Explor Anim Med Res,
Vol. 8, Issue - 1, 2018, p. 59-63

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

MINERAL STATUS IN FEMALE DOGS WITH MALIGNANT MAMMARY GLAND TUMORS FED WITH DIFFERENT HABITUAL DIETS

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Received 06 February 2018, revised 05 May 2018

ABSTRACT: The present study estimated the level of some macro and micro minerals in female dogs with mammary gland tumors fed with different habitual diets. Female dogs bearing malignant mammary tumors were divided into two groups; group-I (n=12) fed only with balanced commercial diet and group-II (n=12) fed only with homemade diet. Ten physiologically healthy female dogs were taken as control. Trace elements viz. iron, copper, zinc and manganese were estimated in plasma and mammary tumor tissues. Calcium and phosphorus were exclusively estimated in plasma. Plasma concentrations of iron, copper and calcium were found to be significantly ($p<0.05$) elevated in homemade diet group as compared to control. However, the level of zinc, manganese and phosphorus in plasma of female dogs fed only with homemade diet was decreased ($p<0.05$) in comparison to control. The mammary gland tissue analysis of iron, copper, zinc and manganese has revealed that their level was found to be significantly ($p<0.05$) increased in mammary gland tumor tissues of both commercial and homemade diet group as compared to normal mammary tissues. The study concluded that, homemade diet significantly influence the plasma mineral status in the tumor bearing female dogs. However, mineral levels in mammary tumor tissues were altered irrespective of diet types.

Key words: Female dogs, mammary tumors, minerals, diet.

INTRODUCTION

Mammary gland tumors are most commonly diagnosed neoplasia in female dogs. It is second in line after skin cancer in canines (Kumaraguruparan *et al.* 2005). The incidence of the disease is increasing in canine population. The increasing prevalence of mammary tumours has been attributed to change in habitat and dietary pattern of female dogs from free ranging to the home made diet as consumed by human (Karayannopoulou *et al.* 2013). The minerals present in diet viz. iron, copper, zinc, manganese, calcium, phosphorus etc. are derived from the natural sources from which the diet is prepared and influenced by mineral content of water, soil and plants (Becker *et al.* 2007). Hence deficiency or excess of minerals in the natural source is reflected in the food. The common misconception among people is that by supplementing homemade diet, they are feeding a healthy diet to their bitches; however, key minerals may be missing or are fed in excess through homemade diet. Homemade food is not necessary for good health of female dogs rather food with appropriate proportion of nutrients is the requirement (Karayannopoulou *et al.* 2013).Some deficiency and excess has been so severe that mineral concentrations did not reach 50% of the National Research Council (NRC) recommendations or are more than the maximum limit suggested by NRC respectively (Perez *et al.* 2000). Hence, dietary control of these minerals is important with respect to canine health and disease. It is important that during health problem like cancer, diet of female dogs must "complete and balanced," means it should meet all of the dog's nutritional needs which can be achieved by feeding commercially available balanced canine food (Karayannopoulou *et al.* 2013). Commercial pet foods are made from a fixed formula of ingredients and preparation methods. These foods are supplemented with appropriate proportion of canine grade minerals and vitamins required for dogs providing them a balanced diet (Perez *et al.* 2000).Mammary cancers like any other diseases are associated with imbalanced mineral status

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Table 1. Types of malignant mammary tumors observed in the study.

Diet groups	Tumor types (n)		
	Simple carcinoma	Squamous cell carcinoma	Carcino-sarcoma
Commercial group	2	6	4
Homemade group	2	7	3

n= number of cases.

Table 2. Concentration (Mean ± SE) of macro and micro minerals in plasma of bitches with mammary tumors.

Minerals	Control	Group-I	Group-II
Calcium (mg/dL)	9.68±0.14	9.85±0.22	14.56±0.44*
Phosphorus (mg/dL)	4.73±0.07	4.52±0.07	3.51±0.08*
Iron (µg/dL)	87.25±2.06	88.31±2.11	167.61±3.02*
Copper (µg/dL)	105.11±2.12	106.04±2.52	191.67±2.88*
Zinc (µg/dL)	64.23±1.04	65.71±2.01	36.71±2.08*
Manganese (µg/dL)	17.25±0.22	17.69±0.35	12.61±0.33*

* Significantly ($p<0.05$) differ from the control.

Table 3. Concentration (Mean ± SE) of micro minerals in mammary tumor tissues of bitches.

