

Haemato-biochemical, cytological, histopathological and immuno-histochemical studies on canine mammary gland tumors

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ABSTRACT

The present study was carried out to investigate paraneoplastic syndromes along with histopathological and immunohistochemical characterization. A total of 55 tumors cases were evaluated and about 10 canine mammary tumor cases were recorded at Veterinary Clinical Complex, Tirupati, Andhra Pradesh over a period of 6 months (April 2025-September 2025). General clinical signs shown by these dogs were cancer anorexia, cachexia, inappetence, dullness, dehydration along with pigmented plaques in a few cases. Grossly, the tumor masses were predominantly cystic, nodular and solid, exhibiting a texture that ranged from soft to firm. Some of these masses were pedunculated, either solitary or affecting the entire chain of the mammary gland. Cytological analysis of mammary tumors revealed an acinar pattern of glandular epithelial cells alongside a limited number of myoepithelial cells exhibiting significant anaplasia. Hematological findings indicated the presence of anemia accompanied by neutrophilic leukocytosis, lymphocytosis, monocytosis and thrombocytopenia. Assessment of serum showed reduced levels of alanine amino transferase (ALT) and aspartate amino transferase (AST); elevated protein levels accompanied by increased gammaglobulin, along with hypoglycemia and hypocalcemia. The histopathological examination of the tumors identified simplex cystic papillary and mixed type carcinoma, tubulopapillary carcinoma, microacinar carcinoma, chondrosarcoma, fibrosarcoma, capillary hemangioma, as well as mixed mammary tumors including cystic chondrolipofibroma and hemangiochondrocystic fibrolipoma. Van Geison staining was performed for required set of tissues for differentiation of tissue origin. Immunohistochemistry was conducted with vimentin, cytokeratin for tissue differentiation, estrogen (ER) and progesterone receptor to confirm the presence or influence of hormones for tumor proliferation.

Keywords: Canine mammary tumors, Cytology, Haematology, Histopathology, Immunohistochemistry, Serum biochemistry

INTRODUCTION

The most common neoplasm in female dogs is mammary tumor. They get cancer on their own and have many biological, clinical, pathological, and molecular traits in common with human breast cancer. Additionally, breast cancer represents one of the leading causes of cancer-related in both species¹. In humans, approximately 10 million deaths related to cancer are reported each year, along with 19.3 million new diagnoses, whereas in dogs, there are 4 million new cancer cases diagnosed annually¹. Canine mammary tumors (CMT) pose a serious concern for veterinarians around the world because of their high frequency and low survival outcomes. Around 50% of these tumors are malignant, and due to their similarities with human breast cancer, they are often used as models for studying the disease. These tumors are most commonly found in older dogs, particularly those between 5 to 10 years old and are more prevalent in breeds like Labradors, Golden Retrievers and German Shepherds². While these tumors are prevalent, early diagnosis is essential for formulating treatment plans and avoiding unnecessary surgeries for a better prognosis. Cytology plays a crucial role in the early detection of tumors, and it is also an inexpensive, straightforward, and less painful diagnostic method. Nevertheless, cytology has its limitations, including the challenge of obtaining enough samples and the need for greater expertise in diagnosis. Since histopathology is considered the gold standard for tumor diagnosis, histopathological confirmation is always necessary for precise tumor identification. Additionally, poorly differentiated

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tumors can be challenging to differentiate from other types, and immunohistochemistry is beneficial in distinguishing tumors as well as identifying their nature and origin in such cases. The recent remarkable progress in molecular medicine, genomics, proteomics, and translational research will facilitate the identification of biomarkers, potentially equipping veterinarians and

clinicians with the most effective strategies to address diseases through early diagnosis and the implementation of appropriate treatments. The objective of the present study is to record the paraneoplastic syndromes, analyze haemato-biochemical alterations, pathomorphology of canine mammary tumors and evaluating with few immunohistochemical biomarkers.

MATERIALS AND METHOD

The study was conducted on cases of canine mammary tumors in dogs presented to the Department of Veterinary Clinical Complex (VCC), College of Veterinary Science (C.V.Sc), Tirupati and pet clinics of local veterinary practitioners in and around Tirupati.

