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Determination of Serum Trace Elements and Oxidative Stress in Bitches with Transmissible Venereal Tumor

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ABSTRACT

Background: This study was conducted to compare the changes in oxidative stress levels with some trace elements in blood serum in healthy bitches and bitches with transmissible venereal tumor (TVT).

Methods: A total of 40 bitches were used in the study. According to the genital organ examinations, healthy bitches constituted the control group (n=20) and those with venereal tumors constituted the TVT group (n=20). Total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) were determined spectrophotometrically. In addition, some serum trace element levels such as magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and selenium (Se) were measured using an ICP-MS analysis device.

Result: The serum Se, Mg and Zn levels were lower in the TVT group compared to the control group ($p<0.01$) and serum Fe and Cu levels were higher in the TVT group than in the control group ($p<0.01$). Serum TOS and OSI levels were higher ($p<0.001$) and serum TAS levels were lower ($p<0.001$) in the TVT group compared to the control group. The study results showed that oxidative stress is high in bitches with TVT and in parallel with this, changes occur in the amount of certain trace elements that are associated with oxidative stress.

Key words: Bitches, Oxidative status, Trace element, Transmissible venereal tumor.

INTRODUCTION

Although transmissible venereal tumor (TVT) is widely distributed among dogs throughout the world, it is more common in places with a temperate climate where dogs move around in a crowded and free manner (Booth, 1994). It has been emphasized that the causative agent may be a virus or tumor cell. It has also been suggested that TVT is a naturally occurring example of an allograft that can be transmitted by living cells (Amber *et al*., 1985).

Oxidative stress is the formation of cellular damage to an organism as a result of disruption of the balance between oxidants and antioxidants in favor of the oxidant system, lipid deoxidation and the release of free radical/ reactive oxygen products. Since this is of critical importance in the pathogenesis of many diseases, it leads to increased severity of the disease. This mechanism is responsible for the etiology of many diseases such as the process of aging and cardiovascular diseases, tumor, sepsis, degenerative neurological diseases, kidney failure, infertility, muscle and liver diseases (Ercan and Fidanci, 2012). The enzyme systems in cells are primarily effective in the body's defence system against free radicals, which are defined as antioxidants. In normal conditions, the balance between the amount and activities of antioxidants in the body is necessary for the life and health of the organism (Gutteridge, 1995).

Trace elements are essential for maintaining body homeostasis. In sufficient concentrations, these elements undertake important physiological functions (by activating important enzymes and as a bioactive substance), but a deficiency or excess in the body can cause many diseases, including tumors (Mulware, 2013). They also play important roles in the structural stability and metabolism of both nucleic acids and proteins (Mertz, 1981).

The etiology and development of neoplastic processes are multifactorial. A neoplasm can be formed by physical, biological, chemical, nutritional, therapeutic and environmental agents, or it can be inherited in idiopathic forms. These factors can act at any stage or stages of cell development (Yokota, 2000). Since it is not known whether higher or lower concentrations of trace elements are due to the presence of neoplasms, or whether these changes cause neoplasms, it is important to conduct studies on the formation of neoplasms with the concentration of essential metals (Butik, 2020). From a review of the literature, it was seen that there were no studies examining serum trace element changes in dogs with TVT. Therefore, the aim of this study was to

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investigate the changes in serum Mg, Mn, Fe, Cu, Zn and Se levels and serum oxidative stress levels in bitches with TVT.

MATERIALS AND METHODS

Permission for this study was granted by the Harran University Animal Experiments Local Ethics Committee (HRÜ-HADYEK, no: 2021/008).

Selection of animals and experimental protocol

The study material comprised 40 bitches brought to Harran University Faculty of Veterinary Medicine Animal Hospital from Sanliurfa Metropolitan Municipality Animal Shelter. The study used bitches ranging in age from 3-5 years, selected by a random sampling method under the same feeding and management conditions. All the bitches included had given birth once, had no problems detected in the genital system and a body condition score of around 3. The criteria for this body score are that non-excessive adipose tissue can be palpated on the ribs, the waist is visible behind the ribs when viewed from above and the abdomen is observed to be tight on examination. In the anamnesis of 20 bitches brought in, complaints of bloody discharge from the vulva were recorded. The bitches were divided into two groups according to the genital organ examinations. Group 1 (n=20) consisted of healthy bitches and Group 2 (n=20) consisted of bitches designated TVT. In the examination of the genital organs performed in the group evaluated as TVT; bloating, malformation, excessive licking of the area, abnormal odor and a noticeable cauliflower-like mass were detected (Fig 1). A vaginal smear sample was taken and TVT was diagnosed by staining (Giemsa) and the determination of intracytoplasmic vacuoles and numerous mitotic structures under microscope examination (Fig 2). Blood samples were taken from the bitches in both study groups with a 20 G sterile injector from the cephalic vein and then transferred to both 9 ml K_3EDTA tubes and 10 ml gel vacutainer tubes. The tubes were centrifuged at 3000 rpm for 15 minutes to obtain serum. The samples were stored at -20°C until the analysis of trace elements (Mg, Mn, Fe, Cu, Zn, Se), TAS and TOS levels.

