISA kits. The intra-assay (in labor) and inter-assay (inter labor) coefficient of variation (CV) reported for I-FABP was ≤8% and ≤10%, respectively and the minimum detectable concentration (MDC) was 0.12 ng/mL. The detection range was 0.3 ng/mL-90 ng/mL. The intra-assay (in labor) and inter-assay (inter labor) variation coefficient (CV) reported for TFF-3 was ≤8% and ≤10%, respectively and the minimum detectable concentration (MDC) was 0.29 ng/mL. The detection range was 0.5-100 ng/mL. The reported intra-assay (in labor) and inter-assay (inter labor) coefficient of variation (CV) for CLDN-3 was ≤8% and ≤10%, respectively and the minimum detectable concentration (MDC) was 6.98 ng/L. The detection range was 15 ng/L-300 ng/L.

Serum H-FABP level was measured according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanga-China) using commercially available ELISA kits. The intra-assay (in labor) and inter-assay (inter labor) variation coefficient (CV) reported for H-FABP was ≤8% and ≤10%, respectively and the minimum detectable concentration (MDC) was 0.053 ng/mL. The detection range was 0.1 ng/mL-40 ng/mL. Serum CK-MB level was measured according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanga-China) using commercially available ELISA kits. The intra-assay (in labor) and inter-assay (inter labor) variation coefficient (CV) reported for CK-MB was <8% and <10%, respectively and the minimum detectable concentration (MDC) was 0.028 ng/mL. The detection range was 0.05 ng/mL-30 ng/mL. Serum cTnI level was measured according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanga-China) using commercially available ELISA kits. The intra-assay (in labor) and inter-assay (inter labor) variation coefficient (CV) reported for cTnI was <8% and <10%, respectively and the minimum detectable concentration (MDC) was 2.61 ng/L. The detection range was 5 ng/L-1500 ng/L.

Treatment protocol

Fluid therapy, antibiotics, haemostatics, antimicrobial agents, antiemetics, vitamins, amino acids, and hyperimmune serum were applied to the dogs with parvoviral enteritis. Fluid therapy was performed depending on the results of blood gasometry. A 60-90 mL/kg dose of lactate ringer (Lactate Ringer, Polifarma) and isotonic sodium chloride solution (Isotonic sodium chloride, MS Pharma) was administered intravenously for three days. Three dogs in the experimental group with blood pH <7.1 received 1.3% sodium bicarbonate (NaHCO3) (Bikarvil, Teknovet). Ceftriaxone as antibiotic (Unacefin, ABIS Pharma) at a dose of 20 mg/kg IV, 2 times a day, metoclopramide as an antiemetic (Metpamid, Sifar) at a dose of 0.5 mg/kg IV, twice a day, vitamin K as a haemostatic (Hemadur-K, Alke) at a dose of 2 mg/kg SC twice a day, ascorbic acid 200 mg (Vita-C Vetaquinol,
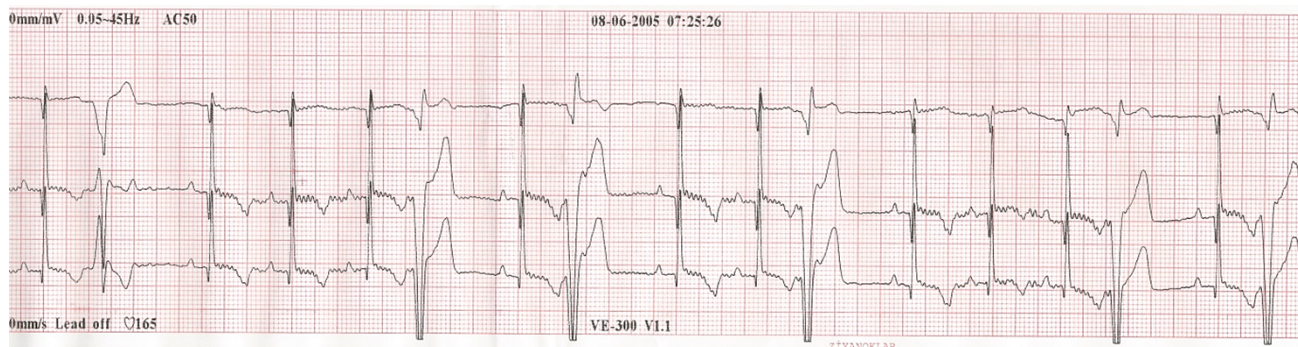
Fig. 1. Sinus tachycardia and ventricular premature complexes in one of the dogs of experimental group (I, II and III; 50 millimeter/ /second; 10 millimeter = 1 millivolt).

