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Evaluation of the relationship between periodontal diseases and oxidative stress parameters in cats

Evaluación de la relación entre las enfermedades periodontales y los parámetros de estrés oxidativo en gatos

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ABSTRACT

Periodontal disease, which is defined as inflammation of the tissues and supporting structures surrounding the teeth, can be observed in cats starting from the age of 2. Periodontal diseases start with Gingivitis, which is the early stage of periodontal disease. Gingivitis can arise from inflammation of the gums due to plaque, a white or yellowish biofilm of bacteria on the tooth surface, and the toxins produced by these bacteria. It can also result from inflammation of the periodontal tissues, including dental calculus or other periodontal tissues. A total of 242 cats were brought to the clinic for surgical diseases and 14 cats (5.78%) were found to have periodontal diseases. Although there have been several studies on oxidative stress, there are very few publications investigating the relationship between oxidative stress and periodontal diseases in cats. The aim of this study is to measure serum MDA, IMA and GSH concentrations and SOD and CAT activities in cats with periodontal diseases and to evaluate the relationship between oxidant and antioxidant status, which are indicators of oxidative stress. In the study, blood samples taken from cats with periodontal disease were centrifuged and serum was removed. MDA and IMA levels of cats with periodontal disease were found to be significantly higher than in cats that recovered after treatment (P<0.001). SOD, CAT and GSH levels were determined to be significantly lower cats with periodontal disease than recovered cats (P<0.001). In conclusion, this study reveals that there is a relationship between periodontal diseases and oxidant/antioxidant balance in cats and it shows that the oxidative stress develop due to the increase of free radicals.

Key words: Cat; dental calculus; Gingivitis; oxidative stress

RESUMEN

La enfermedad periodontal, que se define como la inflamación de los tejidos y estructuras de soporte que rodean los dientes, se puede observar en gatos a partir de los 2 años. Las enfermedades Periodontales comienzan con la Gingivitis, que es la etapa inicial de la enfermedad Periodontal. La Gingivitis puede surgir de la inflamación de las encías debido a la placa, una biopelícula de bacterias blanca o amarillenta en la superficie del diente y las toxinas producidas por estas bacterias. También puede resultar de la inflamación de los tejidos periodontales, incluido el cálculo dental u otros tejidos Periodontales. Un total de 242 gatos fueron llevados a la clínica por enfermedades quirúrgicas y se encontró que 14 gatos (5,78 %) tenían enfermedades Periodontales. Aunque se han realizado varios estudios sobre el estrés oxidativo, existen muy pocas publicaciones que investiguen la relación entre el estrés oxidativo y las enfermedades Periodontales en gatos. El objetivo de este estudio es medir las concentraciones séricas de MDA, IMA y GSH y las actividades de SOD y CAT en gatos con enfermedades Periodontales y evaluar la relación entre el estado oxidante y antioxidante, que son indicadores de estrés oxidativo. En el estudio, se centrifugaron muestras de sangre tomadas de gatos con enfermedad periodontaly se eliminó el suero. Se encontró que los niveles de MDA e IMA de gatos con enfermedad periodonta fueron significativamente más altos que en los gatos que se recuperaron después del tratamiento (P<0,001). Se determinó que los niveles de SOD, CAT y GSH eran más bajos en gatos con enfermedad periodontalque en gatos recuperados (P<0,001). En conclusión, este estudio revela que existe una relación entre las enfermedades Periodontales y el equilibrio oxidante/antioxidante en gatos y muestra que el estrés oxidativo se desarrolla debido al aumento de radicales libres.

Palabras clave: Gato; cálculo dental; Gingivitis; estrés oxidativo

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INTRODUCTION

Periodontal disease, which is defined as inflammation of the tissues and supporting structures surrounding the teeth, can be observed in cats (*Felis catus*) starting from the age of 2 old year [1, 2, 3]. Periodontal disease is a patology of the oral cavity caused by the inflammatory response to bacterial plaques on the tooth surface [1, 2, 3].