Micro minerals	Normal mammary tissue (Control)	Group-I	Group-II
Iron (µg/g)	142.28±1.98	178.77±3.66*	257.08±3.18*
Copper (µg/g)	12.32±0.95	20.18±0.93*	28.05±1.02*
Zinc (µg/g)	24.19±0.92	27.55±1.18*	29.37±1.74*
Manganese (µg/g)	2.87±0.09	3.91±0.25*	5.18±0.22*

* Significantly ($p<0.05$) differ from the control.

which is reflected in their plasma and tissue concentrations (Arinola and Charles 2008). Determinations of the plasma and tissue level of minerals in tumour bearing patients provide their status in the diseased state which will help in better diagnosis and prognosis.

The study evaluated the concentration of some minerals in female dogs with malignant mammary gland tumors fed with different habitual diets.

MATERIALS AND METHODS

Animals

Female dogs (aged 6-14 years) bearing malignant mammary gland tumors were selected from the cases presented at Small Animal Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. These animals were clinically examined and diagnosed for malignant mammary tumor (based on histopathology, radiography and ultrasonography) by the Department of Veterinary Surgery and Radiology. Clinically healthy female dogs presented at the clinics for routine checkup were taken as control. The animals were divided into two groups:

Control (n=10): Clinically healthy female dogs

Group-I (n=12): fed only with balanced commercial diet and

Group-II (n=12): fed only with homemade diet.

Dietary history

Based on a questionnaire, the owners were asked about the diet over past years. Focus was given particularly to the dietary regimen in the months preceding the diagnosis. Approximate date of starting of diet was also recorded. The dogs were fed twice daily; morning and evening. Dogs were supplemented with homemade diets usually rich in minerals (calcium, iron, copper, zinc and manganese) that consisted of butter, egg, milk, fish, mineral rich vegetables, fruits and specific foods like

Table 4. Correlation between concentration of micro minerals in plasma and mammary tumor tissues of bitches fed with commercial diet.

Minerals	Pearson's correlation coefficient (r)
Iron $_{(Plasma)}$ Vs. Iron $_{(Tumor\ tissue)}$	0.22*
Copper $_{(Plasma)}$ Vs. Copper $_{(Tumor\ tissue)}$	0.18*
Zinc $_{(Plasma)}$ Vs. Zinc $_{(Tumor\ tissue)}$	0.12*
Manganese $_{(Plasma)}$ Vs. Manganese $_{(Tumor\ tissue)}$	0.11*

*Indicates significant difference at $p<0.05$.

Table 5. Correlation between concentration of micro minerals in plasma and mammary tumor tissues of bitches fed with homemade diet.

Minerals	Pearson's correlation coefficient (r)
Iron $_{(Plasma)}$ Vs. Iron $_{(Tumor\ tissue)}$	0.71*
Copper $_{(Plasma)}$ Vs. Copper $_{(Tumor\ tissue)}$	0.64*
Zinc $_{(Plasma)}$ Vs. Zinc $_{(Tumor\ tissue)}$	-0.55*
Manganese $_{(Plasma)}$ Vs. Manganese $_{(Tumor\ tissue)}$	-0.57*

*Indicates significant difference at $p<0.05$.

chicken and red meat (pork or lamb). Diet was prepared by the traditional method of boiling and in maximum cases butter or palm olive oil was used for cooking these foods. Commercial diet consisted of balanced canned, semi-moist or dry dog food. The brand of canned food was recorded. As mentioned in the label of commercial foods, it consisted of approximately meat or meat derived products (30%), oils or fats (10%), cereals and vegetables (5%) along with required quantity of vitamins and minerals.

Collection and analysis of samples

Blood

Four mililitres of blood were collected in heparinised centrifuge tubes from the cephalic vein of healthy (n=10) and tumor bearing female dogs; commercial (n=12) and homemade (n=12) diet groups. Blood samples were centrifuged at 2500 rpm for 10 minutes and plasma was separated.

Mammary tissues

Five grams of fresh mammary tumor tissues were collected both from commercial (n=12) and homemade (n=12) diet groups of female dogs immediately after surgery. Adjacent normal mammary tissues (n=10) collected from these female dogs were taken as control.

Histopathology

Small portions of tissues were fixed in 10% neutral buffered formalin (NBF), embedded in paraffin and tissue sections were prepared. The tissue sections were stained with Haematoxylin and Eosine (H & E) for microscopic examination.

Estimation of macro minerals

Calcium and phosphorus were determined in plasma using Autopak kits (Siemens Healthcare Diagnostics Ltd.) on semiautomatic biochemical analyzer. All the determinations were performed in duplicate.

