International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; SP-9(1): 452-457

www.veterinarypaper.com
Received: 01-11-2023
Accepted: 06-12-2023

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Diagnosis of canine mammary tumours

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Abstract
The objective of the present study was to determine the various diagnostic modalities for canine mammary tumors. Dogs that were presented to the Veterinary Clinical Complex for a period of six months were screened for the presence of Mammary tumors. Diagnosis of malignant mammary tumors in the present study was made based on clinical signs, FNAC, histopathology, ultrasonography of abdomen and tumour, radiography of thorax and abdomen, haematology, serum biochemistry and immunohistochemistry of tumour.

Keywords: Immunohistochemistry, mammary tumour, dogs

1. Introduction
Cancer can be defined as a disease in which there is uncontrolled multiplication and spread of abnormal forms of the body's own cells within the body which results in a new and diseased form of tissue growth called neoplasm (tumor) and it is one of the major causes of death in human beings and also in dogs (Kumar *et al*., 2017) [5]. Mammary gland tumors are the second most commonly occurring tumors in canines and the incidence is also observed in cats (Nunes *et al*., 2019) [7]. These tumors in female dogs are the most prevalent after skin tumors and can develop anywhere along the mammary chain (Salas *et al*., 2015) [10]. Canine mammary tumors are easily diagnosed through physical examinations, fine-needle aspiration of the tumor mass, aspiration from regional lymph nodes, histopathology of tumor mass, radiography of the thorax and abdomen, ultrasonography of abdomen, tumor mass and computed tomography of the thorax.

2. Materials and Methods
The present research work was carried out on dogs presented to the Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar, Hyderabad and Veterinary Hospital, Bhoiguda during the period from January 2023 to September 2023, P.V. Narsimha Rao Telangana Veterinary University. Dogs presented with the history and clinical signs of the presence of a mass of the mammary gland were selected for the present study. Detailed history regarding the duration and severity of clinical signs was recorded. Suspected cases were subjected to detailed clinical examination (parameters such as temperature, pulse, respiration rate, palpation of lymph nodes and mucous membranes were observed and recorded). Clinical samples such as fine needle aspiration of the tumor mass and biopsy were collected to undertake cytology, histopathology and immunohistochemistry from the dogs suspected of mammary tumors. Fine needle aspiration was taken from the tumors using a 10 ml disposable syringe and 20 gauge needle onto the microscopic slides. Smears were prepared either by blood smear technique or squash method according to the consistency of mass as described by Valenciano and Cowell (2014) [12]. Fixed smears were stained by Giemsa and Field staining methods to examine under the light microscope. Biopsy punches of 4 mm gauge were used for the collection of the biopsy samples from superficial tumors like mammary tumors which can be used for histopathology and immunohistochemistry. Two ml of whole blood was withdrawn from the cephalic or saphenous vein in a vacutainer with K-EDTA for the estimation of hematological parameters with the help of ABX Micro ESV 60 fully automated veterinary hematology analyzer manufactured by Horiba Pvt. Ltd, India. For serum separation, blood was collected in a vacutainer containing a clotting accelerator.

The serum was separated immediately after clotting by centrifugation at 3000 rpm for 5 minutes and collected in Eppendorf tubes. All the serum biochemical parameters such as ALT, AST, ALP, BUN, creatinine and total proteins were estimated by an auto analyzer (EM DENSITY 180), supplied by M/s Erba Mennheim Pvt. Ltd., Germany) employing the kits supplied by Span Diagnostics Pvt. Ltd.

3. Results and Discussion

Out of 28,350 dogs presented to the hospitals, 1350 were suspected of various tumors. Out of these, 273 dogs of different age, breed and gender were diagnosed with various malignant tumors with an incidence of about 0.96%. Among these 273 dogs with malignant tumors, mammary tumours were found to be 24.91% (68 dogs).

3.1 Clinical signs

Among the 68 dogs with malignant mammary tumors, the most common clinical sign observed was palpable masses in mammary glands (100%). Among them, the most affected mammary glands were inguinal i.e., in 29 dogs (42.65%) followed by caudal abdominal glands in 18 dogs (26.47%), cranial abdominal in 10 dogs (14.71%), caudal thoracic in 6 dogs (8.82%) and least in cranial thoracic i.e., 5 dogs (7.35%). These findings were in accordance with Kumar and Parasar (2020) [4], Devarathnam *et al*. (2021) [1] and Rokad *et al*. (2023) [9]. A higher percentage of mammary tumors in the inguinal and the caudal abdominal mammary glands, probably due to the greater volume of mammary tissue susceptible to tumour transformation and also these two glands they maintain their secretary activity longer than other pairs (Sanchez and Guarin, 2014) [13]. The results are depicted in table 1 and fig 1.