Periodontal disease is reported to be the disease that most frequently causes health problems in cats, with a prevalence of 2.8–24% [4]. Clinical signs of periodontal disease in cats include anorexia, halitosis, salivation, difficulty swallowing, Gingivitis, dental calculus accumulation, mobile teeth, and tooth loss [5, 6].

Periodontal diseases start with Gingivitis, which is the early stage of periodontal disease. Gingivitis can arise from inflammation of the gums due to plaque, a white or yellowish biofilm of bacteria on the tooth surface, and the toxins produced by these bacteria. It can also result from inflammation of the periodontal tissues, including dental calculus or other periodontal tissues [7, 8]. Gingivitis and dental calculus can cause destruction of teeth and surrounding tissues, leading to serious periodontal diseases and tooth loss [9].

Active oxygen derivatives of free radicals are called oxidants [10]. In living organism, oxidants and antioxidants are in balance [10]. This balance is disturbed in favour of oxidants in conditions that prevent the uptake of antioxidants, such as malabsorption, inflammation, infection, stress and environmental factors, and leads to cellular damage in the organism, this situation is defined as oxidative stress [11].

Various defence mechanisms have been developed in the organism to prevent damage to cellular structures by free radicals, more specifically (Reactive Oxygen Species) ROS, These mechanisms are called antioxidant defence systems [10, 11].

This study aimed to determine the oxidative stress status of dental calculus and Gingivitis, which cause periodontal diseases in cats.

MATERIAL AND METHODS

Animal material

Between July 2023 and January 2024, a total of 242 cats with various complaints, different ages, races, and genders were brought to the Surgical Clinic of the Animal Hospital at Cumhuriyet University Faculty of Veterinary Medicine in Sivas. The study material consisted of a total of 14 cats of different weights (3–6 kg, PNR, Istanbul, Turkey), races and genders, aged between 2–5 years, with complaints of halitosis, salivation and anorexia, out of 242 cats. Information about the cat was recorded according to the clinical findings obtained by gathering disease occurrence details from the owners of the patients.

Each case was examined in terms of body temperature, pulse and respiration. In accordance with the anamnesis obtained from the patient owner, the oral cavity was examined clinically for the presence of halitosis, plaque, dental calculus and gingival lesions. Detailed examination of the oral cavity of the cats with halitosis, dental calculus and Gingivitis was performed after sedation following intramuscular injection of Xylazine hydrochloride (1.1–2.2 mg·kg^{-1}, Alfazyne, Ege Vet, Turkey) and Ketamine (0.2–0.5 mg·kg^{-1}, Alfamine, Ege Vet, Turkey). The cats under sedation with detected dental calculus underwent dental scaling using a cavitron device (Hasvet VDU Portable Dental Unit, Shanghai Greeloy Industry Co., Ltd., Shanghai, China) to remove the dental calculus (FIGS. 1 and 2). Each case was classified in terms of detailed calculus and Gingivitis (TABLE I). Gingivitis cases were treated with oral 0.2% chlorhexidine gluconate spray (Kloroben 30 mL, Drogsan A.Ş, Ankara, Turkey) at 12 hour intervals for one week. In the post-treatment controls, it was determined that the Gingivitis cases were cured.

Collection of blood samples

Pre-post treatment, 2 mL blood samples were taken from the cats' antebrachial cephalic vein of cats and added to sterile tubes without anticoagulant. The samples taken were kept at room temperature for

FIGURE 1. Gingivitis in the upper premolars of case 4, appearance before and after scaling of dental calculus

FIGURE 2. Gingivitis in the upper premolars of case 9, appearance before and after scaling of dental calculus

30 min and then centrifuged at 3000 G·15 min^{-1} (Nüve NF 800, Nüve Laboratory & Sterilization Technology, Turkey), and the resulting sera were stored at -20°C until analysis (Haier, DW-86L828S, China).

Serum biochemical analysis

The levels of MDA, IMA, GSH, SOD and CAT from the serums obtained were measured using a biotek ELISA reader (Bio Tek µQuant MQX200 Elisa reader/USA) in accordance with the procedure.

