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## Occurrence, clinico-Haemato-biochemical and histopathological studies on mammary gland tumor in geriatric dogs



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**Abstract**

Mammary gland tumor was recorded in 18.2% of female geriatric dogs of Jammu. Grossly, red, purple or flesh color, irregular shaped, soft and hard masses, firmly attached to the skin on different pairs of teats were recorded in affected dogs. On histopathological examination, all the six cases of mammary gland tumor were identified as adenocarcinoma i.e. 100% mammary tumors were found to be malignant. Haemato-biochemical profile of the affected dogs revealed significantly ( $P<0.05$ ) lower mean values of haemoglobin, higher total leucocyte counts ( $17.59\pm2.45 \times 10^3/\mu\text{L}$ ) and neutrophil counts ( $79.5\pm1.99\%$ ) whereas lymphocyte count was lower ( $12.72\pm1.68\%$ ) as compared to the healthy control group. Platelet count was also significantly higher in the affected dogs ( $453.00\pm25.18 \times 10^6/\mu\text{L}$ ). No significant deviation was recorded in biochemical parameters

**Keywords:** Dogs, geriatric, Haemato-biochemical, mammary gland tumor, histopathology

**Introduction**

Mammary gland tumors are frequent in dogs and cats, appearing extremely sporadically in other domestic animals; being the second most common neoplasms after skin tumors in dogs (Rezia *et al.*, 2009) [24]. The mean age of onset for mammary tumors is between 8-11 years. Mammary tumors can vary in size from a few millimeters to over a few centimeters, and about 50% of cases have multiple masses mainly located at the caudal glands (Wey *et al.*, 2000) [30]. The majority of these tumors occur in intact female dogs (Khimta *et al.*, 2010) [15]. As in humans, canine mammary cancer is a heterogeneous group of diseases linked to morphology and biological behavior. Gonadal hormones induce the hypertrophy of the mammary parenchyma, which is obvious after estrus (Baba and Câtoi, 2007) [1]. Mammary tumors in the bitch are hormone dependent, and the risk of appearance increases after each estrous cycle. Hematologic abnormalities have frequently been encountered in small animal cancer patients with neoplasms, and may result from the direct effects of tumor growth or from paraneoplastic syndromes. They may be characterized by decreases or increases in the absolute number of circulating formed elements of the blood, alterations of hemostasis or plasma protein dyscrasias (Childress, 2012) [6]. The present investigation was undertaken to study the status of occurrence of canine mammary tumor in geriatric dogs of Jammu and also to determine histopathological findings in mammary tumors along with hemato-biochemical changes.

**Materials and Methods**

Thirty three female geriatric dogs of  $\geq 9$  years of age presented to TVCC of SKUAST-J, R.S. Pura, Jammu for routine check-ups/vaccination or for varying ailments were studied. Eight apparently healthy geriatric dogs were placed in control group (Group I). Six dogs of different breeds aged 9-13 years old showed mammary gland swelling, red, purple or fleshy color, irregular shape, soft and hard masses, firmly attached to the skin on varying pairs of teats (Table 1 and Fig. 1) were diagnosed as cases of canine mammary tumors and placed in Group II. Tissue samples from six mammary tumor cases were collected in 10% neutral buffered formalin solution at the time of surgery and histopathological evaluation was done using H&E stain (Bancroft and Gamble, 2002) [2].

Approximately 8 ml of blood was aseptically collected in a sterile disposable plastic syringe from dogs of both the groups. Of this, 3 ml blood was transferred immediately into a K<sub>3</sub> E.D.T.A anticoagulant containing vacutainer for haematological studies. Rest of the blood

sample was transferred in clot activator vacutainer (BD vacutainer® Serum, BD Franklin Lakes, NJ, USA). Serum was extracted and stored at -20 °C for estimation of biochemical parameters.

The haematological parameters i.e. haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated as per the methods described by Jain (1986). Biochemical analysis of all biochemical parameters (AST, ALT, total protein, albumin,

globulin, A:G ratio, BUN, creatinine, Ca and P) was carried out using semi-automatic biochemical analyzer (ERBA Chem-7 plus V<sub>2</sub>, Transasia Biomedicals Limited, India) and standard biochemical kits (ERBA diagnostics Mannheim GmbH, Germany). Statistical analysis was done by independent t-test to determine the differences between the groups and a P value of <0.05 was considered to be significant.