The other clinical signs such as enlargement of lymph nodes was seen in 40 dogs (58.82%). Among them, pre-scapular, axillary, inguinal and popliteal lymph node enlargement was seen in 4, 9, 17 and 10 dogs depicting 5.88%, 13.23%, 25.00% and 14.71%, respectively. There was no involvement of any lymph node in 28 dogs (41.17%) with malignant mammary tumors.

Regarding the consistency of tumors, soft consistency of tumour mass was seen in 2 dogs (2.94%) and the consistency was found to be hard in 17 dogs and semisolid in 7 dogs with 25.00% and 10.30%, respectively. The tumour mass showed nodular/cauliflower-like growth in 25 dogs (36.76%). The masses were maggoted in 8 dogs and ulcerated in 9 dogs with an incidence of 11.77% and 13.23%, respectively. Dileep Kumar *et al*. (2016) [14] stated that among all canine mammary tumour cases, ulcerated and infected types were seen in 33.3% of cases. Kumar *et al*. (2018b) [15] and Patel *et al*. (2019) [8] explained that dogs with mammary tumors showed mammary gland swelling, red, purple, or fleshy colour, irregular shape, soft and hard masses, firmly attached to the skin on varying pairs of teats.

Other general clinical signs like anorexia, weight loss, lethargy, dehydration and vomiting were also noticed in 29 dogs (42.64%), 14 dogs (20.60%), 18 dogs (26.47%), 19 dogs (27.94%) and 35 dogs (51.47%), respectively.

3.2 Staging of Mammary Tumours by TNM Classification

In the present study, malignant mammary tumors diagnosed in 68 dogs were further classified based on the TNM classification of WHO. Among these 68 dogs diagnosed with malignant mammary tumors, the tumour size was less than 3 cm in 17 dogs i.e., (T_1), 15 dogs had tumors of 3-5 cm (T_2) size, 17 dogs had tumors of 5-10 cm (T_3) size and 19 dogs had >10 cm (T_4) size. Further, 28 out of 68 dogs with malignant mammary tumors of the study did not show any regional lymph node involvement (N_0), whereas 40 dogs showed lymph node involvement i.e., N1 were 38 dogs and N2 were 2 dogs. Similarly, metastasis was not seen in 49 dogs (M_0) but seen in 17 dogs (M1) and 2 dogs (M2). Among these, 15 dogs with T_{1a}, b, c, N_0 and M_0 were considered as Stage-I and 17 dogs with T_{2a}, b with N_0, N1a and M_0 were considered as Stage-II whereas, 17 dogs with any T any N, M_0 considered as Stage-III and 19 dogs with any T any N, M1 or M2 were considered as Stage-IV. Silva *et al*. (2019) [11] conducted studies on mammary tumors in dogs which revealed 46% had T_1 tumors, 25% T_2 tumors and 29% T_3 tumors and further stated that increased tumour size was associated with increased malignancy in canine mammary tumors. As mammary carcinoma spreads through the lymphatic channel, knowledge of lymphatic drainage of the five pairs of mammary carcinomas is highly essential for deciding the stages of metastasis and clinical staging of the tumour into Stage-I to Stage-V. The results are depicted in table 2.

3.3 FNAC (Fine needle aspiration cytology)

Examining stained smears taken by FNAC from dogs with malignant mammary tumors has revealed that the cytoplasm and nuclear ratio were varying (anisocytosis) and also the nuclear size was showing alterations (anisokaryosis). The cytoplasm was filled with vacuoles and the nucleus showed varied granular staining properties (Fig 2).

