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CHANGES IN THE OXIDATIVE STATUS AND SERUM TRACE ELEMENT LEVELS IN DOGS WITH MAMMARY TUMOURS

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The purpose of the study was to evaluate the changes in oxidative status and serum trace element levels by determining the concentrations of malondialdehyde (MDA), nitric oxide (NO), ascorbic acid, and certain trace elements. The study included 15 clinically healthy female dogs and 15 dogs with malignant mammary tumour. Blood samples were taken from the jugular vein of the patient dogs just before the surgical treatment. The patients exhibited a significant increase in MDA ($P<0.001$), NO ($P<0.001$), Fe ($P<0.001$), and Cu ($P<0.01$) levels and a significant decrease in levels of ascorbic acid ($P<0.001$) and Zn ($P<0.01$). A strong correlation was observed in the patient group for ascorbic acid and Fe ($r=-0.87$) concentration, but there was a weak correlation between ascorbic acid and Cu ($r=-0.69$), and Zn ($r=0.49$) concentrations. Also there was a strong correlation between Zn and MDA ($r=-0.84$) concentrations, indicating oxidative stress. The results suggest that ROS generated by trace elements, like Fe and Cu may cause the mammary tumours.

Key words: dog, mammary tumour, oxidative stress, trace elements

INTRODUCTION

Mammary tumours are the most common tumours in female dogs (Kumaragrupan *et al*., 2005). All dog breeds can be affected, but certain breeds, such as pointers, Irish setters, Springer spaniels and Labrador retrievers, show a higher prevalence (Zatloukal *et al*., 2005). The etiology of mammary tumours in dogs is multifactorial. It may include the participation of sex hormones, and genetic and immunological factors, as well as oncogenic viruses and environmental carcinogens of a physical and chemical nature (Szczubial *et al*., 2004; Kumaragrupan *et al*., 2005).

The imbalance between oxidative and antioxidative reactions, which leads to an excess of reactive oxygen species (ROS) is known as oxidative stress (Szczubial *et al*., 2004). ROS can cause damage to membranes, mitochondria,

and macromolecules, including proteins, lipids, and DNA. Accumulation of DNA damage has been suggested to contribute to carcinogenesis (Yilmaz and Ozan, 2003; Szczubial *et al*., 2004). On the other hand, cells have the ability to defend themselves against ROS by way of their own antioxidant mechanisms, like enzymes and trace elements and vitamins (Huang *et al.*, 1999).

The usefulness of biochemical tumour markers in cancer patients is well established and there is a need for new sensitive and specific tumour markers for detecting malignant disease, staging prognosis and evaluating response to treatment. There is limited information about the exact role of oxidative stress and trace elements in canine mammary tumours. The aim of the present study was to evaluate the changes in oxidative status and serum trace element levels in canine mammary tumours by determining the concentrations of malondialdehyde (MDA), nitric oxide (NO), ascorbic acid, and certain trace elements, such as iron (Fe), copper (Cu) and zinc (Zn).

MATERIAL AND METHODS

Animals: The study included 15 female dogs of different breeds that had malignant mammary tumours (patient group). The animals, ranging in age from 7-12 years, were patients of the Reproduction Clinic of the Faculty of Veterinary Medicine, Ankara between 2003 and 2004. The control group consisted of 15 clinically healthy female dogs over 6 years of age. Patient animals were clinically examined and surgically treated.

Histopathological examination: For histopathological analysis, fresh tumour tissues were obtained after surgery, fixed in 10% formaldehyde. Histopathological examination and tumour classification was performed by the Pathology Department of the Ankara University Veterinary Faculty, according to World Health Organization (WHO) nomenclature (Owen, 1980).

Biochemical Analysis: For biochemical analysis, blood samples were collected from the jugular vein of female dogs into serum and lithium-heparin tubes. In the patient group, blood samples were taken from the patients just before surgery. Then, the blood samples were centrifugated at 2000 rpm for 5 min to obtain plasma and serum. Samples were analyzed for MDA, NO, ascorbic acid, and trace elements such as, Fe, Cu and Zn.

