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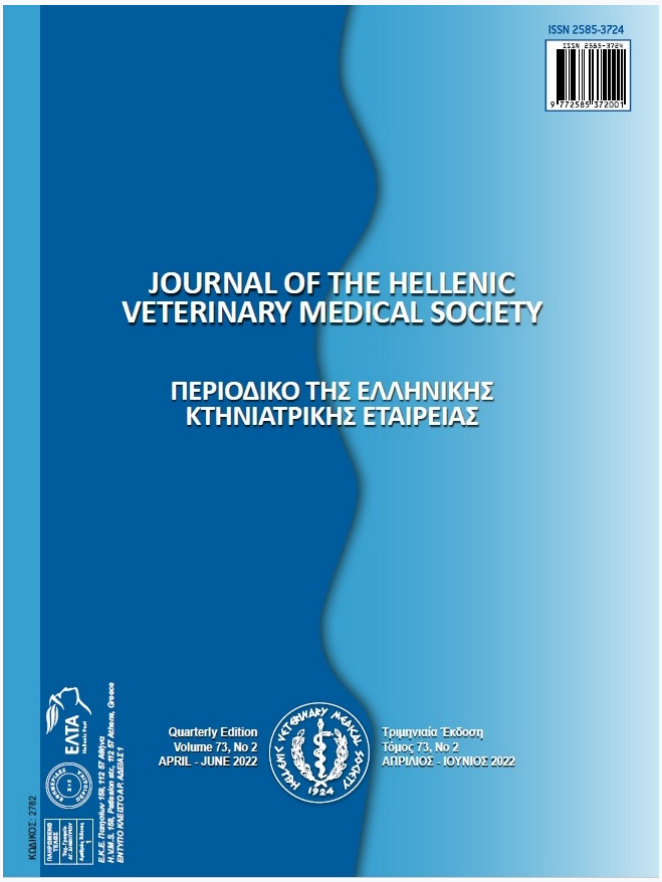

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Research article
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The role of oxidative biomarkers in feline panleukopenia

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ABSTRACT: Feline Panleukopenia is an important deadly gastrointestinal disease of cats. It is most prevalent in kittens with declining maternal antibodies as well as unvaccinated cats. Vitamin D and E, glutathione peroxidase and superoxide dismutase are essential enzymatic and non-enzymatic components of defensive system involve in defending the body against oxidative radicals. Malondialdehyde is a reliable commonly used marker of overall lipid peroxidation level showing the presence of oxidative stress. Measurement of the total serum antioxidant capacity seems to represent a suitable biochemical parameter for evaluating the overall antioxidant status resulting from antioxidant intake or production, and their consumption by the increasing levels of oxidative stress.

The purpose of this study was to investigate the changes in serum levels of Vitamins D and E, glutathione peroxidase, superoxide dismutase, total antioxidant capacity and malondialdehyde in cats infected with panleukopenia virus.

Ten Persian male cats with clinical and laboratory symptoms of panleukopenia on the second day of the disease, (n=10) and ten healthy Persian male cats (n=10) with a mean age of 6±2 months (minimum and maximum age were 3 and 8 months respectively) were selected. Superoxide dismutase, total antioxidant, malondialdehyde and glutathione peroxidase levels were assayed by ELISA, and vitamin E and D were measured by chromatography methods.

There were no statistically significant differences between the mean serum levels of vitamin E, superoxide dismutase and total antioxidant in healthy cats and those with feline pan leukopenia infection ($P>0.05$), while a statistically significant decrease in glutathione peroxidase and vitamin D levels, and an increase in malondialdehyde level were observed ($P<0.05$).

A condition of oxidative stress appears in feline panleukopenia, possibly associated with the virus infection. This oxidative stress may play a role in the prevention/treatment of feline panleukopenia.

Keywords: Antioxidant, Feline panleukopenia, Malondialdehyde, Vitamin.

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INTRODUCTION

Feline panleukopenia (FPL) is a serious contagious viral disease of cats capable of being prevented by vaccination [1]. Kittens are routinely vaccinated repeatedly during their first month of live, and thus, feline panleukopenia virus (FPV) is usually found in unvaccinated cats [2,3]. FPV is a member of the Parvovirus genus of the Parvovirideae family [4]. This virus is highly stable in the environment, and is endemic in many cat populations throughout the world [5].

The severity of clinical signs of feline panleukopenia is related to age, immune status and concurrent infections [6]. Clinical Feline panleukopenia ranges from subclinical infections to a per-acute syndrome with sudden death. Typical early signs include fever, lethargy and anorexia [7]. Affected cats may initially show vomiting and, with lower frequency, develop watery to hemorrhagic diarrhoea. Patients die from complications associated with secondary bacterial infection, sepsis, dehydration, and disseminated intravascular coagulopathy (DIC) [8].