Haematology: Two millilitre whole blood samples were collected in EDTA for haematological parameters such as haemoglobin(Hb) via Sahli's method²³, total leucocyte count (TLC), total erythrocyte count (TEC) using the dilution method⁹, differential leucocyte count (DLC), total platelet count following the Battlement method⁹.

Biochemistry: Blood samples obtained from dogs, collected in clot activating vacutainers for separation of serum and analysis of alanine transaminase (ALT), aspartate amino transferase (AST), gamma-glutamyl transferase (GGT), serum blood urea nitrogen (BUN), serum creatinine, serum total protein, serum calcium, serum alkaline phosphatase (ALP), glucose, albumin and globulin were conducted using an auto span semiautomatic biochemical analyser along with commercial biochemical kits (ERBA and Biosystem kits)

Cytology: Fine needle aspiration cytology (FNAC) and impression smears of tumor tissue samples were obtained. Smears were stained with with Field's A and B stains for examination²⁶.

Histology: Tumor tissue samples obtained after surgical mastectomy were collected and fixed in 10% neutral buffered formalin (NBF) for histopathological examination using routine Hematoxylin & Eosin stain after obtaining 3-4µm thin paraffin sections.³

Special stains: van Gieson staining was conducted as required on tumor tissue sections to distinguish collagen fibres from the surrounding stroma²⁴.

Immunohistochemistry: Immunohistochemistry was performed on tumor tissue cut sections onto APES-coated slides and incubated overnight at 37°C. After deparaffinization and rehydration, sections were immersed in a preheated antigen retrieval solution and cooled. The slides were then rinsed in Tris Ethylene Diamine Tetra-acetic Acid (EDTA) buffer, peroxidase blocking solution, and power block solution included in kit. Primary antibodies (Vimentin, Cytokeratin, Estrogen receptor, Progesterone receptor) obtained

from BioGenex Company in Bangalore, with 1:100 dilution were introduced, and the slides were kept at room temperature. Secondary antibody conjugated with poly Horseradish peroxidase obtained from BioGenex Company, were applied for 30 minutes and followed by tris EDTA buffer wash. The sections were then stained with DAB (Diamino benzidine), counterstaining with Harris hematoxylin and treated with lithium carbonate. The sections were air dried, mounted in Dibutylphthalate Polystyrene Xylene (DPX) and air dried again.

RESULTS

The present study has focused on the evaluation of different canine mammary tumors that were brought to the Veterinary Clinical Complex (TVCC), College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, between April 2025 and December 2025 for assessing paraneoplastic syndrome using clinical signs, haemato-biochemistry and histological classification. The research encompassed thorough patient histories, which entailed gathering crucial information such as breed, age, body weight and specifics about the quantity and location of the impacted mammary glands. The analysis of the findings included investigating the frequency of mammary tumor occurrences in relation to several factors, such as their overall appearance, age, breed, glandular involvement, cytological traits, histopathological characteristics and immunohistochemical profiling.

Occurrence of CMTs: The breed most frequently affected was the Pomeranian, accounting for 50% (5 cases), followed by the Labrador Retriever at 30% (3 cases). The other breeds included German Shepherds (10%) and Spitz (10%), with only isolated cases reported.

The occurrence of canine mammary tumours (CMT) across different age groups is a significant factor to take into account when researching this condition in dogs. Age of affected dogs varied from 1 to 15 years. The highest occurrence of CMT was noted in the age range of 10-12 years (50%), followed by the 13-15 years group (30%) and the 7-9 years group (20%). In contrast, there were no reported cases of CMT in dogs younger than 6 years of age.

Dogs weighing between 10-15 kg had highest (40%) occurrence of CMT, followed by dogs weighing between 30-35 kg (20%) and 35-40 kg (20%), least occurrence in 5-10 kg (10%) and 20-25 kg (10%) each. In dogs bearing weight of 0-5 kg, 15-20 kg, 25-30 kg no cases recorded.

The most common site of occurrence was caudal abdominal followed by inguinal, cranial abdominal and cranial thoracic mammary glands. In few cases more than one mammary gland was affected, where whole side mastectomy was conducted to excise the tumor mass.