Biochemical, trace element and haemogram analysis

Serum TAS (Rel Assay, Gaziantep, Turkey) and TOS (Rel Assay, Gaziantep, Turkey) levels were determined spectrophotometrically using commercial kits and EL0SA plate reader (Molecular Device SpectraMax M5 Plate Reader, Pleasanton, CA, USA). Trace elements (Mg, Mn, Fe, Cu, Zn, Se) were analyzed using an ICP-MS device (Thermo Scientific, iCAP™ TQ, Germany). For the oxidative stress index (OSI) value, the formula was used of TOS (mol H_2O_2 equivalent/L)/TAS (mmol Trolox equivalent/L X 10)= OSI (AU) (Erel, 2005; Erel, 2004). Haemogram was measured in anticoagulant venous blood samples with an automatic blood count device (Sysmex Europe, pocH-100iV Diff, Germany).

The serum samples were heated in a microwave system (Milestone Ethos Easy Advanced Microwave Digestion System model, Italy) with the addition of 0.5 ml of H_2O_2 and 1 ml of 65% HNO_3 to 0.5 ml of serum, slightly modifying the previously mentioned method (Laur *et al*., 2020). The final product was made up to 20 ml with ultrapure water. A calibration chart is provided for each element and parameters were measured using standard solutions (standard concentrations: 0.1-0.5-1-5-20-50 and 100 ppb, respectively) and ultrapure water as a blank. To obtain 10 ppb, 5 mL of the final product was withdrawn and 10 µL of the mixing standards were added. Trace elements (Mg, Mn, Fe, Cu, Zn, Se) in the samples were then analyzed using the ICP-MS instrument. The prepared serum samples were sorted and each sample was read twice. The accuracy of the instrument was checked by reading the standard solution with some sample intervals (Fig 3).

Statistical analysis

Data were analyzed statistically using SPSS for Windows version 24.0 software (Statistical Package for the Social Sciences). Conformity of the variables to normal distribution was examined using visual (histogram and Q-Q Plot) and analytical methods (Shapiro-Wilk tests). Descriptive analyses were reported as mean±standard error (SEM) for normally distributed variables. Since the data were in accordance with normal distribution, serum TAS, TOS, OSI and trace element levels were compared between the groups using the Independent Samples t-test. Homogeneity of the variances was determined using the Levene test. A value of $p<0.05$ was considered statistically significant for all analysis.

RESULTS AND DISCUSSION

The changes in serum trace element levels between the groups

The changes in serum trace elements of both groups are shown in Table 1. Serum Se, Mg and Zn levels were significantly lower in the TVT group than in the control group ($p<0.01$). Serum Fe and Cu levels were significantly higher in the TVT group than in the control group ($p<0.01$). There was no difference between the groups in respect of the serum Mn levels ($p>0.05$).

Epidemiological studies have identified magnesium deficiency as a risk factor for some types of human cancer and impaired magnesium homeostasis has been reported in cancer patients (Castiglioni and Maier, 2011). Serum Mg concentrations often decrease in patients with neoplasia regardless of treatments (Sartori *et al*., 1992). One of the possible reasons for this is that tumors act as Mg traps, thereby disrupting the Mg balance of the body, resulting in a lower serum Mg concentration (Parisse *et al*., 2021). Low serum Mg status has also been associated with increased inflammation in combination with increased oxidative stress in humans (Nielsen, 2010). In the current study, the serum Mg levels were found to be low in bitches with TVT. The reason for this may have been the damage to the cell caused by oxidative stress and the subsequent development of inflammation. It could also have been caused by the fact that the neoplastic cell behaves like an Mg trap.

Iron (Fe) is an important element involved in many cellular processes, which is used by living cells. Studies have associated the element Fe with various diseases, including cancer (Abbaspour *et al*., 2004). Excess Fe can lead to an increase in cell oxidative stress, resulting in accelerated damage to tissues and DNA (Ames, 2001). In studies conducted on humans, it has been reported that increased oxidative stress caused by excess iron may increase the risk of liver, pancreatic and skin cancer (Crawford, 1995). It has also been reported that low Fe concentrations can reduce the incidence of cancer (Saleh *et al*., 2020; Zacharski *et al*., 2008). The presence of a higher serum Fe concentration in bitches with TVT than in the healthy group in the current study is consistent with the findings of previous human studies (Saleh *et al*., 2020; Zacharski *et al*., 2008).