Table 1. Intestine and heart damage biomarker values (Mean ± standard error) and significance in dogs with parvoviral enteritis and healthy dogs.

Parameters	Hour	Control group (n:10)	Experimental group (n:30)	p-value
CLDN-3 (ng/L)	0	81.4±12.3	80.2±3.54	0.927
	48	104±8.32	88.3±3.01	0.087
I-FABP (ng/mL)	0*	2.58±0.17	3.08±0.15	**0.039**
	48	2.74±0.20	2.93±0.05	0.388
TFF-3 (ng/mL)	0*	2.71±0.19	3.34±0.15	**0.020**
	48*	2.65±0.19	3.69±0.11	**0.000**
H-FABP (ng/mL)	0	1.05±0.09	1.19±0.03	0.199
	48	1.12±0.20	1.31±0.05	0.106
CK-MB (ng/mL)	0*	29.6±2.60	41.0±2.62	**0.005**
	48	32.5±2.58	30.4±2.37	0.569
cTnI (ng/mL)	0*	0.004±0.0006	0.02±0.003	**0.000**
	48*	0.004±0.0008	0.01±0.002	**0.002**

CLDN-3=Claudin 3, I-FABP=Intestinal Fatty Acid Binding Protein, TFF-3=Intestinal Trefoil Factor 3 H-FABP=Heart Fatty Acid Binding Protein, CK-MB=Creatine kinase Myocardial band, cTnI=Cardiac troponin I * $p<0.05$ (statistically significant).

Vetaquinol), 50 mg thiamine and 5 mg pyridoxine (Nervit, Vetaş) once a day, and 2 mg hydroxycobalamin (Dodeks, Vetaş) once a day IV were administered, and amino acid supplements (Duphalyte, Zoetis) were administered once a day at 5-10 mL/kg dose. Mixed immunoglobulins (Polyglob, Bioveta) as hyperimmune serum were administered in 2 mL every two days and in two doses SC. In addition to the treatment protocol, atenolol (Tensinor, AstraZaneca) at a dose of 0.5 mg/kg twice a day was administrated to the dog with ventricular premature complexes. Total of 25 dogs responded to treatment while five dogs did not.

Statistical analysis

One sample Kolmogorov-Smirnov test was used to determine whether the data were parametric or non-parametric. Because all data were parametric, they were evaluated by Student independent t-test as mean± ±standard deviation. Multivariate regression analysis of the mortality was made to determine independent predictors of mortality. The prognostic value of I-FABP, TFF-3, cTnI and CK-MB for 0th hour and TFF-3 and cTnI for 48th hour were evaluated using receiver operating characteristic (ROC) curve analysis to determine for the differentiation between survivors and non-survivors. Statistical significance was considered as $p<0.05$ (SPSS 21.00).