Clinical signs: General signs of cancer anorexia, cachexia, inappetence, dullness and dehydration were observed. Pigmented plaques of melanin deposition in skin appearing brown to black color were also noticed. Tumor masses were ulcerated and necrosed with foul smell emitting in few mammary tumors.

Haematology: Haematology revealed anaemia with neutrophilic leucocytosis, lymphocytosis, monocytosis and thrombocytopenia compared with normal reference values (Table 1).

Biochemistry: Serum evaluation revealed decreased levels of ALT, AST; hyperproteinemia with hypergamma-globulinemia, hypoglycemia and hypocalcemia compared with normal reference values (Table 2).

Cytology of CMTs: FNAC and impression of different mammary tumors revealed predominance of malignant tumors over benign tumors. Cytological examination of cells from benign tumors showed minimal variation in both shape and size, with nuclear characteristics remaining unchanged. The cells exhibited strong adhesion

to one another and were found in clusters, indicating a high level of cohesion within the cellular mass. In contrast, smears from malignant tumors displayed signs of cellular anisocytosis (Fig.1a), pleomorphism, an increase in cell exfoliation, reduced cohesion among cells, hypercellularity, anisokaryosis (Fig.1b), an elevated nuclear to cytoplasmic ratio, nuclear moulding, a coarse chromatin pattern and presence of clumped chromatin (Fig.1c) with characteristic myoepithelial cells (Fig.1d) and mammary epithelial cells showing round to polyhedral shapes with single individual cells or clusters.

Histopathology of canine mammary tumors: In current study, a total of 10 cases of canine mammary tumors were recorded. The most frequently documented were malignant tumors, accounting for 7 cases (70%), while benign tumors were recorded in 3 cases (30%). These are classified according with WHO classification⁴.

Among the malignant tumors, simple cystic-papillary carcinoma was common, which was grossly located on left inguinal, right caudal abdominal and cranial abdominal mammary gland in one case; left

Table 1. Haematological parameters of different canine mammary tumors

Mammary tumors	Hb (g/dL)	TEC (millions/ μ l)	PLT (Thousands/ μ l)	TLC (Thousands/ μ l)	N (%)	L (%)	E (%)	M (%)
Ma1	8.6	5.45	669	33.5	80	15	3	2
Ma2	11	5.2	165	16	76	22	1	1
Ma3	11.2	6.87	149	12	86	12	1	1
Ma4	13.6	6.45	50	13.19	67	18	4	11
Ma5	14.6	7.06	177	12.42	7	81	2	10
Ma6	9.6	5.1	179	8	81	17	1	1
Ma7	11.8	6.07	198	17.08	67	19	3	11
Ma8	12	7.01	197	9	89	8	2	1
Ma9	22.2	10.6	194	12.22	72	16	5	7
Ma10	9	5	90	14	59	39	1	1
MEAN $\pm$ SE	12.36 $\pm$ 1.25	6.48 $\pm$ 0.52	206.8 $\pm$ 53.64	14.74 $\pm$ 2.26	68.4 $\pm$ 7.42	24.7 $\pm$ 6.77	2.3 $\pm$ 0.45	4.8 $\pm$ 1.50