Statistical analysis

Statistical analyses were performed using the 23.0 SPSS package programme (Statistical Package for Social Sciences, Chicago, IL). Shapiro Wilk-Smirnov was used to assess the study's numerical data's distribution. Since the data did not show a normal distribution, they were analyzed with the Wilcoxone test. For all comparisons, *P* values less than 0,05 were considered as significant (TABLE II).

RESULTS AND DISCUSSION

A total of 242 cats were brought to the clinic for surgical diseases and 14 cats (5.78%) were found to have periodontal diseases. When assessed based on races for periodontal disease, it was observed that the domestic cat breed had 8 cases (57.14%), the British race had 5 cases (35.71%), and the Scottish race had 1 case (7.14%). In terms of gender, 7 were males (50%) and 7 were females (50%). Regarding body weight, it was found that Periodontally affected cats weighed between 3.15 and 6 kg (TABLE I).

TABLE I
Distribution and treatment of cases according to race, age, weight and gender

Case	Race	Age (year)	Gender	Weight (kg)	Diagnosis	Treatment
1	Tekir	2	Female	3.15	Gingivitis	Kloroben spray for 7 days
2	Tekir	3	Male	4.00	Gingivitis	Kloroben spray for 14 days
3	British	2	Female	4.20	Calculus+ Gingivitis	Dental scaling + Kloroben spray 14 days
4	Tekir	4	Male	3.50	Calculus+ Gingivitis	Dental scaling + Kloroben spray 14 days
5	Tekir	2	Male	3.50	Gingivitis	Kloroben spray for 7 days
6	British	4	Female	4.00	Gingivitis	Kloroben spray for 14 days
7	British	2	Male	5.40	Gingivitis	Kloroben spray for 7 days
8	Tekir	3	Female	3.50	Calculus+ Gingivitis	Dental scaling + Kloroben spray 7 days
9	British	2	Male	3.50	Calculus+ Gingivitis	Dental scaling + Kloroben spray 7 days
10	Tekir	4	Female	5.20	Calculus+ Gingivitis	Dental scaling + Kloroben spray 14 days
11	Tekir	3	Female	4.30	Calculus+ Gingivitis	Dental scaling + Kloroben spray 7 days
12	British	5	Male	6.00	Calculus+ Gingivitis	Dental scaling + Kloroben spray 7 days
13	Scottish	4	Male	5.10	Calculus+ Gingivitis	Dental scaling + Kloroben spray 7 days
14	Tekir	4	Female	4.20	Calculus+ Gingivitis	Dental scaling + Kloroben spray 7 days

In only 5 cases, Gingivitis was identified, while in 9 cases, both dental calculus and Gingivitis were diagnosed together (FIGS. 1 and 2). Dental calculus was frequently detected in premolar and molar teeth in the lower and upper jaw. Lesions related to Gingivitis cases were identifiedas hyperaemic areas with smooth borders at the gum margin of the teeth where the dental calculus was located.

Cases diagnosed with dental calculus and Gingivitis were underwent cleaned for dental calculus under sedation. For the treatment of Gingivitis, 0.2% Chlorhexidine gluconate spray (Kloroben 30mL, Drogsan A.Ş, Ankara, Turkey) was recommended for at 12-hour intervals for one week. In cases where Gingivitis did not healing during the one-week follow-up, this duration was extended to two weeks, examinations at the end of this period revealed that all cases had recovered.

Serum MDA, IMA, CAT, GSH and SOD levels of periodontal disease cats diagnosed with dental calculus and Gingivitis before and after treatment are given in TABLE II.

TABLE II
Significance of Differences Between Groups that before and after treatment in cats

	Pre treatment Group (n:14; x̄ ± Sd)	Post treatment Group (n:14; x̄ ± Sx)	*P* value
MDA (mmol·mL^{-1})	23,09 ± 0,92^{b}	13,65 ± 0,29^{a}	***
IMA (ABSU)	1,51 ± 0,10^{b}	1,01 ± 0,077^{a}	***
GSH (mmol·mL^{-1})	1,046 ± 0,05^{b}	1,83 ± 0,039^{a}	***
SOD (U·mL^{-1})	30,73 ± 0,53^{b}	33,34 ± 0,33^{a}	***
CAT (kU·L^{-1})	60,01 ± 1,22^{b}	72,81 ± 0,49^{a}	***

^{a,b}:Different superscripts letters indicate statistically significant differences (***P< 0.001)

The biochemical analysis revealed that the serum levels of MDA and IMA in cats diagnosed with periodontal disease significantly increased before treatment compared to after treatment (P<0.001) (FIG. 3).

