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Research article
Ερευνητικό άρθρο

Paraoxonase activity assessment in dogs suffering from *Parvovirus* infection

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ABSTRACT: Canine *Parvovirus* enteritis is one of the most common causes of diarrhea and death in dogs younger than 6 months of age. Clinical gastroenteritis in parvo-positive dogs is associated with increased levels of lipid peroxides and alteration in antioxidant enzymes. Paraoxonase/arylesterase 1 is considered as an antioxidant enzyme and acute phase protein in laboratory animals and human. The present study evaluated paraoxonase activity in 27 dogs suffering from *Parvovirus* infection and compared with 9 normal dogs. Blood samples were taken from all of the dogs and were sent to the laboratory for complete cell blood count and also biochemical factors assessment (paraoxonase/arylesterase 1 activity, liver, kidney and metabolic profiles). Infected dogs significantly showed decrease in paraoxonase/arylesterase 1 enzyme activity, an increase number of neutrophils and lymphopenia compared with parvo-negative dogs. Significant decrease in the enzyme activity was also observed in dogs with neutropenia or leukocytosis when compared to control group. It seems that in dogs paraoxonase/arylesterase 1 enzyme activity is decreased as a part of acute phase response in *Parvovirus* infection.

***Keywords*:** Paraoxonase; *Parvovirus* enteritis; acute phase proteins

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INTRODUCTION

Canine *Parvovirus* infection (CPV) is one of the major causes of morbidity and mortality in puppies and young dogs for more than three decades (Appel et al., 1979; Decaro and Buonavoglia, 2012; de Oliveira et al., 2019). High mitotic rate tissues such as thymic cortex and germinal epithelium of the intestinal crypts are the excellent targets for *Parvovirus* to lead lymphocytolysis, lymphopaenia, and collapse in the absorptive capacity of enterocytes (Greene, 2012). Clinicopathological biomarkers in this disease such as haematology or serum biochemistry and endocrine changes guide clinicians making right decision and help predicting outcomes (Goddard et al., 2008; Dossin et al., 2011; McClure et al., 2013). Recently, new renal biomarkers such as urinary immunoglobulin G (uIgG), C-reactive protein (uCRP), neutrophil gelatinase associated lipocalin (uNGAL) and urinary protein/ creatinine ratio (UPC) have been detected in parvo infected dogs suffering from acute kidney injury (Van den Berg et al., 2018). **S**erum paraoxonase or (PON1) (aryldialkylphosphatase, EC 3.1.8.1) is an antioxidant enzyme belonging to hydrolase family which is synthesized by the liver and causes degradation of lipid oxidation products and lactones such as homocysteine thiolactone (Rosenblat et al., 2006; Weijun et al., 2008; Altenhöfer et al., 2010; Ciftci et al., 2015). Alteration in level of PON1 had been approved in various diseases involving inflammation and oxidative stress such as cardiovascular, renal and liver diseases or obesity (Camps et al., 2007; Ferretti et al., 2010; Zhao et al., 2012; Zhang et al., 2015; Dalal et al., 2018). For these properties, this enzyme attracted scientists' attention in human and veterinary medicine as a novel biomarker of oxidative stress or acute phase response (Rossi et al., 2013; 2014[a,b]; Tvarijonaviciute et al., 2015; Moya and Manez, 2018). The current study was conducted to evaluate the PON1 activity in dogs suffering from *Parvovirus* infection.

MATERIALS AND METHODS

Animals

This retrospective study performed on 27 *Parvovirus* infected dogs (test group) and 9 healthy dogs (control group) that were admitted to the Hospital of Veterinary School of Shahid Bahonar University of Kerman in Iran for treatment and routine check-ups respectively. Animals were client-owned dogs aged between 2- to14-month-old from both genders and mixed breed.

Animal ethics

The study was conducted in accordance to the guidelines for use of animals in research and approved by the State Committee on Animal Ethics, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Blood samples

Blood samples were taken from cephalic vein (2.5 ml) and were divided into two parts. 0.5 ml of the blood sample was anticoagulated with EDTA for complete cell blood count (CBC) analysis and 1.5 ml of the blood sample was centrifuged at 750 g for 15 min in order to separate sera for measuring PON1 activity, liver, kidney and metabolic profiles.

Complete blood count parameters

Immediately after blood collection, samples with EDTA were used to prepare blood smear for measuring white blood cell (WBC), red blood cell (RBC) count and hematocrit.