3.4 Histopathology

In dogs with mammary tumors, biopsy samples stained with H&E revealed epitheloid cells with a large nucleus, one or two nucleoli and numerous vacuoles upon histopathological examination. In the present study, a total of 68 canine malignant mammary tumors were studied of which 53 (77.95%) were simple malignant and 15 (22.05%) were mixed mammary tumors. Among 53 simple malignant canine mammary tumors, adenocarcinomas were 21 (30.90%), tubulo-papillary adenocarcinomas were 16 (23.52%), ductular adeno carcinoma were 11 (16.18%) and solid carcinomas were 5 (7.35%). Mixed mammary carcinomas were diagnosed in 15 dogs (22.05) out of which myxochondro adenocarcinoma and fibrosarcoma were 6 each (8.82%), myxolipo adenocarcinoma were 2 (2.94%) and liposarcoma were 1 (1.47%). The findings of the present study were in accordance with Krishna *et al*. (2022) [2] who reviewed the types of mammary tumors observed were, tubular adenocarcinoma (9.52%), papillary adenocarcinoma (14.28%), papillary cystic adenocarcinoma (4.76%), solid carcinoma (9.52%), squamous cell carcinoma (4.76%), fibrosarcoma (4.76%), malignant mixed mammary tumors (19.04%), cavernous haemangioma (9.52%), fibroadenoma (4.76%), lipoma (9.52%) and soft tissue hyperplasia (9.52%) (Table 3 and Fig 3).

3.5 Radiography

In the present study the radiograph of the thorax didn't reveal any pulmonary metastatic lesions in dogs with malignant mammary tumors of Stage-I and Stage-II whereas the radiograph of the thorax revealed the presence of pulmonary metastasis in 3 out of 17 dogs with Stage-III and 6 out of 19 dogs with Stage-IV, respectively. Pulmonary radiographic examination revealed various types of metastatic lesions in the lung parenchyma which can be enumerated as the

presence of fine dots of miliary nodules in 3 dogs (4.41%), extended larger metastatic masses in 1 dog (1.47%), single/solitary nodules in 2 dogs (2.94%), diffuse interstitial pattern of metastasis in 3 dogs (4.41%) suffering with malignant mammary tumors. Similar results were also observed by Nair *et al.* (2022) [6] who stated that early pulmonary metastasis signs were characterized by lesions such as fine dots of miliary nodules, pulmonary micronodules, pulmonary nodules, pulmonary mass, multiple solid nodules, single solid mass, cavitary nodules and single cavity mass in bitches with malignant mammary tumors (Fig 4).

3.6 Ultrasonography

Abdominal sonographic examination in dogs with malignant mammary tumors did not reveal any abnormal echo pattern of abdominal organs suggesting metastasis which implies that there are no secondary malignancies induced via metastasis of the primary malignant tumors of the mammary glands. Ultrasonographic examination of the canine malignant mammary tumour mass revealed the presence of anechoic fluid-filled cystic collections, hyperechoic areas with diffuse margins and anechoic areas with hyperechoic margins (Fig 5).

3.7 Hematology and Serum Biochemistry

The mean hematological parameters of dogs with malignant mammary tumors revealed that there is significant (p<0.05) decrease of hemoglobin (12.43±0.09g/dl), TEC (5.87±0.13x10^6/µl) (p<0.01), insignificant decrease of PCV (37.41±1.49%), lymphocytes (21.80±1.45%) and platelets (2.87±0.11x10^5/dl) and insiginificant increase of TLC (13.74±0.28x10^3/µl), neutrophils (73.05±2.24%), monocytes (3.31±0.13%) and eosinophils (1.84±0.08%) when compared to hematological parameters of apparently healthy dogs such as haemoglobin (13.66±0.25%), PCV (41.06±0.79%), TEC (7.09±0.21 x10^6/µl), TLC (13.09±0.65x10^3/µl), Neutrophils (71.91±0.48%), Lymphocytes (23.26±0.41%), Monocytes (3.12±0.18%), Eosinophils (1.71±0.09%) and Platelets (3.20±0.22 x10^5/dl) (Table 4).

The mean serum biochemical parameters of dogs with malignant mammary tumors revealed that there is significant (p<0.01) decrease of BUN (13.15±0.38 mg/dl), significant increase of ALT (46.20±0.81 IU/L), insignificant decrease of AST (43.28±1.37 IU/L) and insignificant increase of creatinine (0.96±0.10 mg/dl), ALP (57.21±1.23 IU/L) and total protein (7.38±0.11 g/dl) when compared to serum biochemical parameters of apparently healthy dogs such as BUN (19.04±1.35 mg/dl), Creatinine (0.89±0.10mg/dl), ALT (36.4±2.19 IU/L), AST (47.12±1.14 IU/L), ALP (52.61±3.49 IU/L) and total protein (6.81±0.20 g/dl) (Table 5).