As an integral part of metabolism and part of a controlled inflammatory reaction, exposure to environmental factors induces a constant generation of a wide range of substances, known as Reactive Oxygen Species (ROS), consisting of free radicals such as O2, OH andother non-radical oxygen derivatives such as hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and singlet Oxygen (1O2) [9].

ROS circulate freely in the body with access to all organs and tissues. They cause tissue damage by a variety of different mechanisms including DNA damage, lipid peroxidation (through activation of cyclooxygenases and lipoxygenases), protein damage, oxidation of important enzymes, e. g. anti-proteases such as α 1-antitrypsin, and stimulation of pro-inflammatory cytokine release by monocytes and macrophages [10]. Since ROS production and reduced enzyme activity cause oxidative stress [11], so it's not surprising that all oxygen-consuming organisms have developed complex antioxidant systems to deal with ROS and reduce their damage [12].

Enzymatic and non - enzymatic antioxidants are integral parts of the defense systems, naturally neutralizing the oxidative damage caused by free radicals [13-15]. Vitamins D, C and E are the most important constituents of non-enzymatic antioxidant system. Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase are the most important antioxidant enzymes [16, 17,18]. A trace amounts of antioxidant enzymes are found in serum [12,14, 17].

Malondialdehyde (MDA) is a reliable and commonly used marker of overall lipid peroxidation levels and the presence of oxidative stress [19].The level of malondialdehyde (MDA) is served as a reliable biomarker of lipid peroxidation (LPO) and usually served as a marked of LPO [20].The changes in plasma antioxidants and malondialdehyde can be the results of cellular damage caused by the activity of free radical molecules. The unstable free radical compounds induced by infections can react with cellular lipids, proteins, nucleic acids and carbohydrates, among which lipids are the most sensitive. Membrane structures are sensitive to ROS, and its generation during oxidative stress induces lipid peroxidation [21] leading to irreversible damages to the cell membrane [22].

The pathological increase of ROS generation has already been recognized in over one hundred human and animal diseases including cancer, cardiovascular disease, diabetes mellitus, male infertility, renal disease and dialysis, cataracts, neurological, liver, periodontal, lung and inflammatory diseases [9, 10,23-26].

It has been shown that Feline parvovirus severely reduces white blood cells, which are essential in immune system [2].Also, in the retrospective studies, FPV has been identified as the causative agent of death in %25 of kittens sent for pathological examination [27,28]. Hence, its control and treatment is very important in veterinary science. The aims of this study are to investigate the changes in the serum levels of total antioxidant capacity (TAC), SOD, GPX, MDA, vitamin E and D in cats infected with feline panleukopenia virus.

MATERIALS AND METHODS

This study was conducted on cats referring to the Veterinary Hospital. All the procedures employed were approved by the Institutional Animal Care. 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 specialist to diagnose and treat their diseases. The clinical panleukopenia virus infection was defined based on Kruse et al, (2010), including ELISA of faeces, polymerase chain reaction of faeces or blood samples [29], or some combination of these procedures in cats with

fever and gastrointestinal symptoms [30].

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 age were 3 and 8 months respectively) showing signs of panleukopenia virus infection, were selected. These signs included fever, lethargy, anorexia, vomiting and diarrhoea with at least two positive PCR or ELISA tests of their blood and faeces. The infected cats showed symptoms for 4-7 days and blood samples were collected on the second day from the symptoms onset. 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 condition, Blood samples were collected from all the cats from the cephalic vein. Serum was isolated by centrifugation at 2500 RPM for 15 min at 4°C, and stored at -20°C to be analyzed later.

Total antioxidant capacity was assayed using diagnostic kit made by RANDOX (Randox Laboratories Ltd., Crumlin, Country Antrim, UK). The assay is based on the reduction of free radicals (ABTS•+ - 2,2'-azinobis (3-ehylbenzothiazoline-6-sulfonate) measured as a decrease in absorbance at 600 nm at 3 min by antioxidants. The ABTS•+ radical cation is formed by the interaction of ABTS with ferryl myoglobin radical species, generated by the activation of met myoglobin with hydrogen peroxide. The suppression of the absorbance of the ABTS•+ radical cation by serum antioxidants was compared with that from a Trolox (6-hydroxy-2, 5, 7, -tetramethylchroman-2-carboxylic acid), which is included as part of the TAC kit. The results are expressed as nmol/ml of Trolox equivalents. In addition, control serum (Randox, Crumlin, UK) with TAC value of 1.0 to 1.36 nmol/ml was assayed in each batch of samples for the estimation of analytical imprecision (between-batch coefficient of variation).