Estimation of micro minerals

Plasma (2ml) and mammary tissue (3g) samples were digested after adding 15 ml of tri-acid mixture (HNO_3, H_2SO_4 and $HClO_4$ in 10:4:1 ratio) until a transparent solution was obtained (Ludmila 1976). After cooling, the digested samples were filtered and the volume was made to 10 ml with doubled distilled water. Iron Copper, Zinc and Manganese concentration in digested plasma and mammary tissue samples were estimated using atomic absorption spectrophotometer (AAS 4139, Electronics Corporation of India Limited, Hyderabad).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using statistical package for Social Sciences (SPSS) software (Version 16.0). The results were expressed as mean ± SE of mean (SEM). Correlation between different parameters was determined by Karl Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Histopathological analysis of tumor tissue samples revealed that both commercial and homemade diet groups of female dogs were having simple carcinoma, adenocarcinoma and carcino-sarcoma of mammary gland (Table 1). In the homemade diet group, plasma concentration of calcium, iron and copper has been found to be significantly ($p<0.05$) increased compared to control. This was accompanied by significant ($p<0.05$) decline in plasma level of phosphorus, zinc and manganese in the homemade diet group (Table 2). Food prepared in home may be treated as a balance diet for human; but may not be balanced in all respect for canines (Karayannopoulou *et al.* 2013). Minerals like zinc, copper, iron, manganese, calcium and phosphorus may be deficient or excess in the homemade diet depending on the type of diet and geographical region from which

the raw materials used for preparation of these diets were derived (Arinola and Charles 2008).

Female dogs fed with unbalanced homemade diet have been observed to have compromised health (Vannucchi *et al.* 2007). Calcium, iron, copper and phosphorus are essential for numerous biochemical processes in the cell. However, excess serum calcium with low phosphorus level is associated with different types of malignancies (Nathaniel *et al*. 2010). Hypercalcemia and hypophosphatemia observed in mammary tumour patients can be attributed to enhancement in bone osteolysis and disturbances in the reabsorption and excretion of calcium and phosphate ions (Esbrit 2001). The most common cancer associated with high calcium and low phosphorus is mammary cancer (Nathaniel *et al.* 2010) which is in agreement with our study. Increased plasma levels of calcium, iron and copper observed in the homemade diet group might be a consequence of intake of more butter, milk, fish and vegetables in diet. Zinc and manganese are required for proper growth and division of cells. However, neoplastic cells can also use these elements for their proliferation (Smith *et al.* 2007). Lowered level of plasma zinc and manganese in tumour bearing female dogs might be due to the consumption of these elements by the malignant mammary tissues for their growth and proliferation (Arinola and Charles 2008). In our study level of copper, iron, zinc and manganese were elevated in mammary tumor tissues of both homemade and commercial diet groups (Table 3). Copper and iron are essential elements for health. These elements serve as cofactors of the enzyme superoxide dismutase However, excess iron and copper can be toxic and generate reactive oxygen species through Fenton reaction causing oxidative stress (Kumaraguruparan *et al.* 2005). Studies suggested that antioxidant enzymes are over expressed in tumor tissues to provide protection against the oxidative stress (Kumaraguruparan *et al.* 2005, Filomeni *et al.* 2007). Elevated levels of iron, copper, zinc and manganese in mammary tumor tissues of both homemade and commercial diet groups might be due to increased expression of superoxide dismutase enzyme and uptake of these elements from the plasma by the tumour cells for their proliferation and conversion into a malignant phenotype (Arinola and Charles 2008) which has been supported by the significant correlations observed between their plasma and tumour tissue levels in both commercial diet (Table 4) and homemade (Table 5) diet groups. Previously conducted studies suggested that female dogs bearing mammary tumors fed with homemade diet were observed to be associated with imbalanced plasma and tissue minerals (Perez *et al.* 1998, Perez *et al.* 2000 and Karayannopoulou *et al.* 2013) which is in line with the results of the present study.

CONCLUSION

The study concluded that, homemade diet significantly influence the plasma mineral status in the tumor bearing female dogs. However, mineral levels in mammary tumor tissues were altered irrespective of diet types.

ACKNOWLEDGEMENT

Authors are thankful to the Director of Research, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for providing funds for this study.

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***Cite this article as:** Dash SK, Singh C, Singh G (2018) Mineral status in female dogs with malignant mammary gland tumors fed with different habitual diets. Explor Anim Med Res 8(1): 59-63.