Results

Clinical examination findings

In dogs with parvoviral enteritis, anorexia, stagnation, depression, fever (20 cases > 39.5 °C), tachypnea (33±2.1/mins), tachycardia (106.26±14.46/mins), lethargy, vomiting, dehydration (>9%), elongation at capillary filling time (>3 sec), tachycardia, haemor-
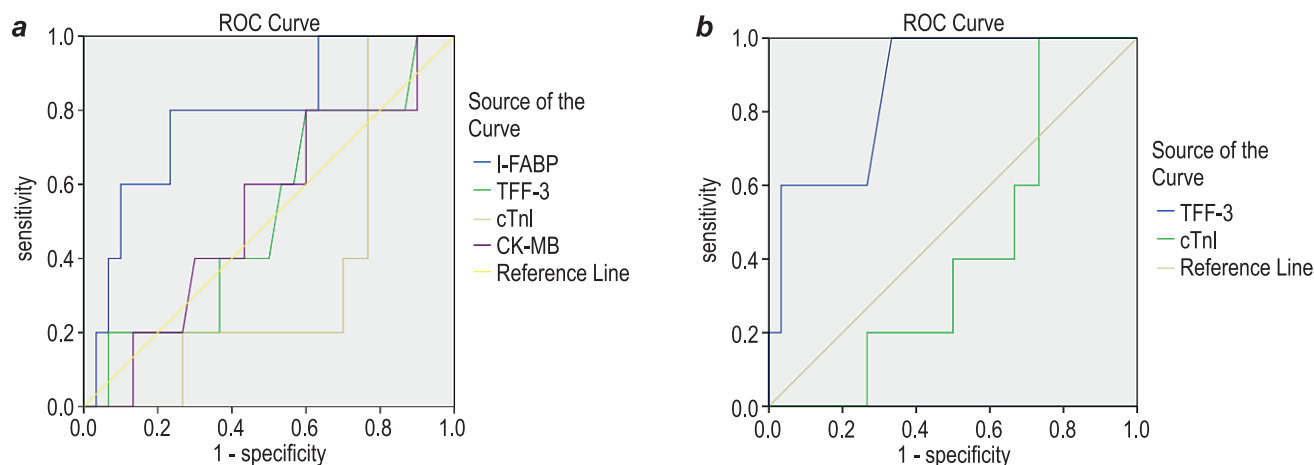
Fig. 2. (*a*) ROC curve of intestinal-related and cardiac-related biomarkers (I-FABP, TFF-3, cTnI and CK-MB) on the 0th hour of experimental group. (*b*). ROC curve of intestinal-related (TFF-3) and cardiac-related (cTnI) biomarkers on the 48th hour of experimental group.

rhagic diarrhea (25 cases), and shock (three cases) were observed. Twenty two of 30 dogs with parvoviral enteritis were unvaccinated, eight of them had been vaccinated previously once (five dogs) or twice (three dogs). As a result of the treatment, 25 dogs (83.3%) completely recovered and were discharged from the hospital. Five dogs (16.6%) did not respond to the treatment and died between 50th and 56th hour.

Cardiac examination findings

In the experimental group, during the auscultation according to the point of maximal intensity (PMI) there were no detectable heart murmurs. In control group heart auscultation findings were normal. All dogs of control group had normo-dynamic femoral pulse. Some of the dogs of experimental group had normo-dynamic (66.6%) and hypo-dynamic (33.3%) femoral pulse due to cardiovascular deterioration. Electrocardiographic examination revealed sinus tachycardia and ventricular premature complexes in one of the dogs which died in experimental group (Fig. 1).

Intestinal and cardiac biomarker findings

As shown in Table 1, serum I-FABP and CK-MB levels was found to be statistically significantly higher in dogs with parvoviral enteritis at 0th hour than in healthy dogs ($p<0.05$), whereas no statistically significant difference was found at 48th hour between the groups (Table 1). Serum TFF-3 and cTnI levels were significantly higher in dogs with parvoviral enteritis at 0th and 48th hour than in the healthy dogs ($p<0.05$) (Table 1). No significant difference was found in dogs with parvoviral enteritis at 0th and 48th hour of serum CLDN-3 levels compared to the healthy dogs (Table 1).

Multivariate regression analysis of mortality at 0th hour showed that I-FABP has mortality prediction importance. Receiver operating characteristic curve (ROC) analysis on 0th hour for the utility of I-FABP in differentiating in the experimental group between the survivor and non-survivor dogs estimate an area under the curve (AUC) of 0.787 ($p=0.043$, 95% CI=0.571-1.000) and sensitivity of 80% and specificity of 77% for prediction of mortality. ROC analysis on 48th hour for the utility of TFF-3 in differentiating in the experimental group between the survivor and non-survivor dogs estimate an AUC of 0.867 ($p=0.010$, 95% CI=0.720-1.000) and sensitivity of 80% and specificity of 70% for prediction of mortality. Other intestinal-related biomarker (CLDN-3) and none of the cardiac-related biomarkers (H-FABP, CK-MB and cTnI) were not high enough for prediction of mortality (Fig. 2a,b).