Serum biochemical analysis

The sera were analyzed for total protein (Biuret method), albumin (Bromocresol green), alkaline phosphatase enzyme activity (ALP), blood urea nitrogen or BUN (Urease method), creatinine (Jaffe method), cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG) using commercial kits (Pars Azmoon Co., Tehran, Iran). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using Reitman and Frankel colorimetric method (Reitman and Frankel, 1957). Analysis procedure was carried out using automated analyzer (Ames, Rome, Italy). PON1 enzymatic activity was assessed using commercial kit (ZellBio GmbH, Germany) according to manufacturer instruction briefly by measuring in vitro hydrolysis of paraoxon to ρ-nitrophenol. Product absorbance measurement (412 nm) was carried out using microplate spectrophotometer (Epoc, BioTek Instruments Inc., USA). Based on the results of the study by Rossi and her colleagues, paraoxon- based methods were accurate and precise for evaluating the esterase activity of PON1 in canine serum (Rossi et al., 2013).

Statistical Analysis

Statistical analysis was performed using the SPSS software® (Version 17 for Windows; SPSS Inc., Chicago, IL, USA). Data was checked for normal dis-

tribution using the Kolmogorov-Smirnov test. Comparison of mean values within groups was performed using Independent Samples T-Test. *P* value less than 0.05 was considered statistically significant. Potential correlation between PON1 and other parameters was determined using the Pearson Correlation test.

RESULTS

In this study, blood samples selected from healthy dogs and dogs suffering from *Parvovirus* infection. A complete evaluation of the history, physical examination findings, hematology and biochemical analysis were performed on all animals. *Parvovirus* infection was admitted by both clinical examination and a point-of-care test (ICT test, Quicking Biothech Co., Ltd, China). In order to prevent misdiagnosing *Parvovirus* infection, we excluded dogs that had been vaccinated two weeks earlier. Anorexia, vomiting and diarrhea were reported in the history of the dogs in the test group. Healthy dogs (n=9) were presented to clinic for routine check-ups. They did not present any clinical evidence of disease and absence of CPV was confirmed by a point-of-care test. Blood samples were taken within 2 hours of admission before any treatment. Data were presented as means ± standard errors (X ± SE) in Tables (1, 2) and Figures (1-5). As shown in Table 1, significant differences were observed in lymphocyte, segmented and band neutrophil between healthy and infected dogs. There was significant difference in PON1 activity between healthy and parvo-positive dogs (Fig 1); however, there was no significant difference in PON1 activity between infected dogs without neutropenia in comparison with neutropenic dogs (Fig 2). In Fig 3, lower PON1 activity also was seen in neutropenic dogs in comparison with healthy ones (*P=0.03*). There was no significant difference in PON1 activity between infected dogs without cytosis in comparison with dogs which had cytosis; in contrast, there was significant decrease in PON1 activity in dogs that had cytosis compared with healthy dogs (*P=0.02*) (Fig 4, 5). According to the results in Table 2, significant differences were observed in creatinine, albumin, total protein concentrations and ALT activity between two groups. There were no significant differences between triacylglycerol (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and cholesterol between groups.

Table 1. The Mean ± SE of blood components in healthy and infected dogs

Parameters	Healthy dogs	Infected dogs
Hematocrit	40.62±9.11	61.38±98.57
RBC	6.03±1.34	6.63±1.37
WBC	10.00±1.96	13.15±14.39
Segmented neutrophil	4.78±1.47	10.37±13.50*
Band neutrophil	0.09±1.13	0.63±0.77*
Lymphocyte	4.83±1.28	1.88±1.71*
Monocyte	0.05±0.13	0.12±0.23
Eosinophil	0.18±0.11	0.09±0.19

*Asterisk showed significant differences between groups (*P<0.05*).

Table 2. The concentration of biochemical factors (Mean ± SE) in healthy and infected dogs

Parameters	Healthy dogs	Infected dogs
AST (U/ml)	40.33±21.27	72.44±86.61
ALT (U/ml)	29.33±13.72	82.70±110.26*
ALP (U/ml)	436.67±320.28	492.37±466.46
BUN (mmol/L)	21.84±17.50	28.31±18.02
Creatinine (µmol/L)	102.54±19.44	79.56±32.70*
TG (mmol/L)	0.90±0.26	1.02±0.40
Cholesterol (mmol/L)	5.91±0.37	3.61±1.56
HDL(mmol/L)	2.85±0.70	3.12±0.92
LDL (mmol/L)	1.55±0.84	2.20±1.45
Albumin (g/L)	36.2±3.7	30.1±6.9*
Total protein (g/L)	720±63.6	711±168.8*

*Asterisk showed significant differences between groups (*P<0.05*).