Fig 1: Transmissible venereal tumour in a bitch.

Fig 2: Vaginal smear obtained from a bitch with transmissible venereal tumour. Typically round to polyhedral tumour cells, hyperchromatism and cytoplasm with punctate vacuoles (arrow).

The average Cu concentration of cancerous tissues has been shown to be significantly different from the normal tissue average (Gregoriadis *et al*., 1983). Increased Cu levels in cancer tissue support tumor development by angiogenesis (Wang *et al*., 2010). In addition, it has been reported that Cu can stimulate the proliferation and migration of endothelial cells (Hu, 1998). Askar *et al*. (2009) reported higher serum Cu levels in dogs with breast tumors. Enginler *et al*. (2015) revealed a significantly higher level of Cu in malignant breast tissue and Skibniewska *et al*. (2010) found a significantly higher Cu content in neoplastic tissue in dogs compared to healthy mammary glands. In the current study, serum Cu levels were found to be higher in bitches with TVT than in healthy animals. This result shows that Cu plays an important role in the tumor development process, consistent with the findings of previous studies.

Cancer cells can consume circulating Zn to maintain growth and membrane integrity (Schwartz, 1975). In a previous study, Enginler *et al*. (2015) reported that serum Zn levels were low in dogs with breast tumors. In meta-analysis studies conducted on serum Zn levels in humans, it has been reported that the serum Zn levels of patients with cervical and prostate cancer were lower than those of the control group (Xie *et al*., 2018; Zhao *et al*., 2016). Serum Zn levels in the current study were found to be low in bitches with TVT, consistent with previous studies. The decrease in serum Zn concentration may contribute to the formation and progression of the tumor due to the impaired activity of Zn

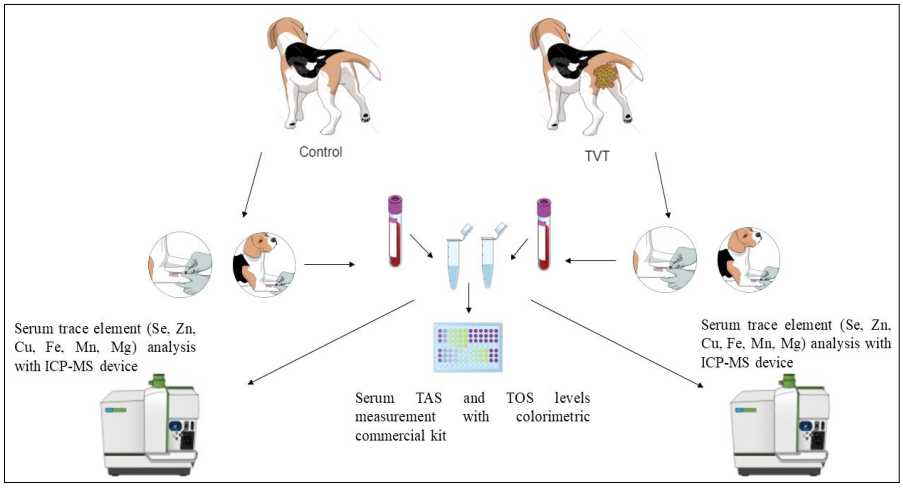

Fig 3: Study design.

in the immune and antioxidant response (Murakami and Hirano, 2008).

Selenium (Se) participates in the structure of various selenoproteins, such as glutathione reductase, which plays an important role as an antioxidant. Other selenoproteins have roles that maintain immune functions. Therefore, as it can play a protective role in the formation and development of certain malignancies through certain cellular pathways, low Se levels may induce tumor formation (Behne and Kyriakopoulos, 2001). In the current study, low serum Se levels were determined in the bitches with TVT compared to the healthy animals. It has been reported that low levels of Se may be associated with carcinogenesis and is a risk factor for cancer. It has also been reported that high levels of Se have a beneficial effect on the prevention of cancer and that Se has clear anti-tumorigenic and chemo-preventive effects in various malignant tumors. Therefore, it is not surprising that a decrease in erythrocyte, serum and urinary Se levels has been reported in these cancers compared to the control groups (Li *et al*., 2004).