3.8 Immunohistochemistry

In the present study, a total of 24 canine malignant mammary tumors were studied for immunohistochemistry findings of which 19 dogs (77.95%) were simple malignant and 5 dogs (22.05%) were mixed mammary tumors. Dark brown-coloured nuclear immunostaining was considered positive for Ki-67. Tumor cells showed distinct nuclear staining and were considered positive and counted. Ki-67 scoring was done according to Kandefer-Gola *et al.* (2013) [16] by using image 'J' software, based on the percentage of Ki-67 positive cells (Fig 6).

Table 1: Clinical signs of dogs with malignant mammary tumors (n=68)

S. No	Observation	Clinical signs	Number	Percentage
1	Palpable masses in mammary glands	Cranial thoracic	05	7.35
		Caudal thoracic	06	8.82
		Cranial abdominal	10	14.71
		Caudal abdominal	18	26.47
		Inguinal	29	42.65
		TOTAL	68	100.00
2	Enlargement of lymph nodes	Prescapular	04	5.88
		Axillary	09	13.23
		Inguinal	17	25.00
		Popliteal	10	14.71
		No lymph node involvement	28	41.18
		TOTAL	68	100.00
3	Consistency of tumor	Soft	02	2.94
		Hard	17	25.00
		Semisolid	07	10.30
		Nodular/Cauliflower	25	36.76
		Maggoted	08	11.77
		Ulcerated	09	13.23
		TOTAL	68	100.00

Fig 1: Gross pictures of clinical Signs of mammary gland tumors in dogs

Table 2: Staging of canine malignant mammary tumors(n=68)

S. No	TNM Classification	No. of tumors in stages				
		Stage-I	Stage-II	Stage-III	Stage-IV	Total
1	$T_{1a}N_0M_0$	9	-	-	-	9
2	$T_{1b}N_0M_0$	6	-	-	-	6
3	$T_{1c}N_{1a}M_0$	-	2	-	-	2
4	$T_{2a}N_0M_0$	-	8	-	-	8
5	$T_{2b}N_0M_0$	-	5	-	-	5
6	$T_{2b}N_{1a}M_0$	-	2	-	-	2
7	$T_{2b}N_{1b}M_0$	-	-	-	-	-
8	$T_{3a}N_{1b}M_1$	-	-	3	2	5
9	$T_{3b}N_{1b}M_0$	-	-	6	-	6
10	$T_{3b}N_{2b}M_2$	-	-	-	6	6
11	$T_{4a}N_{1a}M_1$	-	-	-	4	4
12	$T_{4a}N_{1b}M_0$	-	-	8	7	15
Total		15	17	17	19	68

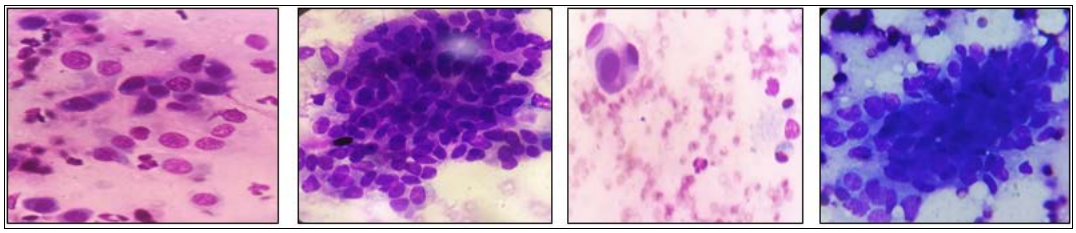

Fig 2: FNAC pictures of malignant mammary tumors in dogs

Table 3: Histopathological types of canine malignant mammary tumors (n=68)

Type of neoplasm	No. of animals	Percentage
I. Simple malignant tumors:	53	77.95
Adenocarcinoma	21	30.90
Tubulo-Papillary adenocarcinoma	16	23.52
Ductular adenocarcinoma	11	16.18
Solid carcinoma	05	7.35
II. Mixed mammary tumors:	15	22.05
Myxo Chondroadenocarcinoma	06	8.82
Fibrosarcoma	06	8.82
Myxo lipoadenocarcinoma	02	2.94
Liposarcoma	01	1.47
TOTAL	68	100.00