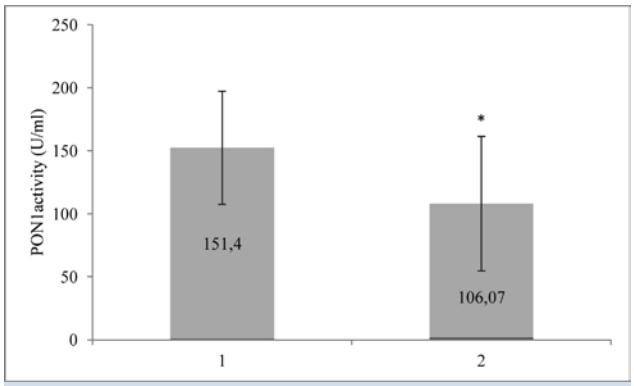

Fig 1. The activity of PON1 (U/ml) in healthy (1) and infected dogs (2)

*Asterisk showed significant differences between groups (*P<0.05*).

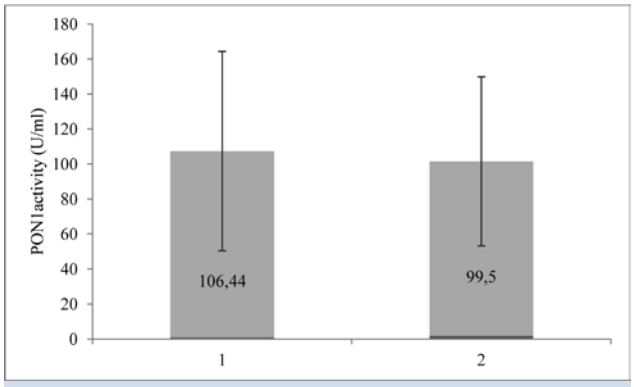

Fig 2. The activity of PON1 (U/ml) in dogs without neutropenia (1) in comparison with infected neutropenic dogs (2)

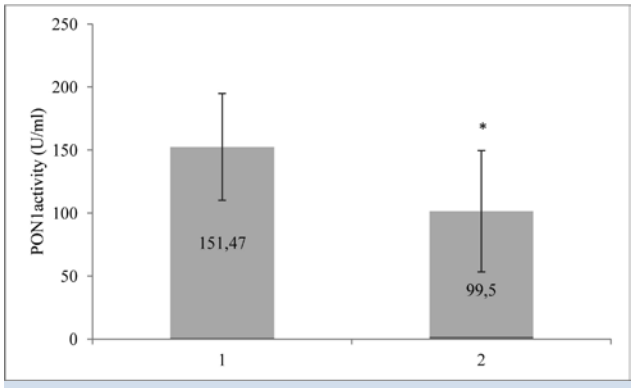

Fig 3. The activity of PON1 (U/ml) in healthy dogs (1) in comparison with neutropenic infected dogs (2)

*Asterisk showed significant differences between groups (*P=0.03*).

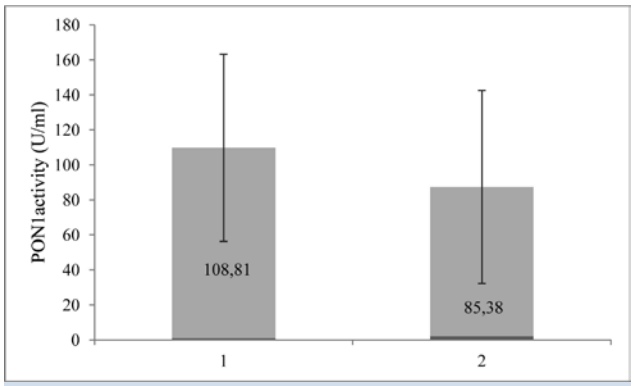

Fig 4. The activity of PON1 (U/ml) in infected dogs without cytosis (1) in comparison with infected dogs with cytosis (2)

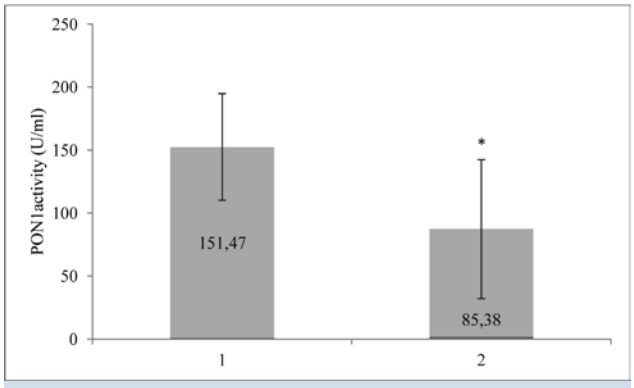

Fig 5. The activity of PON1 (U/ml) in healthy dogs (1) in comparison with infected dogs with cytosis (2)

*Asterisk showed significant differences between groups (*P=0.02*).