Plasma lipid peroxidation was determined by a previously determined method (Yoshoiko *et al*., 1979) in which MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex with a maximum absorbance at 535 nm. Plasma NO concentration was determined indirectly by measuring the nitrite levels, based on the Griess reaction (Cortas and Wakid, 1990). Plasma ascorbic acid was measured by the phosphotungstic acid method of Kway (1978).

The concentrations of Fe, Cu, and Zn in the serum samples were determined with atomic absorption spectrophotometry (Elmer, 1973).

Statistical analysis: Data for biochemical analyses are presented as mean ± SD. Statistical analysis was performed using Student's *t*-test; P values < 0.05 were

considered statistically significant. The correlation of the ascorbic acid concentration with MDA, NO, Fe, Cu, and Zn concentration was analyzed using Pearson's rank correlation coefficient. And the correlation of Zn concentration with MDA, NO, ascorbic acid, Fe, and Cu concentration was analyzed using Spearman's rank correlation coefficient.

RESULTS

According to the results of the histopathological analysis, histological type and clinical stage of malignant mammary tumours are given in Table 1. Among the 15 female dogs in the patient group, the most often diagnosed malignant tumour was malignant mixed tumor (n=10) and the most freqently seen clinical stage of malignant tumour was stage II (n= 9).

Table 1. Histological type and clinical stage of 15 dogs with malignant mammary tumours

Histological Type of Mammary Tumours	Number of Dogs
Malignant mixed tumour	10
Squamous cell carcinoma	2
Adenocarcinoma	3
Clinical Stage of Mammary Tumours	
Stage I $T_2N_0M_0$	6
Stage II $T_2N_0M_0$	9

[a]T, primary tumour. Size T_1 < 3 cm, T_2 3-5 cm, T_3 > 5 cm.
[b]N, regional lymph node metastasis. N_0 reginal lymph node not involved, N_1 regional lymph node unilateral involved, N_2 regional lymph nodes bilateral involved.
[c] M, distant metastasis, M_0 no evidence of distant metastasis, M_1 distant metastasis including distant nodes.

The results of oxidative stress and trace element parameters in the patient and control groups are presented in Table 2. We observed a significant increase in MDA ($P<0.001$), NO ($P<0.001$), Fe ($P<0.001$) and Cu ($P<0.01$) levels in the patient group. As seen in Table 2, the patient group had significantly lower levels of plasma ascorbic acid ($P<0.001$) and serum Zn ($P<0.01$) levels than the control group.

The correlations of ascorbic acid concentration with MDA, NO, Fe, Cu, and Zn concentrations were examined in the study. By using Pearson's rank correlation coefficient, a strong correlation was observed with Fe (r = -0.87, $P<0.001$) concentration, but there was a weak correlation with Cu (r = -0.69, $P<0.01$) and Zn (r = 0.49, $P<0.01$) concentrations. The negative correlations of ascorbic acid concentration with Fe, and Cu is shown in Figure 1.

Table 2. The results of oxidative stress and trace element parameters in dogs with malignant mammary tumours and healthy controls

Parameters	Control group (n=15)	Dogs with Malignant Mammary Tumour (n=15)
Malondialdehyde (μmol/L)	19.5±2.0[a]	31.7±3.8[b]
Nitric oxide (μmol/L)	18.8±2.3[a]	27.9±3.1[b]
Ascorbic Acid (mg/dL)	0.48±0.02[a]	0.35±0.03[b]
Iron (ppm)	209.3±11.5[a]	303.5±29.6[b]
Copper (ppm)	100.3±31.1[a]	203.4±53.8[b]
Zinc (ppm)	230.4±41.7[a]	131.7±23.2[b]

[a] All values are expressed as mean ± SD.
[a, b] differences are statistically significant in groups marked with different letters in the same row ($P<0.05$).