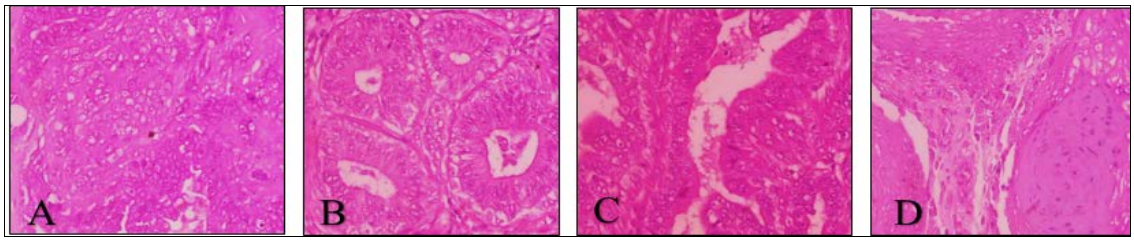

Fig 3: A: Adenocarcinoma characterized by closely packed cells form dense irregularly sized lobules without lumina supported by fine fibrovascular stroma. B. Tubular adenocarcinoma showing mitotic figures, double layered tubular epithelium. C. Papillary adenocarcinoma characterized by pockets of proliferative cells invading the parenchyma in a papillary pattern with numerous mitotic figures. D. Myxo chondro adenocarcinoma characterized by proliferation of luminal epithealial cells admixed with spindle myoepithelial cells and presence of neoplastic cells in the cartilaginous matrix

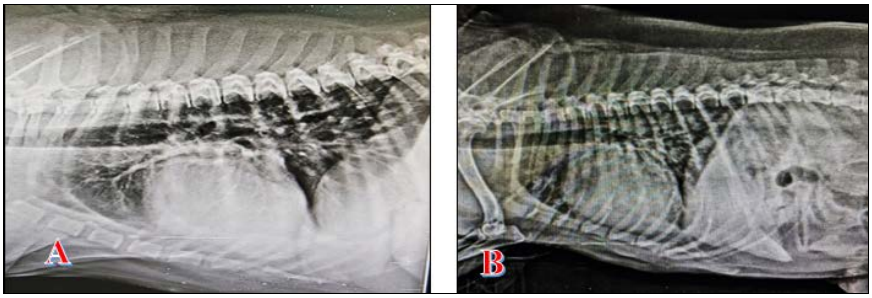

Fig 4: Radiograph of thorax showing diffuse interstitial pattern with metastatic nodules indicating malignancy of mammary tumour. B. Radiograph showing the severe diffuse metastatic lesions occupied entirely in the lung parenchyma