Haemogram and blood gas findings

As shown in Table 2, the WBC count was statistically significantly lower in dogs with parvoviral enteritis compared to the healthy dogs at 0th and 48th hour ($p<0.05$). It was determined that in dogs with parvoviral enteritis, the THR count was significantly higher than that of the healthy dogs at 0th and 48th hour ($p<0.05$) (Table 2). There was no statistically significant difference in RBC, Hct and Hb levels in dogs with parvoviral enteritis at 0th and 48th hour compared to the healthy dogs. 11 of 30 patients were with leukopenia (<4000 m/mm³) and 19 were found to have normal leukocytes counts (12000-17000 m/mm³). Leukocyte counts of four dogs that died were significantly low (<760 m/mm³). As it can be seen in Table 3, the levels of pH, pO_2, sO_2, Na, and HCO_3 in dogs with parvoviral enteritis at 0th hour were found to be significantly lower than in healthy dogs ($p<0.05$) and the levels of pCO_2, K, Ca, Cl and lactate were not statistically different

Table 2. Mean and significance of haemogram of dogs with parvoviral enteritis and healthy dogs (Mean±standard error).

Parameters	Hour	Control group(n:10)	Experimental group (n:30)	p-value
WBC (m/mm³)	0*	12.4±0.50	7.93±1.20	**0.001**
	48*	12.8±0.67	8.62±1.06	**0.002**
RBC (m/mm³)	0	7.33±0.18	7.36±0.35	0.936
	48	7.04±0.20	6.75±0.19	0.309
MCV	0	62.62±0.74	60.23±0.90	0.163
	48	61.21±0.63	60.20±0.73	0.985
MCH	0	18.32±0.29	17.89±0.22	0.462
	48	18.61±0.26	18.52±0.32	0.118
MCHC	0*	29.30±0.16	29.77±0.23	**0.001**
	48*	30.49±0.30	30.86±0.44	**0.034**
Hct (%)	0	45.8±1.34	44.1±2.39	0.541
	48	42.3±1.28	40.7±1.24	0.378
Hb (g/dL)	0	13.4±0.43	13.6±0.62	0.845
	48	12.6±0.37	12.3±0.37	0.646
THR (m/mm³)	0*	242±16.5	344±23.1	**0.001**
	48*	251±27.3	344±24.0	**0.018**

WBC=Leukocyte, RBC=Erythrocyte, MCV=Mean corpuscular volume, MCH=Mean cellular haemoglobin, MCHC=Mean haemoglobin concentration, Hct=Hematocrit, Hb=Haemoglobin, THR=Platelet, * $p<0.05$ (statistically significant).

(Table 3). All dogs with parvoviral enteritis had mild metabolic acidosis.

Discussion

In the present study biomarkers specific for the detection of intestinal damage were used, for the first time, in parvoviral enteritis of dogs. Biomarkers measured to determine intestinal damage were I-FABP, TFF-3, and CLDN-3. Many of the markers used are derived from enterocytes. I-FABP is synthesized only by enterocytes while L-FABP is released from hepatocytes as well as enterocytes. In an earlier study, it was found that intestinal I-FABPs produced in stomach, small and large intestines are biomarkers that can be used to determine acute ischemic bowel injury (Gollin et al. 1993). Abdel-Haie et al. (2017) reported that serial measurements of serum I-FABP levels may be a useful marker for predicting severity and early diagnosis of NEC. Yildiz et al. (2018) found that serum I-FABP and L-FABP levels were significantly higher in calves with atresia coli than that of the unaffected calves. In this present study, serum I-FABP level was significantly higher in dogs with parvoviral enteritis at 0th hour compared to the healthy dogs (p<0.05), but no statistically significant difference was determined at 48th hour (Table 1). Also ROC analysis on 0th

hour for the utility of I-FABP in differentiating in the experimental group between the survivor and non-survivor dogs revealed sensitivity of 80% and specificity of 77% for prediction of mortality. As many investigators (Gollin et al. 1993, Thuijls et al. 2010, Ng et al. 2013) reported that I-FABP and L-FABP levels increased significantly in mucosal damage of intestinal barrier proteins in NEC. This finding was consistent with significant increase in the level of I-FABP in dogs with parvoviral enteritis. As Abdel-Haie et al. (2017) reported in humans and Yildiz et al. (2018) reported in calves, in this study we determined that I-FABP is a useful and reliable biomarker for determining the intestinal damage and prediction of mortality in dogs with parvoviral enteritis.