SOD activity was assayed using 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 [31].

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 [32].

MDA was measured by a commercially-available assay based on the method of Esterbauer and Cheeseman, (1990) [33] and the results were expressed as nmol/ml. In brief, preformed malondialdehyde was reacted with N-methyl-2-phenyl-indole and methane sulfonic acid.

Vitamin E and 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.

The results were statistically analyzed using SPSS statistical package (Version 10.0, SPSS Inc). Duncan and student's tests were used to find statistical significant differences between healthy and infected groups. Pearson's correlation coefficient was evaluated to determine statistical significant relationships among different parameters. Statistical significance was accepted at $P< 0.05$.

RESULTS

Findings of this study are presented in Tables 1 to 3. Although the mean levels of vitamin E (P=0.702) and TAC (P=0. 53) declined, and SOD (P=0.243) increased, As seen, there were no statistically significant differences between the mean serum levels of these parameters in healthy cats and those with feline pan leukopenia infection. There are significant decreases in the mean levels of vitamin D (P=0.000), GPX (P=0.009) and WBC (P=0.000), and a significant increase in the mean level of MDA (P=0.028) in the infected cats compared to the control group (Table 1). There were no significant correlations between the mean number of WBC and other parameters in healthy and infected groups (Tables 2 and 3).

Table 1. Superoxide dismutase (SOD), glutathione peroxidase (GPX),total antioxidant capacity (TAC), malondialdehyde (MDA), white blood cells, vitamin E and vitamin D variables in which a difference between healthy and infected groups

Parameter	Group	Valid Number	Missing Number	Mean	Median	Mode	Std. Deviation	Minimum	Maximum	P-value
SOD (U/ml)	Healthy	10	0	1.43	1.45	1.27[a]	.21	1.05	1.66	0.243
	Infected	10	0	1.68	1.95	.60[a]	.62	.60	2.39	
TAC (nmol/ml)	Healthy	10	0	479.00	460.35	208.60[a]	161.67	208.60	704.60	0.53
	Infected	10	0	351.35	384.35	201.60[a]	108.20	201.60	492.10	
Vitamin D (ngr/ml)	Healthy	10	0	64.66	67.03	38.99[a]	11.79	38.99	77.82	0.000
	Infected	10	0	39.51	36.38	19.23[a]	13.80	19.23	67.85	
MDA (nmol/ml)	Healthy	10	0	8.41	8.30	8.57	1.08	6.99	10.67	0.028
	Infected	10	0	10.67	10.93	8.57	2.65	6.99	14.36	
GPX (U/ml)	Healthy	10	0	63.87	65.28	31.57[a]	24.50	31.57	99.91	0.009
	Infected	10	0	39.65	21.57	17.13	36.92	6.76	114.72	
vitamin.E (ngr/ml)	Healthy	10	0	261.83	265.87	152.63[a]	74.48	152.63	372.06	0.702
	Infected	10	0	246.17	234.66	96.36[a]	103.21	96.36	422.57	
WBC (1000/ µl)	Healthy	10	0	6.588	6.663	5.500[a]	0.822	5.500	7.648	0.000
	Infected	10	0	1.860	1.713	1.525[a]	0.353	1.525	2.603	

a. Multiple modes exist. The smallest value is shown

Table 2 Investigation the correlation between the mean number of white Blood Cells and other parameters in healthy cats

Group			SOD	TAC	Vitamin D	MDA	GPX	Vitamin E	WBC
Healthy	SOD	Pearson Correlation	1	.554	.090	-.086	-.249	-.168	.249
		Sig. (2-tailed)		.096	.804	.814	.489	.642	.489
		N	10	10	10	10	10	10	10
	TAC	Pearson Correlation	.554	1	-.249	-.207	-.287	-.043	-.126
		Sig. (2-tailed)	.096		.487	.567	.421	.907	.728
		N	10	10	10	10	10	10	10
	Vitamin D	Pearson Correlation	.090	-.249	1	.407	.519	-.201	.280
		Sig. (2-tailed)	.804	.487		.243	.124	.579	.433
		N	10	10	10	10	10	10	10
	MDA	Pearson Correlation	-.086	-.207	.407	1	.116	-.349	.072
		Sig. (2-tailed)	.814	.567	.243		.749	.324	.843
		N	10	10	10	10	10	10	10
	GPX	Pearson Correlation	-.249	-.287	.519	.116	1	-.144	-.115
		Sig. (2-tailed)	.489	.421	.124	.749		.692	.751
		N	10	10	10	10	10	10	10
	vitamin.E	Pearson Correlation	-.168	-.043	-.201	-.349	-.144	1	.476
		Sig. (2-tailed)	.642	.907	.579	.324	.692		.164
		N	10	10	10	10	10	10	10
	WBC	Pearson Correlation	.249	-.126	.280	.072	-.115	.476	1
		Sig. (2-tailed)	.489	.728	.433	.843	.751	.164	
		N	10	10	10	10	10	10	10