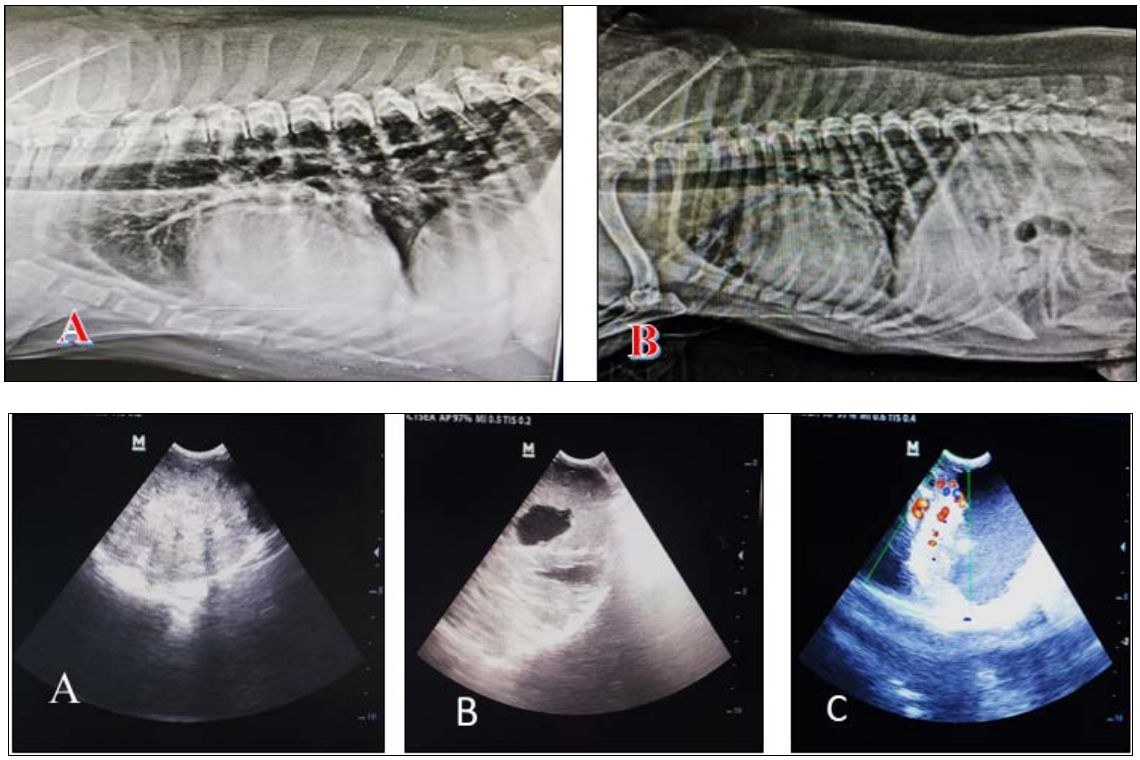

Fig 5: Mammary tumor mass showing hyperechoic areas with shadowing. B. Mammary tumor showing complex cystic and solid echopattern. C. Colour doppler assessment of mammary tumour showing presence of blood flow in selected region of interest

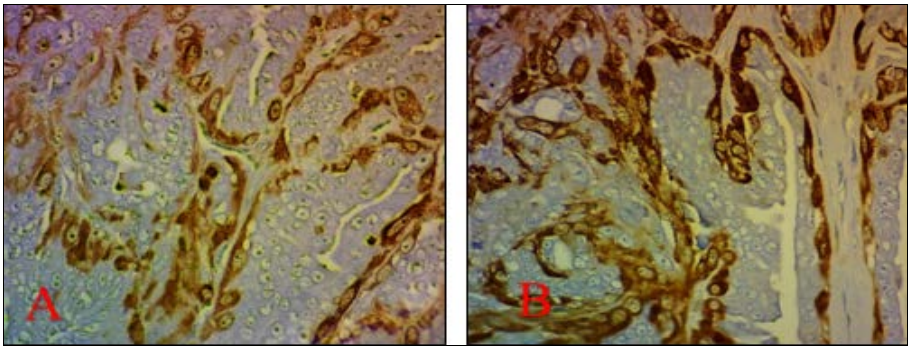

Fig 6: Immunoreactivity of Ki-67 in tubular adenocarcinoma. B. tubulo-papillary adenocarcinoma (400x).

Table 4: Mean haematological values of apparently healthy dogs and dogs with malignant mammary tumors

Parameter	Units	Apparently Healthy dogs (n=10)	Dogs with mammary tumour
Haemoglobin	(g/dl)	13.66±0.25	12.43±0.09*
PCV	%	41.06±0.79	37.41±1.49
TEC	(x 10^6/ µl)	7.09±0.21	5.87±0.13**
TLC	(x 10^3/ µl)	13.09±0.65	13.74±0.28
DLC	(%)		
Neutrophils	(%)	71.91±0.48	73.05±2.24
Lymphocytes	(%)	23.26±0.41	21.80±1.45
Monocytes	(%)	3.12±0.18	3.31±0.13
Eosinophils	(%)	1.71±0.09	1.84±0.08
Platelets	(x 10^5/dl)	3.20±0.22	2.87±0.11

**: Significant at $p<0.01$ when compared to apparently healthy dogs; *: Significant at $p<0.05$ when compared to apparently healthy dogs

Table 5: Mean serum biochemical values of apparently healthy dogs and dogs with malignant mammary tumors

Parameter	Units	Apparently Healthy dogs	Dogs with mammary tumour
BUN	(mg/dl)	19.04±1.35	13.15±0.38**
Creatinine	(mg/dl)	0.89±0.10	0.96±0.10
ALT	(IU/L)	36.4±2.19	46.20±0.81**
AST	(IU/L)	47.12±1.14	43.28±1.37
ALP	(IU/L)	52.61±3.49	57.21±1.23
Total Protein	(g/dl)	6.81±0.20	7.38±0.11