Table 2. Biochemical parameters of different canine mammary tumors

Mammary tumors	ALT (U/L)	AST (U/L)	TP (g/dL)	Creatinine (mg/dL)	Alb (mg/dL)	Glob (mg/L)	Glucose (mg/dL)	Calcium (mg/dL)	GGT (U/L)	ALP (U/L)	BUN (mg/dL)
Ma1	16.72	33.33	5.1	0.57	2.88	2.22	23.77	4.97	4.35	40.42	28.8
Ma2	26.5	17.13	5.76	0.79	3.23	2.53	56.86	5.7	0.85	55	14.3
Ma3	17.91	34.64	7.12	0.68	4.04	3.08	50.81	4.28	0.69	35	15
Ma4	60	41.3	7.3	1.19	3.5	3.8	78.2	8.8	9	133	17
Ma5	32.09	46.95	6.66	0.68	3.76	2.9	40.17	4.37	1.46	43.5	13.2
Ma6	60.14	31.48	5.96	0.71	2.84	3.12	67.14	6.94	5.54	66	18
Ma7	22.1	26.52	6.44	1.22	1.84	4.6	62.67	10.2	3.4	55	14.2
Ma8	42	28	7.3	1.7	2.7	4.6	105	7.8	0.58	34	11
Ma9	7.01	20.1	8.26	0.68	2.92	5.34	64.83	4.24	1.2	55	18.4
Ma10	25.4	16.66	8.64	0.63	2.95	5.69	81.21	7.05	5.48	103	18
MEAN $\pm$ SE	30.99 $\pm$ 5.67	29.61 $\pm$ 3.17	6.85 $\pm$ 0.35	0.88 $\pm$ 0.11	3.06 $\pm$ 0.19	3.79 $\pm$ 0.38	63.7 $\pm$ 21.3	6.43 $\pm$ 0.65	3.25 $\pm$ 0.89	61.99 $\pm$ 10.11	16.79 $\pm$ 1.53

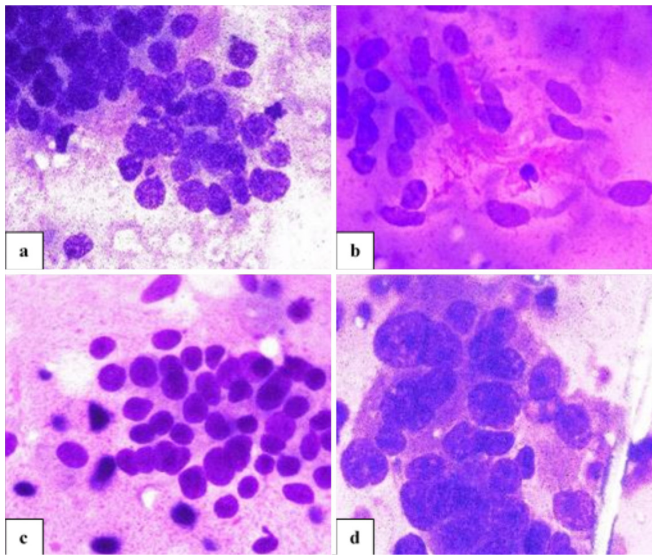


Fig.1. a. Round to polyhedral cells arranged in linear to cluster pattern with moderate amount of eosinophilic cytoplasm and round to oval nucleus with clumped chromatin. Field's stainx1000; b. Fusiform cells showing oval to spindle shaped nucleus with whispy borders and had clumped chromatin. Field's stainx1000; c. Mammary epithelial cells arranged in acinar pattern with prominent nucleoli and chromatin clumping. Field's stainx1000; d. Polyhedral cells with anisocytosis and anisokaryosis showing prominent nucleoli. Field's stainx1000.

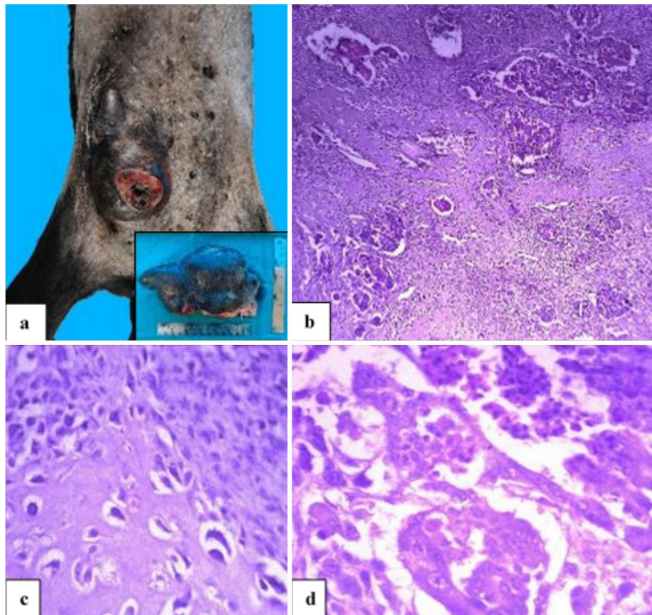


Fig.3. Chondrosarcoma with adenocarcinoma a. Ulcerated, hyperaemic and necrotic mass present on the right inguinal mammary gland; Inset: Hard mass of size 15cmx15cm after resection; b. Intermixed chondroid and glandular tissue. H&Ex40; c. Chondroid matrix showing cells with single to bi nucleus and cytoplasmic vacuolation H&Ex400; d. Carcinomatous cells with anisokaryosis and anisocytosis and few vacuolations. H&Ex400.