Table 3 Investigation of the correlation between the mean number of white Blood Cells and other parameters in infected cats

group			SOD	TAC	Vitamin D	MDA	GPX	Vitamin E	WBC
Infected	SOD	Pearson Correlation	1	.512	.543	-.116	.466	-.501	.349
		Sig. (2-tailed)		.130	.105	.750	.174	.140	.322
		N	10	10	10	10	10	10	10
	TAC	Pearson Correlation	.512	1	.383	-.434	.121	-.505	.286
		Sig. (2-tailed)	.130		.275	.210	.738	.137	.423
		N	10	10	10	10	10	10	10
	Vitamin D	Pearson Correlation	.543	.383	1	-.089	-.126	.042	.119
		Sig. (2-tailed)	.105	.275		.806	.729	.909	.743
		N	10	10	10	10	10	10	10
	MDA	Pearson Correlation	-.116	-.434	-.089	1	-.020	-.285	.215
		Sig. (2-tailed)	.750	.210	.806		.956	.424	.550
		N	10	10	10	10	10	10	10
	GPX	Pearson Correlation	.466	.121	-.126	-.020	1	-.379	.119
		Sig. (2-tailed)	.174	.738	.729	.956		.280	.743
		N	10	10	10	10	10	10	10
	Vitamin E	Pearson Correlation	-.501	-.505	.042	-.285	-.379	1	-.504
		Sig. (2-tailed)	.140	.137	.909	.424	.280		.137
		N	10	10	10	10	10	10	10
	WBC	Pearson Correlation	.349	.286	.119	.215	.119	-.504	1
		Sig. (2-tailed)	.322	.423	.743	.550	.743	.137	
		N	10	10	10	10	10	10	10

DISCUSSION

In the present study, we investigated enzymatic and non-enzymatic antioxidants as biomarkers for detecting the severity of feline panleukopenia**.** Animals are constantly bombarded by toxic exogenous free radicals (ROS) such as superoxide and peroxides; they are also generated in the body as by-products of oxidative metabolism, like those of long-chain fatty acids in peroxisomes [30]. Various enzymatic and non-enzymatic antioxidant systems are developed to scavenge ROS, and prevent cellular damage [34] .It is known that excessive number of free radicles, 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 radicle level) may result in the reduction of antioxidant enzyme activity [35].

The main enzymes involved in antioxidant defense and ROS detoxification are catalase, Glutathione peroxidase and superoxide dismutase [36]. SOD converts superoxide anions to H2O2, which is then further degraded to H2O by catalase or GPX [30].

Many diseases such as chronic renal failure [37], diabetes mellitus [38], feline infectious peritonitis [39] and immunodeficiency virus infection in cats [40] also atopic dermatitis , monocytic ehrlichiosis ,gastroenteritis and inflammatory bowel disease in dogs are directly related to oxidative stress [40-44].

Depending on the severity of ROS production and antioxidant defense system, the levels of serum antioxidants and oxidation products may change significantly during resistance of animals to different diseases.

In the present study, MDA level was significantly increased in cats with feline pan leukopenia, demonstrating the role of ROS in pathogenicity of FPL infection. Similarly, a significantly higher serum MDA levels were found in animals with feline coronavirus infection ($P < 0.001$) than in healthy animals [19], but Michałek et al. (2020) did not observe statistically significant differences in the concentration of malondialdehyde in cats with hypertrophic cardiomyopathy in comparison to healthy cats[45].

In another study the acute cases of gastroenteritis in dogs were associated with altered erythrocytic lipid peroxidation as evident by estimation of malonaldehyde (MDA) concentration [44]. Kapun et al. (2012) were found a significantly higher plasma MDA levels in dogs infected to atopic dermatitis in comparison to healthy dogs [41].

According to our findings, the level of vitamin D in infected cats shows a significant difference in comparison to the control group, but the decrease in the level of vitamin E in cats with this disease wasn't significant. These findings reveal that vitamin D is used more than vitamin E in neutralizing oxidative stress resulting from feline pan leukopenia infection.