**Table 1:** Age, Breed and Parts (s) of mammary gland involved in cases of mammary tumor in geriatric dogs

S. No.	Breed	Age (year)	Parts (s) of mammary gland involved
1	German Shepherd	13	Inguinal teat involved
2	Mongrel	10	One pair of abdominal teat involved
3	Pomeranian	12	Inguinal teat involved
4	Mongrel	12	Multiple teats involved
5	Mongrel	12	Two pair of abdominal teat involved
6	Labrador	9	One pair of inguinal and one pair thoracic teats involved

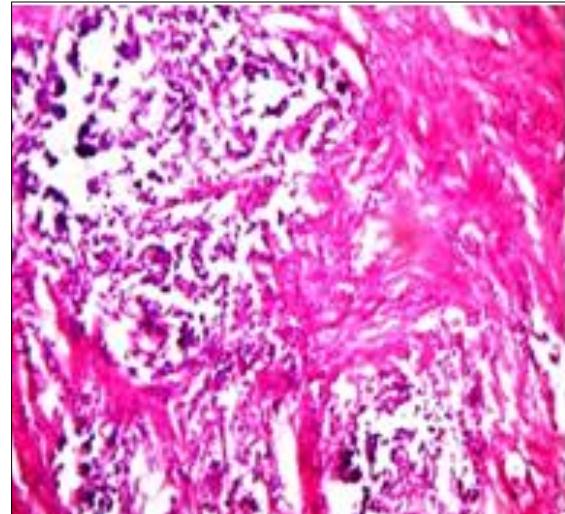


**Fig 1:** Mammary tumor

## Results and Discussion

The study records an occurrence of mammary tumor 18.2% (6/33) in female geriatric dogs of Jammu. An incidence of 8 to 52% has been reported from various parts of India (Singh *et al.*, 1998, Srivastava *et al.*, 2009, Dhami *et al.*, 2010, Simon *et al.*, 2017) [27, 28, 7, 26]. Variable incidence of canine mammary tumor has been reported from other parts of the world; from as low as 0.7% in Hungary (Boldizsar, 1992) [4] to 15.27% in Germany (Kessler *et al.*, 1997). According Perez Alenza *et al.* (2000) [23], mammary tumours were the most frequent neoplasms in the bitch and their incidence was extremely high in those areas where early ovarioectomy of bitches (before two years of age) was not practiced. The incidence usually peaks at the age of 10- 12 years followed by subsequent decrease (Zatloukal *et al.*, 2005) [31].

On histopathological examination, all of the six cases of mammary gland tumor were identified as adenocarcinoma (Fig. 2). Extensive proliferation of fibrous connective tissue with desquamation of duct epithelial cells and mild nuclear pleomorphism was recorded. Similar gross morphology and histopathological findings have also been reported by others (Jones *et al.*, 1997, Kumar *et al.*, 2010, Kimura *et al.*, 2012) [13, 15, 17]. Previous studies suggest that nearly 41% to 53% of the mammary tumors that occur in the bitch are malignant (Misdorp, 2002) [20]. In the present study, we recorded that the mammary tumors in all six cases were malignant. This might be due to the fact that percentage of malignant tumors increase with age (Gupta *et al.*, 2012; Kim *et al.*, 2016) [9, 16] and that geriatric dogs were the subjects of present study.



**Fig 2:** Mammary gland adenocarcinoma

Hematological observation of dogs affected with canine mammary tumor (CMT) depicted significantly lower haemoglobin level (although within normal range) as compared to the control group whereas total erythrocyte count showed no alteration in canine mammary tumor affected dogs (Table 2). These findings are in agreement with those of Gupta *et al.* (2014) [10] and Lallo *et al.* (2016) [19]. A normocytic, normochromic anemia is one of the most frequent paraneoplastic signs (Finora, 2003 and Bergman,

2013) [8, 3]; and may be associated with the secretion of cytokines [such as interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF- $\alpha$ )] by tumor cells, which sequester iron, reduce erythrocyte half-life (IL1), and also reduce erythropoietin release (TNF and IL-6). However, the most relevant cause of non-regenerative anemia in cancer patients is believed to be anemia of chronic disease, also known as anemia of inflammation (Childress, 2012) [6].

The mean TLC in CMT affected dogs was significantly higher ( $17.59 \pm 2.45 \times 10^3/\mu\text{L}$ ) as compared to that of control group ( $7.75 \pm 0.45 \times 10^3/\mu\text{L}$ ); with significantly higher neutrophils ( $79.5 \pm 1.99$  vs  $68.75 \pm 2.43\%$ ) and significantly lower lymphocytes ( $12.72 \pm 1.68$  vs  $22.99 \pm 1.78\%$ ). The monocyte, eosinophil and basophil counts in CMT affected dogs showed no difference compared to the control. These results were in agreement with those of Munkhtuul *et al.* (2014), Gupta *et al.* (2014) [10] and Lallo *et al.* (2016) [19]. This neutrophilic leukocytosis may be attributed to inflammation or tissue necrosis associated with mammary cancer or triggered by chronic stress (Childress, 2012) [6]. Moreover, IL-1 and TNF- $\alpha$  also can induce neutrophilia by stimulating the production of growth factors (Harvey, 2012) [11].

