Short Communication

Evaluation of changes in some enzymatic and non-enzymatic antioxidants in cats with feline panleukopenia

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Abstract

Feline panleukopenia is a serious contagious viral disease of cats that has been identified as the causative agent of death in 25% of kittens sent for pathological examination. Antioxidant systems are one of the very important defense systems of body. The aim of the current study was to evaluate changes in serum levels of Vitamin D, glutathione peroxidase and superoxide dismutase in cats infected with panleukopenia virus. Ten healthy male cats and ten male cats with clinical and laboratory symptoms of panleukopenia with a mean age of 6±2 months were selected. The Glutathione peroxidase and superoxide dismutase were measured by the ELISA method and vitamin D was assayed by chromatography in serum. There was no statistically significant difference between the level of superoxide dismutase in the healthy cats and those with feline panleukopenia virus infection (P=0.243) Significant decreases were observed in the levels of vitamin D (P=0.000) and glutathione peroxidase enzyme (P=0.009) in the infected cats in comparison to the healthy group. These findings demonstrate an association between oxidative stress and Feline panleukopenia disease. It is possible to prevent panleukopenia virus infection in cats and accelerate its treatment by strengthening the components of the host antioxidant system.

Keywords: Antioxidant, Vitamin D, Glutathione peroxidase, superoxide dismutase, Feline Panleukopenia

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Introduction

Feline panleukopenia is a serious contagious viral disease of cats capable of being prevented by vaccination (Kruse *et al*. 2010). Kittens are routinely vaccinated repeatedly during their first month of life and, thus, feline panleukopenia virus (FPV) is usually found in unvaccinated cats (Barrs 2019).

The causative agent of feline panleukopenia is a member of the Parvovirus genus of the Parvovirideae family (Di Serio *et al*. 2012). Feline panleukopenia virus is highly stable in the environment and is endemic in many cat populations throughout the world (Scott *et al*. 2007). Clinical disease is categorized from subclinical infection to a per-acute syndrome with sudden death. Typical early signs include fever, lethargy and anorexia (Addie *et al*.1996). Affected cats initially may show vomiting and, with lower frequency, develop watery to hemorrhagic diarrhea. Patients die from complications associated with secondary bacterial infection, sepsis, dehydration and disseminated intravascular coagulopathy (DIC) (Mantione and Otto 2005). In a retrospective study, FPV was identified as the causative agent of death in 25% of kittens sent for pathological examination (Cave *et al*. 2002). Hence, the control and treatment of feline panleukopenia is very important in veterinary science. It has been proven that feline parvovirus severely reduces white blood cells that are one of the very important elements of the immune system. Enzymatic and non - enzymatic antioxidants are another defense system of the body. The oxidative damage caused by free radicals is naturally neutralized by the antioxidant defense system. The components of this system include a series of enzymatic and non-enzymatic antioxidants (Halliwell 1997).

Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase are the most important antioxidant enzymes (Kohn,1996) and vitamins C and E are the most important constituents of the non-enzymatic antioxidant system.

An essential mechanism for the protection of host cells against excess levels of free radicals is the enzymatic antioxidant defense system, of which SOD is a key enzyme that appears to act as the first line defense against ROS (Dringen and Hamprecht 1997). Changes in plasma antioxidants and malondialdehyde can be the results of cellular damage caused by the activities of free radical molecules.

Free radicals are chemically active molecules, mostly ROS (superoxide and hydrogen peroxide), normally produced during biological processes in various organisms (Hussain *et al*. 2004). It is known that excessive amounts of free radicals, particularly hydrogen peroxide, in cells induces elevation of antioxidant enzymes activity to neutralize ROS and prevent internal cellular damage. Conversely, decreasing the severity of oxidative stress (and thus the free radical level) may result in the reduction of antioxidant enzyme activity (Hussain *et al*. 2004).

Therefore, the aims of this study were to investigate the changes of the serum levels of SOD, GPX and vitamin D in cats infected with feline panleukopenia virus.