In the study, serum CAT, GSH, and SOD levels were found to be significantly lower in cats diagnosed with periodontal disease before treatment compared to those without the condition (P<0.001) (FIG. 3).

Periodontal disease in cats; It is reported to be the disease that most frequently causes health problems, with a prevalence rate of 2.8 to 24% from the age of 2 [4]. In the current study, periodontal disease was diagnosed in 14 of 242 cats in terms of dental calculus and Gingivitis. According to these data, the prevalence of periodontal diseases in cats was determined as 5.78%.

It has been reported that the incidence of periodontal diseases is directly proportional to the aging of the patient and is more common in premolar and molar teeth [12, 13]. In this study, calculus on premolars and molars and Gingivitis at the level of these teeth were found in all of the cases, which supports that periodontal diseases are more frequently in premolars and molars, and the fact that all of the cats except one case (no: 12) were between 2–4 years old supports that periodontal diseases will increase with age.

Among the cats showing periodontal disease according to races, Tekir (domestic) race (57.4%), British race (35.71%) and finally Scottish race (7.14%) were the most common. In the present study, the disease was very common in Tekir (Domestic) races cats (57.4%). It is thought

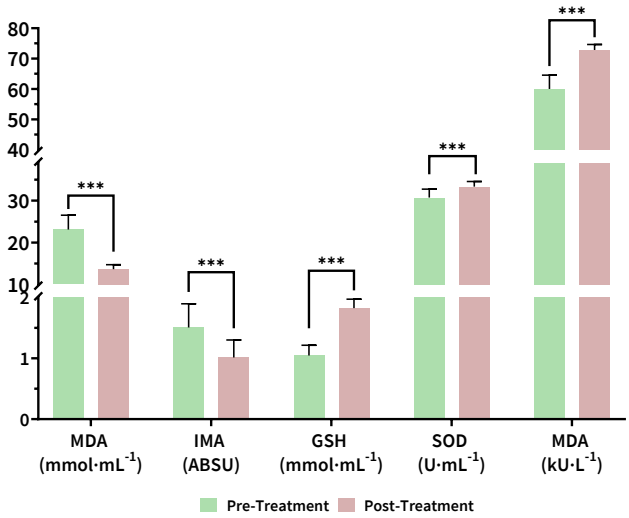

FIGURE 3. Data are presented as mean and standard deviation. Statistical evaluation of the data was performed with the Wilcoxon test and *P* values (* *P*<0.001) are depicted above the bars**

that this situation may be related to the fact that there are many Tekir (Domestic) cats in this Country and cats with different homeland such as British and Scottish cats are slowly being adopted in Turkey.

It has been reported that oxidative stress, resulting from the increase in excessive free radical production that causes cellular damage in the organism and the weakening of antioxidant defense as a result of exposure of living beings to various stress factors such as infection, inflammation, and unbalanced nutrition plays an important role in the pathogenesis of many diseases [13]. Although there are various studies on oxidative stress, there are very few publications investigating the relationship between oxidative stress and periodontal diseases of cats. Therefore, this study was conducted to measure serum MDA, IMA and GSH concentrations, SOD and CAT activities and to evaluate oxidative stress status in cats with periodontal diseases.

When oxidant substances are overproduced in the organism for any reason, they cause cell damage and cell death by accelerating the peroxidation of lipids, which are very important for the biological membrane [10, 11, 13].