Thus, prescribing vitamins, particularly vitamin D, may contribute to the prevention of cats from being infected by feline pan leukopenia virus; it may also be added to therapeutic protocols of the disease. Of course, more targeted studies have to be performed in order to confirm the validity of these first indications.

Barros et al. (1999) reported low plasma vitamin C level in cataractous dogs relative to healthy dogs. The decreased plasma level of vitamin C may indicate a decline in the antioxidant capacity of aqueous humour [46]. Some studies have demonstrated that treatment with antioxidants, particularly vitamin C, can reduce both oxidative stress and protein glycation [46]. Heliovarra et al. (1994) found low levels of vitamin E, β-carotene and selenium to be associated with increased risk of rheumatoid arthritis [47].

SOD is a key enzyme that appears to act as the first line defense against ROS [48]. In this study, GPX level was significantly reduced, but the decreased in the levels of TAC and increased in the levels of SOD was not significant. In this regards Keegan and Webb (2010) failed to find significant differences in SOD between cats with chronic kidney disease and normal cats ,but they reported that the higher GSH : GSSG ratio and lower antioxidant capacity in chronic renal failure cats is consistent with activation of antioxidant defense mechanisms[37]. Our results indicate that the enzymatic antioxidant production, specially SOD concentration, is increased in feline panleukopenia disease, and their induction is to such an extent that despite their consumption in ROS neutralization, there still is no significant differences between the TAC and SOD levels in infected cats relative to the healthy animals.

In contrast, serum TAC level was found to be significantly lower in cats with feline coronavirus compared to healthy cats ($P < 0.001$) [19] . Michałek et al. (2020) results indicate that the activities of superoxide dismutase and catalase are different in cats with hypertrophic cardiomyopathy in compared to healthy cats, however the activity of the latter was only lower in asymptomatic stage of the disease[45]. Crnogaj et al. (2017) cited SOD, GPX and TAC as biomarkers for detecting the severity of normal Babesiosis in dogs [26]. In line with this, cats with feline peritonitis infection (FPI) showed a significant decrease in paraoxonase-1 (PON1) and TAC concentrations, and demonstrated the presence of oxidative stress [39]. Comparison of our results and those of Teclesa et al. (2015) shows that the oxidative stress severity in FPL disease is less than FIP [39].

The Elsayed et al. (2020) study showed in dogs with canine parvovirus infection, MDA, and H_2O_2 elevation contributed to oxidative stress state and alteration in antioxidant biomarkers as SOD, GPX, catalase, and trace minerals as Zn, Cu, and iron to counteract the expected damage of cells were reported. They concluded that antioxidant supplementation might bolster body defense mechanism and decreases stress condition[49].

The role of glutathione peroxidase is the elimination and detoxification of hydrogen peroxide and reactive lipids [50] protecting the cells against oxidative stress [51]. The results of this study demonstrate that GPX and vitamin D levels decline significantly in cats with panleukopenia infection. Interestingly, glutathione peroxidase concentrations were found to be significantly increased in the course of acute feline immunodeficiency virus infection [40].

Insignificant decrease activity of TAC and increased level of MDA in cats with pan leukopenia virus infection (Table 1) indicate that the disease increases the production of ROS to consume 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 endocytosis [52]. 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 in cats [53].

In this study sever leukopenia was observed that is expected in feline pan leukopenia disease. Even though the antioxidant system reaction is affected by leukocyte numbers, there were no significant correlations between the mean number of WBC and other parameters in healthy and infected groups (Tables 2 and 3).

Candellone et al. (2019) results showed that the dietary supplementation of antioxidants in hyperthyroid cats receiving methimazole (MMI) exerts a protective effect against oxidative stress, likely contributing to the reduction of MMI-related side effects [51].

The inhibition of intracellular free radical formation can 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. Despite the obvious merit potential of using antioxidants along with current therapies, the safety and efficacy of antioxidant supplementation in any future treatment remains to be established.

Conclusion

This paper reports a significant decrease in GPX and vitamin D levels, and a significant increase in MDA concentration in cats with FPV infection compared with healthy animals, while the levels of vitamin E, SOD and TAC remained unchanged. It seems that a condition of oxidative stress appears in FPL disease possibly associated with FPV activity that is responded well by the body's antioxidant system. The oxidative status might play a role in prevention/treatment of feline pan leukopenia infection.

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Conflict of interest

All the authors declare that this study was conducted in the absence of any commercial or financial supports that could be considered as a potential conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analysis or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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