Statistically significant ( $P < 0.05$ ) higher mean value of platelet count was observed in affected dogs ( $453.00 \pm 25.18 \times 10^3/\mu\text{L}$ ) when compared with the control group ( $374.88 \pm 19.67$

$\times 10^3/\mu\text{L}$ ). Present findings corroborate with those of the Lallo *et al.* (2016) [19]. In human medicine, thrombocytosis is a clinically important finding often detected in cancer patients, but there is a paucity of reports on this condition in veterinary oncology (Childress, 2012 [6] and Bergman, 2013) [3]. Although its mechanism is not well-known in veterinary medicine, it has been suggested that epinephrine-induced splenic contraction and the release of pro-angiogenic tumor proteins and inflammatory cytokines (e.g., IL-1 and IL-6) or hematopoietic growth factors (Saavedra *et al.*, 2011, Childress, 2012) [6, 25] secreted or induced by the tumor may contribute to thrombocytosis.

No significant difference was observed between the control and mammary tumour affected geriatric dogs with respect to serum biochemical parameters. This is similar to the findings reported by others (Todarova *et al.*, 2005; Gupta *et al.*, 2014 and Mohapatra *et al.*, 2016) [10]. Numerically lower ( $P > 0.05$ ) levels of AST, total protein, albumin, globulin, A:G ratio, creatinine, BUN and inorganic phosphorus were recorded in CMT affected dogs than control group. Affected dogs showed higher ( $P > 0.05$ ) ALT and calcium than control. Hypoalbuminemia may be related to the inflammation caused by the neoplastic process, as albumin is a negative acute-phase protein that tends to reduce its serum levels after an inflammatory stimulus (Cerón *et al.*, 2005) [5].

**Table 2:** Blood profile of geriatric dogs suffering from mammary gland tumor

Parameters	Group I (Control, n=8)	Group II (Mammary gland tumor, n=6)	Reference value
Hb (g/dL)	$15.41 \pm 0.4^a$	$13.25 \pm 0.95^b$	11.9-18.9
TEC ( $\times 10^6/\mu\text{L}$ )	$6.30 \pm 0.36$	$6.94 \pm 0.58$	4.95-7.87
TLC ( $\times 10^3/\mu\text{L}$ )	$7.75 \pm 0.45^a$	$17.59 \pm 2.45^b$	5.0-14.1
Neutrophils (%)	$68.75 \pm 2.43^a$	$79.5 \pm 1.99^b$	58-85
Lymphocytes (%)	$22.99 \pm 1.78^a$	$12.72 \pm 1.68^b$	8-21
Monocytes (%)	$3.94 \pm 0.39$	$4.42 \pm 0.67$	2-10
Eosinophils (%)	$4.21 \pm 0.85$	$3.37 \pm 0.60$	0-9
Basophils (%)	$0.11 \pm 0.07$	$0.00 \pm 0.00$	0-1
Platelets ( $\times 10^3/\mu\text{L}$ )	$374.88 \pm 19.67^a$	$453.00 \pm 25.18^b$	211-621
AST (U/L)	$40.57 \pm 3.98$	$35.10 \pm 3.37$	10-50
ALT (U/L)	$48.39 \pm 3.71$	$52.28 \pm 8.48$	10-109
Total protein (g/dL)	$8.44 \pm 0.52$	$7.23 \pm 0.25$	5.4-7.5
Albumin (g/dL)	$2.95 \pm 0.26$	$2.91 \pm 0.15$	2.3-3.1
Globulin (g/dL)	$5.48 \pm 0.55$	$4.32 \pm 0.17$	2.7-4.4
A:G ratio	$0.59 \pm 0.09$	$0.68 \pm 0.04$	0.50-1.11
BUN (mg/dL)	$18.83 \pm 2.02$	$18.23 \pm 0.84$	8-28
Creatinine (mg/dL)	$0.95 \pm 0.08$	$0.88 \pm 0.12$	0.5-1.7
Ca (mg/dL)	$9.54 \pm 0.53$	$10.40 \pm 0.36$	8.86-10.3
P <sub>i</sub> (mg/dL)	$5.27 \pm 0.31$	$4.50 \pm 0.29$	2.5-5

Different superscripts <sup>a,b</sup> indicates significant difference within row at  $P < 0.05$

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