MDA, which is an important biochemical marker in determining the degree of cellular damage in tissues, increases in the presence of various stress factors such as unbalanced nutrition, inflammation and infection [14, 15]. Wu *et al.* [16] stated that MDA level will increase due to oxidative stress in periodontal diseases. In the present study, serum MDA levels were significantly (P<0.001) increased in cats with periodontal disease compared to cats that recovered after treatment. This increase in MDA levels can be interpreted as an indicator of excessive free radical production that causes cellular damage in tissues.

Albumin is a plasma protein synthesized primarily in the liver. Many conditions such as ischemia cause a decrease in the metal ion binding capacity of albumin and metabolically different protein production [17, 18]. This change is known as ischemic modified albumin (IMA) [17]. IMA levels increase due to inflammation and reactive oxygen radicals (ROS) production and are shown as a marker of oxidative

stress [17, 18, 19]. Tayman *et al.* [20] reported that IMA levels increased in periodontal diseases and IMA levels decreased in healed cases.

In this study, IMA levels in cats with periodontal disease increased statistically significantly (P<0.001) compared to recovered cats. This increase in IMA levels can be interpreted as due to inflammation and the production of reactive oxygen radicals (ROS) that cause cellular damage in tissues.

Free radicals constantly produced in the cell are destroyed by antioxidant defense systems produced during normal metabolism in the body [21]. Antioxidants prevent or delay cell damage by scavenging free radicals in the cell [21]. Enzyme systems in cells are effective in the defense system against free radicals in living organisms. SOD (Superoxide dismutase) and CAT (Catalase) are important enzymatic antioxidants that prevent the accumulation of free radicals and the onset of lipid peroxidation in the organism [22].

The first defense process in the organism against free radicals occurs with the SOD enzyme and protects the organism from the harmful effects of oxidants [14]. Shang *et al.* [23] reported that the activity of various enzymatic antioxidants such as SOD will decrease in periodontal diseases. In this study, serum SOD levels were found to be decreased in cats with periodontal disease.

In this study, serum SOD levels were significantly (P<0.001) increased in cats recovered after treatment compared to cats with periodontal disease before treatment, and this increase in SOD levels can be interpreted as an increase in SOD levels to compensate for the harmful effects of excessive free radicals that cause cellular damage in tissues.

Catalase is an enzymatic antioxidant that plays an important role in the oxidative defense of tissues by catalyzing the reduction of hydrogen and lipid peroxides in living organisms [24]. Changes in CAT enzyme activity are considered as an indicator of oxidative stress [25, 26]. Almerich-Silla *et al.* [27] reported that the activity of enzymatic antioxidants such as CAT decreased in their study on periodontal disease. In this study, serum CAT levels were found to be decreased in cats with periodontal disease.

In this study, a statistically significant (P<0.001) increase in serum CAT levels was detected in cats that recovered after treatment compared to cats with periodontal disease. This increase in serum CAT levels is thought to be an excessive use of CAT activity as an antioxidant to reduce the effects of free radicals that increase due to inflammation in periodontal disease.

Glutathione (GSH) is the most important non-enzymatic soluble antioxidant. GSH is important in protecting the body against oxidative stress by reducing the toxicity of free radicals in the organism [10, 14, 28] and GSH levels decrease during oxidative stress [28]. Fentoglu *et al.* [29] found that GSH levels were lower in patients with Gingivitis and Periodontitis compared to healthy control group. In this study, serum GSH levels were significantly (P<0.001) increased in cats recovered after treatment compared to cats with periodontal disease before treatment. This increase in GSH levels can be interpreted as increased GSH consumption to protect against severe tissue destruction as a result of increased production of oxidants.

CONCLUSIONS

The results show that oxidative stress and lipid peroxidation develop in cats with periodontal disease due to increased free radicals. In addition, it was thought that MDA, IMA, SOD, CAT and GSH activities may be reliable parameters to determine oxidative stress in cats with periodontal disease.

As a result, it was concluded that oxidative damage occurs in cats with periodontal disease and as a result, additional antioxidant administration to sick cats may contribute to the improvement of the disease.

Ethical statement

This study was approved by the Sivas Cumhuriyet University Animal Experiments Local Ethics Board.

Conflict of interest

The authors declare that they have no conflict of interest. Statement of Animal Rights all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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