Some intestinal biomarkers are released to repair the damage in the intestines. The function of TFF-3 is to provide stabilization and stimulation of mucus in the restriction of normal epithelial cells during the repair of tissue damage and the healing of gastrointestinal damage (Emami et al. 2004). It has been reported that TFF-3 concentration increases along with L-FABP, I-FABP in patients with NEC after inflammatory response caused by intestinal mucosal damage (Ng et al. 2013, Srivastava et al. 2015). Yildiz et al. (2018) found that serum TFF-3 level was significantly higher in calves with atresia coli compared to healthy calves. In this study, serum TFF-3 levels were found

Table 3. Mean and significance of haemogram of dogs with parvoviral enteritis and healthy dogs (Mean±standard error).

Parameters	Hour	Control group (n:10)	Experimental group (n:30)	p-value
pH	0*	7.39±0.00	7.31±0.01	**0.000**
	48	7.40±0.00	7.39±0.00	0.149
pCO_2 (mmHg)	0	40.7±1.57	40.8±1.61	0.979
	48	42.1±1.59	39.4±0.98	0.163
pO_2 (mmHg)	0*	42.5±1.60	36.2±1.50	**0.008**
	48	36.7±2.49	36.3±1.59	0.895
sO_2 (%)	0*	69.8±3.75	51.5±3.40	**0.001**
	48	59.8±5.87	54.9±3.35	0.485
K (mmol/L)	0	4.19±0.18	3.88±0.16	0.238
	48	3.8±0.157	3.55±0.10	0.210
Na (mmol/L)	0*	154±1.09	150.1±1.43	**0.015**
	48	150±1.98	148.5±1.24	0.471
Ca (mmol/L)	0	0.98±0.04	0.95±0.04	0.707
	48	1.02±0.05	4.90±3.93	0.332
HCO_3 (mmol/L)	0*	23.7±0.29	19.6±0.55	**0.000**
	48	23.5±0.89	23.1±0.37	0.719
Cl (mmol/L)	0	108±2.67	105±1.27	0.413
	48	105±2.13	103±1.31	0.407
Lactate (mmol/L)	0	1.33±0.17	1.65±0.09	0.113
	48	1.51±0.14	1.58±0.08	0.668

pH=Hydrogen ion concentration, pCO_2=Venous blood partial carbon dioxide pressure, pO_2=Venous blood partial oxygen pressure, sO_2=Venous blood oxygen saturation, K=Potassium, Na=Sodium, Ca=Calcium, Cl=Chlorine, HCO_3=Bicarbonate, * $p<0.05$ (statistically significant).

to be statistically significantly higher in dogs with parvoviral enteritis than the healthy dogs at 0th and 48th hour ($p<0.05$) (Table 1). Also ROC analysis on 48th hour for the utility of TFF-3 in differentiating in the experimental group between the survivor and non-survivor dogs revealed sensitivity of 80% and specificity of 70% for prediction of mortality. As many investigators (Emami et al. 2004, Ng et al. 2013, Srivastava et al. 2015) reported that TFF-3 levels increased significantly in enterocolitis cases, in this study significant increase in intestinal TFF-3 level caused by parvoviral infection was detected. The possible cause of this increase can be attributed to the reparation of the intestinal damage caused by parvovirus (Emami et al. 2004).

When the CLDN-3 levels decrease, the intestinal barrier function becomes impaired (Lu et al. 2013). CLDN-3 levels have been reported to be significantly reduced in humans with inflammatory bowel disease (Ivanov et al. 2004, Zeissig et al. 2007). On the other hand, Mennigen et al. (2009) found that CLDN-3 levels decreased in people with acute colitis. In this study, serum CLDN-3 level at 0th and 48th hour in the dogs

with parvoviral enteritis was not found to be statistically different compared to the healthy dogs (Table 1). The possible reason for the decrease in CLDN-3 level in the dogs with parvoviral enteritis may be related to the early stage of the disease.