Materials and Methods

This study was conducted on cats referred to the Pars Vet Hospital in Shiraz, Fars province in the south of Iran. All the procedures employed were approved by the Institutional Animal Care and Use Committee of Kazerun Islamic Azad University. At their arrival at the hospital, animals were examined by a veterinarian and received veterinary services; if necessary, they were hospitalized. All the routine procedures were employed by different specialists to diagnose and treat their diseases. The clinical panleukopenia virus infection was defined based on Kruse *et al*., (2010) by antigen ELISA of feces, polymerase chain reaction of feces or blood or a combination of these procedures in cats with fever, gastrointestinal symptoms and severe panleukopenia (Kruse *et al*. 2010).

After initial diagnosis with clinical symptoms and definitive diagnosis based on Laboratory reports, 10 Persian male cats with a mean age of 6±2 months (minimum and maximum ages were 3 and 8 months respectively) showing signs of panleukopenia virus infection, were selected. These signs included fever, lethargy, anorexia, vomiting and diarrhea with at least two positive PCR or ELISA tests of their blood and feces, also according to the clinical examinations and laboratory tests they did not show any disease other than feline panleukopenia virus infection. The feline panleukopenia virus infected cats had been showing symptoms for 4-7 days and blood samples were done on the second day of the disease. Also, 10 healthy age-matched Persian male cats were selected and used as the control group.

At the conclusion of the clinical examination and under sterile conditions, blood samples were collected from all the cats from the cephalic vein. Serum was isolated by centrifugation at 2500 RPM for 15 mins at 4°C and stored at -20°C to be analyzed later. SOD activity was assayed using a diagnostic RANSOD kit manufactured by RANDOX (Randox Laboratories Ltd., Crumlin, Country Antrim, UK) according to Arthur and Boyne (1985) and expressed in U of SOD /ml of serum (Arthur and Boyne 1985)

GPX activity was determined using a diagnostic RANSEL kit manufactured by RANDOX (Randox Laboratories Ltd., Crumlin, Country Antrim, UK) according to Paglia and Valentine, (1967) and expressed in U of GPX/ml of serum (Paglia and Valentine 1967).

Vitamin D concentrations were measured by chromatography (ng/ml). The chromatography was carried out using a Shimadzu system (Columbia, MD) composed of two LC-10ADvp pumps, SIL 10ADvp autosampler, CTO-10ASvp column oven, RF-10Axl fluorescence detector and SCL-10ADvp controller. The data was acquired by CLASSvp software, v. 5.03. The separation was achieved on a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm i.d., 5 µm particle size) connected with a guard column RX-C8 (12.5 mm × 4.6 mm i.d., 5 µm particle size) (Agilent Technologies, Palo Alto, CA). A Shimadzu UV-160A spectrophotometer was used to determine the absorbance of standard solutions. In order to ensure the accuracy of the measured values, each parameter was tested twice in

each serum sample and the average of the two measured values was considered as the final value.
The results were statistically analyzed using SPSS statistical package (Version 10.0, SPSS Inc.) for Windows and Duncan and student's t' tests.

Results and Discussion

The findings are presented in Tables 1 and 2. As seen, there was no statistically significant difference between the mean serum level of superoxide dismutase in healthy cats and those with feline panleukopenia virus infection (P=0.243), while significant differences were observed in the mean levels of vitamin D (P=0.000) and glutathione peroxidase enzyme (P=0.009) in the infected cats compared to the control group (Table 2).

Animals are constantly bombarded by toxic exogenous free radicals (ROS) such as superoxide and peroxides; they are also generated in the body as biproducts of oxidative metabolism, like those of long-chain fatty acids in peroxisomes (Li *et al*. 2010). Various enzymatic and non-enzymatic antioxidant systems have been developed to scavenge ROS and prevent cellular damage (Nissen and Kreysel, 1983).