DISCUSSION

Findings of this study demonstrated that all of the *Parvovirus* infected dogs had lower PON1 activity compared with healthy ones. It seemed that PON1 enzyme was deactivated in the course of the *Parvovirus* infection as an acute phase response. Canine *Parvovirus* enteritis is an acute gastrointestinal illness that is seen mostly in puppies and young dogs. Since it's emerging in 1979, numerous scientific efforts were established in order to find reliable clinicopathological biomarkers for this contagious disease (Schoeman et al., 2013; Hoang et al., 2019). Some researches revealed that inflammation state and alteration in profile of serum acute-phase proteins occurred in the time of the canine *Parvovirus* infection; therefore, the anti-inflammatory body's reaction would be as reflex of disease severity (Kocaturk et al., 2010, McClure et al., 2013). PON1 is tightly associated with apolipoprotein A1 in HDL and exerts its protective function through hydrolyzing oxidized lipids (Aviram et al., 2000). During an acute phase response, HDL molecules lose

its associated enzymes, including PON1, which is replaced by serum amyloid A and ceruloplasmin. This phenomenon results in reduced antioxidative properties of HDL (Cabana et al., 2003; Novak et al., 2010). Alteration in serum PON1 had been correlated with inflammation and oxidative damage in many clinical conditions (Rosenblat et al., 2006; Zhao et al., 2012; Tecles et al., 2015; Arenas et al., 2018). The main goal of this experiment was attempting to find whether or not level of PON1 activity would have been changed in dogs that were infected with the *Parvovirus* enteritis. As noted in the results, the significant decrease in PON1 activity was observed in dogs suffering from *Parvovirus* enteritis when it was compared with healthy dogs (Fig 1). This phenomenon probably exhibited that low PON1 activity was linked to a part of acute phase response in the course of the *Parvovirus* infection. This was approved by Rossi and her coworkers (2014[a]) who revealed that PON1 activity was decreased in dogs that were affected by leishmaniasis. After medication of leishmaniasis, normalized PON1 activity was seen earlier than other inflammatory biomarkers in cases with systemic inflammation (Rossi et al., 2014[a]). These researchers concluded that low value of PON1 activity might indicate sever inflammation during leishmaniasis (Rossi et al., 2014[a]). Other investigation also suggested the relationship between reduction in serum PON1 activity and sternness of infection in dogs with acute pancreatitis (Tvarijonaviciute et al., 2015). Sensitivity of PON1 activity to inflammatory conditions and oxidative stress was approved in other small animals by Tecles and his colleagues in 2015. These researchers established that in cats with infectious peritonitis (FIP) not only PON1 level was considerably decreased but total antioxidant capacity was diminished (Tecles et al., 2015). Findings in Table 1 showed that, both segmented and band neutrophil were increased (probably because of secondary infections) and lymphocyte number was decreased noticeably in infected dogs compared with parvo-negative cases. Lower PON1 activity was seen in neutropenic infected dogs in comparison with healthy group ($P=0.03$) (Fig 3). There was significant decrease in PON1 activity in infected dogs that had cytosis compared with healthy dogs ($P=0.02$) (Fig 5). This result proposed the relationship between low PON1 activity and severity of the CPV in dogs. PON1 is an antioxidant enzyme so antioxidant capacity of infected dogs is decreased with lowered PON1 activity. This is in agreement with an investigation which was done by Panda and coworkers in (2009) which revealed that clinical gastroenteritis in parvo-positive dogs was associated with increased levels of lipid peroxides and alteration in antioxidant enzymes in the erythrocytes (Panda et al., 2009). PON1 is a glycoprotein that associated with HDL in circulating system so it was expected that level of HDL would have been declined if PON1 activity was reduced. Surprisingly as shown in Table 2, there were no significant differences in HDL and other metabolic parameters like TG, LDL and cholesterol between groups. This was in conflict with previous study which had shown that serum total cholesterol and HDL levels were decreased and TG level was increased in dogs with *Parvovirus* enteritis (Yilmaz and Senturk, 2007). This study performed on a limited number of samples, however; a large population of dogs with naturally occurring *Parvovirus* infection could lead to a higher generalization of our results.

CONCLUSIONS

It was found that all of the infected dogs and also cases with neutropenia or cytosis had lower PON1 activity compared with parvo-negative cases; therefore, it seemed that PON1 activity level in serum is sensitive to *Parvovirus* infection. This investigation emphasized possible contribution of PON1 level as an antioxidant enzyme and negative acute phase reactant in canine *Parvovirus* infection.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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