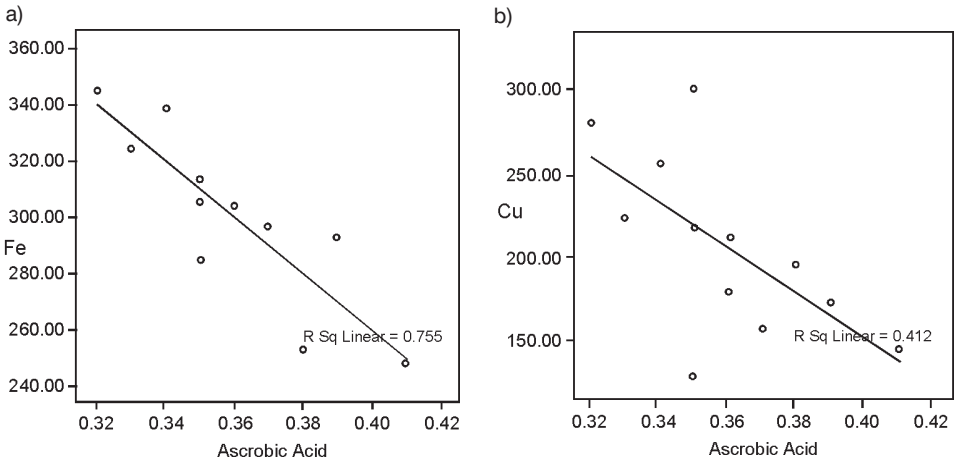

Figure 1. a) The correlation of ascorbic acid and Fe concentration in dogs with mammary tumour. Pearson's rank correlation coefficient was $r = -0.87$, $P<0.001$.
b) The correlation of ascorbic acid and Cu concentration in dogs with mammary tumour. Pearson's rank correlation coefficient was $r = -0.69$, $P<0.01$

On the other hand, we analyzed the correlations between the Zn and MDA, NO, Fe, and Cu concentrations. Spearman's rank correlation coefficient for Zn and MDA concentrations was $r = -0.84$, $P<0.001$, indicating a strong correlation (Figure 2).

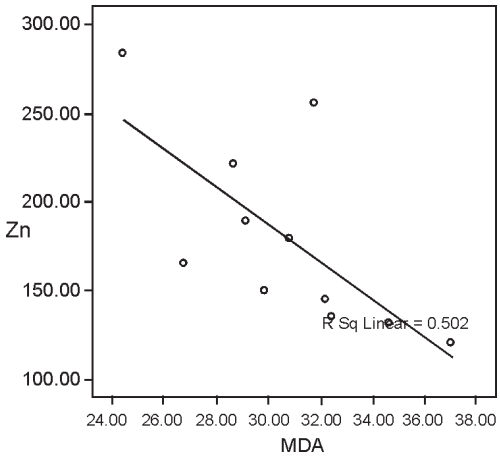

Figure 2. The correlation of Zn and MDA concentration in dogs with mammary tumour. Spearman's rank correlation coefficient for Zn and MDA concentrations was $r = -0.84$, $P<0.001$

DISCUSSION

In the present study we analyzed the MDA, NO, ascorbic acid, Fe, Cu, and Zn levels in female dogs with malignant mammary tumours. Lipids are the most susceptible to the toxic action of ROS (Gupta *et al*., 2004). Peroxidation of lipids induced by ROS generates such products as MDA. Increased lipid peroxidation and MDA-DNA adducts have been detected in human breast cancer (Huang *et al*., 1999) and canine mammary tumours (Kumaragurupan *et al*., 2005). In the present study, the observed higher plasma MDA levels in the patient group suggested that ROS were produced in the tumour tissue.