CK-MB is used to assess the cardiac function in myocardial damage, such as ischemia or myocardial necrosis in both animals and humans (Baisan et al. 2016). Another biomarker used in the diagnosis of heart failure is cTnI. Cardiac troponins provide reliable detection of cardiac damage and even small increases are important to remark myocardial damage (Welsh et al. 2002, Ok et al. 2008, Er and Ok 2015). Serum CK-MB levels begin to increase within four hours following the myocardial damage, reach the highest level at 12th hour and return to normal levels within 24-72 hours (Smithline et al. 2003). Yilmaz and Senturk (2007) found that CK-MB levels increased significantly in dogs with parvoviral enteritis. On the other hand, Burgener et al. (2006) found that in dogs with acute myocardial damage, the cTnI level increased significantly within 24 to 48 hours, and started to return to normal level after 48th hour. In this study, serum

CK-MB levels were significantly higher in the dogs with parvoviral enteritis at 0th hour compared to the healthy dogs, but no statistically significant difference was determined at 48th hour (Table 1). Serum cTnI levels of 0th and 48th hour in the dogs with parvoviral enteritis was found to be statistically significantly higher than the healthy dogs (p<0.05) (Table 1). Increased levels of CK-MB and cTnI in the dogs with parvoviral enteritis could indicate that these dogs may have developed mild or moderate myocarditis along with haemorrhagic enteritis. Er and Ok (2015) found that dogs with parvoviral enteritis developed mild myocardial damage, with a significant increase in CK-MB and BNP levels which showed particularly cardiac damage and a slight increase in cTnI. In this study, the significant increase in cTnI level was consistent with the results of the researchers mentioned above (Welsh et al. 2002, Burgener et al. 2006, Ok et al. 2008, Er and Ok 2015).

H-FABP is used in the early diagnosis of acute myocardial infarction because of its ability to remark sarcolemmal changes during acute myocardial ischemia. Early elevation of H-FABP in blood and urine after acute myocardial infarction suggests that it may be used in the early diagnosis of the acute coronary syndrome (ACS) (Boscheri et al. 2010, Gerede et al. 2013). H-FABP has been reported to be a reliable prognostic marker for predicting mortality from acute heart injury (Dellas et al. 2010). Serum H-FABP levels have been reported to elevate significantly in patients with advanced heart failure and hypertrophic and dilated cardiomyopathy (Komamura et al. 2006, Arimoto et al. 2007, Renaud and Ngako 2007). In addition, H-FABP is accepted as a biomarker that can be used to determine the mortality rate related to cardiovascular diseases in people (Otaki et al. 2014). Increased blood levels have been reported to be in a good correlation with the magnitude of myocardial infarction (Glatz et al. 1994). In this present study, serum H-FABP levels in the dogs with parvoviral enteritis at 0th and 48th hour compared to the healthy dogs were not found to be significantly different (Table 1). The lack of an increase in H-FABP levels may be related to the absence of serious cardiac damage. Komamura et al. (2006) and Arimoto et al. (2007) report that an elevation in H-FABP levels was observed in severe cardiac injuries support our results.

ROC analysis revealed that cardiac-related biomarkers (H-FABP, CK-MB and cTnI) are not high enough for prediction of mortality. This finding may reflect that the deaths in the experimental group were caused by severe dehydration/hypovolemia due to haemorrhagic diarrhea and vomiting, bacterial translocation with subsequent coliform septicemia and endotoxemia, systemic inflammatory response syndrome (SIRS), hypercoagulability or multiorgan dysfunction (Mylonakis et al. 2016) and not due to cardiac damage.

Conclusions

A significant increase in biomarker levels was observed in dogs with parvoviral enteritis showing intestinal (I-FABP, TFF-3) and cardiac (CK-MB and cTnI) damage. It was determined that I-FABP and TFF-3 in the detection of the intestinal injury and prediction of mortality, and CK-MB and cTnI in the detection of the heart injury were useful and reliable biomarkers.

Acknowledgements

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