The changes in TAS, TOS, OSI index levels and blood haemogram levels between the groups

The serum TOS, TAS and OSI levels are shown in Table 2. Serum TOS and OSI levels were seen to be significantly higher ($p<0.001$) and the serum TAS level was significantly lower in the TVT group than in the control group ($p<0.001$). The blood haemogram levels of the bitches are shown in Table 3. There was no difference between the groups in respect of the blood haemogram values (WBC, RBC, HGB, HCT, PLT) ($p>0.05$).

In many studies conducted in the field of humanities and veterinary medicine, increased oxidative damage in patients with tumors has been associated with an increased risk of tumor (Ercan *et al*., 2020; Winter *et al*., 2009). In a study where malondialdehyde (MDA), antioxidant activity and adenosine levels were determined in the serum of dogs with TVT, serum MDA levels were significantly higher and total antioxidant activities were lower in dogs with TVT than in healthy dogs (Aydin *et al*., 2009). In addition, Ercan *et al*. (2020) reported that serum MDA levels and 8-OHdG, a DNA

Table 1: The serum trace elements of both groups.

Serum trace elements	Unit	Groups		P value
		Control	TVT	
		Mean±SEM	Mean±SEM	
Mg	µg/L	19036.62±186.95	14503.96±147.26	0.022
Mn	µg/L	32.13±4.72	29.42±5.49	0.714
Fe	µg/L	1930.53±142.22	3364.82±166.82	0.045
Cu	µg/L	266.56±20.85	344.09±17.90	0.019
Zn	µg/L	2316.65±129.67	1599.92±111.66	0.048
Se	µg/L	178.09±4.38	109.39±7.74	<0.001

*Significance levels according to Independent Samples t-test results. Magnesium (Mg), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Selenium (Se).

Table 2: Comparisons of the serum TOS, TAS and OSI levels between the groups.

Oxidative status	Control group (n=20)		TVT group (n=20)		*P
	Mean	SEM	Mean	SEM	
TAC (mmol/L)	1.79	0.006	1.49	0.007	0.000
TOC (µmol/L)	18.50	0.02	14.08	0.3	0.000
OSI	7.81	0.17	12.36	0.17	0.000

*Significance levels according to the t-test results. Total antioxidant capacity (TAC), Total oxidant capacity (TOC), Oxidative stress index (OSI).

Table 3: Comparsions of the blood haemogram values between the groups.

Blood haemogram	Control group (n=20)		TVT group (n=20)		*P
	Mean	SEM	Mean	SEM	
WBC×10^3/µL	10.45	0.57	13.28	2.12	0.582
RBC×10^6/µL	9.32	0.23	9.29	0.42	0.481
HGB (g/dl)	15.78	0.35	15.52	0.56	0.710
HCT (%)	48.16	1,28	47.31	2.74	0.087
PLT×10^3/µL	128.42	12.26	124.63	15.87	0.054

*Significance levels according to the Independent Samples t-test results. White blood cell (WBC), Red blood cell (RBC), Haemoglobin (HGB), Hematocrit (HCT), Platelet (PLT).

damage marker, were higher in dogs with TVT compared to the control group and antioxidant enzyme levels such as SOD and GPx were lower. Similarly, Macotpet *et al*. (2013) stated that MDA levels were significantly higher in blood samples taken from dogs with tumors and clinically healthy dogs, than in clinically healthy dogs with tumors. In addition, the level of superoxide dismutase (SOD) showing antioxidant activity has been found to be significantly lower in patients with bladder tumors (Dinçer *et al*., 2011). In the current study, in parallel with the previous literature information, TOS and OSI levels, which are oxidative stress markers, were found to be high in bitches with TVT and TAS levels, which are antioxidant markers, were found to be low. Similarly, in studies conducted on humans, it has been reported that serum TAS levels are low and TOS levels and OSI values are high in patients with lung cancer (Zabłocka-Słowińska *et al*., 2018). It has been emphasized that oxidant-antioxidant imbalance is an important factor in the formation and progression of tumors. Thus, it has been concluded that oxidative stress in bitches may be associated with tumor and that increased oxidative damage in TVT bitches leads to a decrease in antioxidants.

CONCLUSION

In conclusion, the results of this study showed that the total redox state is impaired with increasing oxidative stress in bitches with TVT and this change may be associated with an increase in the serum levels of the trace elements of Fe and Cu, together with a decrease in the serum levels of Mg, Co, Zn and Se. There is a clear need for further studies to explain the relationship between trace elements and TVT to be able to determine the effective mechanism and to establish treatment.

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Conflict of interest

The author(s) declared no potential conflict of interests with respect to the research, authorship and/or publication of this article.

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