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 frequently 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

<table>
<thead>
<tr>
<th>Histological Type of Mammary Tumours</th>
<th>Number of Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant mixed tumour</td>
<td>10</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Stage of Mammary Tumours</th>
<th>Number of Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I T1N0M0</td>
<td>6</td>
</tr>
<tr>
<td>Stage II T2N0M0</td>
<td>9</td>
</tr>
</tbody>
</table>

aT, primary tumour. Size T1 < 3 cm, T2 3-5 cm, T3 > 5 cm.
bN, regional lymph node metastasis. N0 regional lymph node not involved, N1 regional lymph node unilateral involved, N2 regional lymph nodes bilateral involved.
cM, distant metastasis. M0 no evidence of distant metastasis, M1 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (n=15)</th>
<th>Dogs with Malignant Mammary Tumour (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>19.5±2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.7±3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nitric oxide (µmol/L)</td>
<td>18.8±2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.9±3.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ascorbic Acid (mg/dL)</td>
<td>0.48±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>209.3±11.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>303.5±29.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>100.3±31.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>203.4±53.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>230.4±41.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>131.7±23.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> All values are expressed as mean ± SD.
<sup>a, b</sup> differences are statistically significant in groups marked with different letters in the same row (<i>P</i>&lt;0.05).

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 <i>r</i> = -0.84, <i>P</i>&lt;0.001, indicating a strong correlation (Figure 2).
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$_2$O$_2$ 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.

ACKNOWLEDGMENTS:
We thank the University of Ankara Department of Pathology, Faculty of Veterinary Medicine for histopathological analysis of the tumour tissues.

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

ASKAR TK, SALMANOGLU B, SALMANOGLU R, ERKAL N i BESKAYA A

**SADRŽAJ**

Cilj ovih ispitivanja je bio da se utvrdi promene u oksidativnom statusu određivanjem koncentracije malonildialdehida (MDA), azot oksida (NO) i askorbinske kiseline kao i koncentracije pojedinih mikroelemenata u krvi kuja bolelih od tumora mlečne žlezde. U ispitivanju 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 oboljelih 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 oboljelih između koncentracija askorbinske kiseline i Fe ($r=-0.87$) a slaba korelacija između koncentracija askorbinske 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.