**: Significant at $p<0.01$ when compared to apparently healthy dogs;
*: Significant at $p<0.05$ when compared to apparently healthy dogs

4. Conclusion

Cytology plays an important role in the diagnosis of malignant mammary tumours but histopathology acts as a gold standard technique and also confirms the pathological type of tumours. Radiography plays an important role in diagnosis of early metastases especially in the lungs. Immunohistochemistry with proliferation biomarker such as Ki-67 might be useful in predicting prognosis of mammary tumours.

5. References

1. Devarathnam J, Suresh Kumar RV, Bharathi S, Anand Kumar A. Epidemiological studies of canine mammary gland tumors. The Pharma Innovation. 2021;10(7):13-17.
2. Krishna KR, Latha MJ, Lakshman M, Rani MU, Kumar YR, Swathi B. Expression pattern of PCNA and Ki-67 biomarkers in canine mammary tumours. The Pharma Innovation Journal. 2022;11(5):233-237.
3. Kumar K, Agrawal R, Pande N, Sharma S, Kumar H. Occurrence, clinico-haemato-biochemical and histopathological studies on mammary gland tumor in geriatric dogs. The Pharma Innovation. 2018;7(5):301.
4. Kumar P, Parasar MC. Occurrence of mammary tumor in dogs. Journal of Agricultural Research Advances. 2020;2(2):29-32.
5. Kumar VVVA, Kumari KN, Kumar KS, Gireesh V. Incidence of certain tumors in dogs. The Pharma Innovation Journal. 2017;6(12):137-141.
6. Nair SS, Narayanan MK, Anoop S, Krishna BD, Narayanapillai U, Martin KDJ. Tumour Node Metastasis Staging as Prognostic Indicator for Surgical Decision-making Tool in Canine Mammary and Superficial Neoplasms: A Survival Analysis Study. Indian Journal of Animal Research. 2022;B-4808:1-5.
7. Nunes FC, Damasceno KA, De Campos CB, Bertagnolli AC, Lavalle GE, *et al*. Mixed tumors of the canine mammary glands: evaluation of prognostic factors, treatment, and overall survival. Journal of Veterinary and Animal Sciences. 2019;7:100039.
8. Patel MP, Ghodasara DJ, Raval SH, Joshi BP. Incidence, Gross Morphology, Histopathology and Immunohistochemistry of Canine Mammary Tumors. Indian Journal of Veterinary Science and Biotechnology. 2019;14(4):40-44.
9. Rokad HA, Bhatt RH, Talekar SH, Vadalia JV, Padaliya NR, Dodia VD. Clinico-Epidemiological Study of Canine Mammary Gland Tumours. Indian Journal of Veterinary Sciences and Biotechnology. 2023;19(1):103-105.
10. Salas Y, Marquez A, Diaz D, Romero L. Epidemiological study of mammary tumors in female dogs diagnosed during the period 2002-2012: A growing animal health problem. Plos one. 2015;10(5):e0127381.
11. Silva DM, Juliani AI, Ribeiro CL, Guerios SD, Froes TR. Abdominal ultrasonographic findings in dogs with mammary tumors: association with tumor characteristics and survival. Turkish Journal of Veterinary and Animal Sciences. 2019;43(6):808-816.
12. Valenciano AC, Cowell RL. Cowell and Tyler's Diagnostic cytology and Hematology of the dog and cat. 4th edn. Elsevier, St. Louis, MO, USA; c2014. p. 118-132.
13. Wang AZ, Saha S, Todi RM, Guarin F, Paul KL, Jindal RP, *et al*. Contributions welcome; c2014.
14. Kumar V, Reinartz W. Creating enduring customer value. Journal of marketing. 2016 Nov;80(6):36-68.
15. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the lactate dehydrogenase assay. Cold Spring Harb. Protoc. 2018b Jun 1;2018(6):465-8.
16. Ciaputa R, Kandefer-Gola M, Nowak M, Madej JA. Prevalence of tumours in domestic animals in the Lower Silesia (Poland) in 2009-2011. Journal of Veterinary Research. 2013 Jan 1;57(1):53-9.