According to our findings, the level of superoxide dismutase in infected cats shows no significant differences in comparison to the control group (P=0.243; Table 2)

Table 1 The mean and standard deviation of superoxide dismutase (SOD), glutathione peroxidase (GPX) and vitamin D levels in healthy cats and those with Feline panleukopenia infection.

group	Animal no.	superoxide dismutase (U/ml)	glutathione peroxidase (U/ml)	vitamin D (ng/ml)
healthy	10	1.43. ±0.07	63.87±7.75	64.66±3.73
infected	10	1.68±0.20	39.65±11.67	39.51±4.36

Table 2 Statistical analysis of mean levels of superoxide dismutase (SOD), glutathione peroxidase (GPX) and vitamin D levels in healthy cats and those with Feline panleukopenia infection showing presence or absence of significant differences.

Parameter	P-value
superoxide dismutase	0.243
glutathione peroxidase	0.009
vitamin D	0.000

Crnogaj *et al*. (2017) cited SOD, GPX and TAC as biomarkers for detecting the severity of Babesiosis in dogs (Crnogaj *et al*. 2017). Cats with feline infection peritonitis (FIP) showed lower paraoxonase-1 (PON1) values and a significant decrease in TAC concentrations compared with healthy cats; these findings demonstrated the existence of oxidative stress in cats with FIP (Crnogaj *et al*. 2017). In the first line of the enzymatic antioxidant system, superoxide dismutase and Glutathione peroxidase prevent cell damage by neutralizing free radicals (Kohn *et al*. 1996).

In the current study the lack of a significant reduction in SOD in cats with panleukopenia infection indicates that the host defense system increases the production of SOD to such an extent that (despite consuming it) its level still remains high. In this way, the host tries to fight against pathogens as much as possible by keeping its immune system at a normal level so that it does not lose its resistance to other infections.

Our results demonstrate that Glutathione peroxidase and vitamin D levels significantly decline in cats with panleukopenia virus infection (P =0.000; Table 2). Similarly, patients with multiple myeloma have lower levels of vitamins A, C, E, SOD, GPX and catalase than healthy individuals (Teclesa *et al*. 2015). Fengel *et al*. (2015), found that mastitis induces the release of free radicals and reduces the serum levels of antioxidants (Fengle and Li, 2015).

Decreased levels of vitamin D and GPX in cats with panleukopenia virus (Table 2) indicate that the infection increases the production of free radicals to consume the enzymatic and non-enzymatic components of the antioxidant system, thereby weakening the host defense mechanisms. Indeed, studies have shown that vitamins protect cell membranes against oxidative agents like free radicals and play a major role in maintaining the function of endocytes (Baker *et al*. 2001). Jewell *et al*., (2002) investigated the effects of vitamins in the prevention of disease and reported the positive effect of vitamin E on the prevention and treatment of skin diseases as well as limiting their spread (Jewell *et al*. 2002). Of course, our findings are related to the early stages of the virus infection and the later stages are unclear. It is possible that if the infection is left untreated and the disease becomes more severe, the level of SOD will also decline significantly similar to GPX and vitamin D. In this case, it is possible to prevent this disease and speed up its treatment by prescribing vitamins and strengthening the immune system.

The inhibition of intracellular free radical formation would provide a therapeutic strategy to prevent oxidative stress. Antioxidants may act at different levels, inhibiting the formation of ROS or scavenge free radicals or increase the antioxidants defense enzyme capabilities (Bajaja and khan,2012).

In conclusion, this paper shows two antioxidant biomarkers GPX and vitamin D decrease in cats with FPV compared with healthy animals, while the level of SOD remains unchanged in this disease. Furthermore, whether the observed level of SOD at the onset of the infection actually continues in the later stages of the disease requires further investigations. This indicates an association between oxidative stress and Feline panleukopenia disease. Further research should be

performed to explore the possible practical applications that monitoring the oxidative stress could have in this disease, as well the possibility of antioxidants, especially by prescribing vitamins D and E, having preventative or therapeutic potential.

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