NO is a gaseous mediator that plays a role in many pathologic conditions. Furthermore, it is well known that NOS activity increases in some invasive tumours (Alagöl *et al*., 1999). NO has been shown to render cells resistant to oxidative stress (Kiral *et al*., 2005). It can be suggested that increased formation of NO in female dogs with malignant mammary tumours strengthens the host defence mechanism against tumor growth.

The increased lipid peroxidation observed in the present study was accompanied by a significant decrease in ascorbic acid levels. Ascorbic acid is a powerful antioxidant that reacts rapidly with a variety of oxidants and represents the first line of antioxidant defense (Bildik *et al*., 2004). Although Kumaragurupan *et al*. (2005) found high levels of tissue ascorbic acid in malignant mammary tumours, decreased antioxidant levels in the target cells and tissues has been suggested to play an important role in carcinogenesis (Huang *et al.*, 1999). In the present study, the observed lower plasma ascorbic acid levels in the patient group, may be a result of the host defense mechanism against the increased

oxidation caused by tumour growth. It is possible that ascorbic acid in plasma is first used against oxidants.

There is limited data available concerning the serum levels of Fe, Cu, and Zn in canine mammary tumours. A correlation between tumour induction and the changes in trace elements has been described in various cancers (Huang *et al*., 1999; Geraki and Farquharson, 2001). Fe is known to promote the formation of hydroxyl radicals through Fenton's reaction (Kiral *et al*., 2005). Also the interaction of Cu with H_2O_2 generates more reactive species, such as hydroxyl radicals (Huang *et al*., 1999). Yücel *et al*. (1994) showed that the mean serum Cu level was higher and the mean Zn level lower in patients with breast cancer, and our results are similar to those of previous studies. In the present study there was a significant increase in Cu and Fe concentrations, and a significant decrease in Zn concentrations in the patient group, indicating an increase in the oxidative status. Observed negative correlations with ascorbic acid and Fe (r = -0.87), and Cu (r = -0.69) concentrations (Figure 1), and the Zn and MDA (r = -0.84) concentrations (Figure 2) in the patient group, supporting the increase in the oxidative status.

In conclusion, the results of the present study provide evidence of enhanced oxidative stress in dogs with mammary tumours, and it can be suggested that Cu and Fe play a role in the generation of ROS and the enhancement of lipid peroxidation in dogs with mammary tumours.

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PROMENE U OKSIDATIVNOM STATUSU I KONCENTRACIJI MIKROELEMENATA KOD KUJA SA TUMOROM MLEČNE ŽLEZDE

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SADRŽAJ

Cilj ovih ispitivanja je bio da se utvrde promene u oksidativnom statusu određivanjem koncentracije malonildialdehida (MDA), azot oksida (NO) i askorbinske kiseline kao i koncentracije pojedinih mikroelemenata u krvi kuja obolelih od tumora mlečne žlezde. U ispitivanja je bilo uključeno 15 klinički zdravih kuja i 15 kuja sa malignim tumorom mlečne žlezde. Uzorci krvi su od bolesnih kuja uzimani neposredno pre izvođenja operativnog zahvata. U grupi obolelih kuja, zapažen je značajan porast koncentracije MDA ($P<0,001$), NO ($P<0,001$), Fe ($P<0,001$) i Cu ($P<0,01$) kao i značajan pad koncentracija askorbinske kiseline ($P<0,001$) i Zn ($P<0,01$). Dokazana je visoka korelacija u grupi obolelih između koncentracija askorbinske kiseline i Fe ($r=-0,87$) a slaba korelacija između koncentracija askorbinke kiseline i Cu ($r=-0,69$) i Zn ($r=0,49$). Takođe je dokazana i visoka korelacija između koncentracija MDA i Zn ($r=-0.84$) što je jedan od indikatora oksidativnog stresa. Ovi rezultati ukazuju da ROS (reactive oxygen species) nastali usled prisustva viška mikroelemenata mogu da imaju uticaj na nastanak tumora mlečne žlezde.