caudal abdominal and right inguinal mammary gland in other case. A white solid mass characterized by its pedunculated appearance few cystic structures and

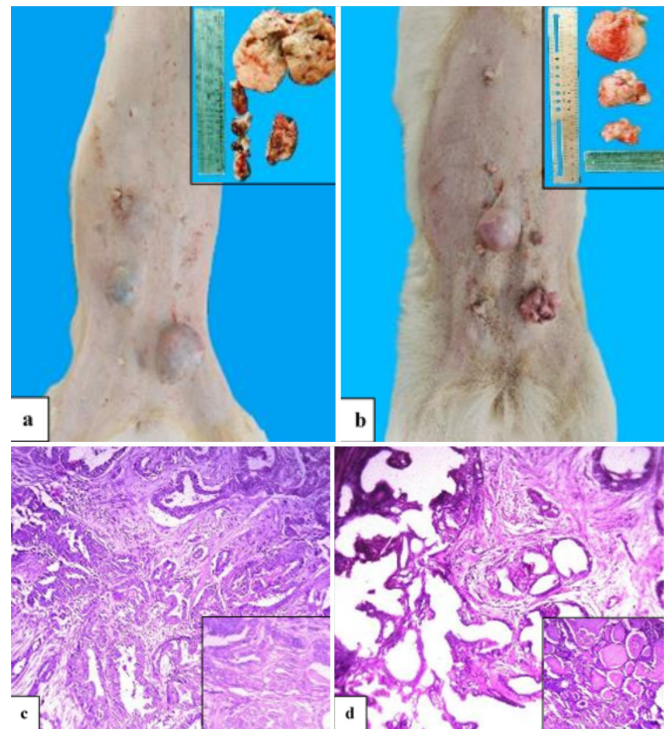


Fig.2. Simple cystic papillary carcinoma a. Solid masses on left inguinal, right caudal abdominal and cranial abdominal mammary gland. Inset: Cut section of mass with white solid and cystic areas; b. Solid multinodular masses left caudal abdominal and right inguinal mammary gland. Inset: White, solid, pedunculated mass with few cystic structures and nodular growth of varying sizes (7cm x 5cm; 5cm x 4cm); c. Glandular epithelium formed papillae that extended into dilated and cystic lamina. H&E x100; Inset: Tubules with proliferation of neoplastic epithelial cells with eosinophilic cytoplasm and nucleus appeared hypochromic with prominent nucleoli. H&E x400; d. Multiple cystic spaces along with projections into lumen. H&E x100. Inset: Glands dilated and cystic with few spaces filled with serous eosinophilic material H&E x10

nodular growth of varying sizes (7cm x 5cm; 5cm x 4cm) papillae that extended into dilated and cystic lamina (Fig.2 a). Many glands were dilated and cystic with few spaces filled with serous eosinophilic material (Fig.2b and 2c). Tubules were lined by multiple layers of columnar epithelial cells with eosinophilic cytoplasm and hypochromic nucleus with multiple nucleoli. Intertubular stroma had few congested blood vessels and infiltration of mononuclear cells.

A case of chondrosarcoma with adenocarcinoma was noted. It was located in right inguinal mammary gland with 15cmx15cm size, appeared ulcerated with hyperaemic and necrosed areas (Fig.3a). Histology revealed blending of chondroid tissue with glandular tissue. Neoplastic cells showed small round hyperchromatic nucleus with occasionally bi or multinucleate cells. The outline of nucleus was irregular with prominent nucleoli. Chondroid matrix was having cells with single to double nucleus, cytoplasmic vacuolation (Fig. 3b).

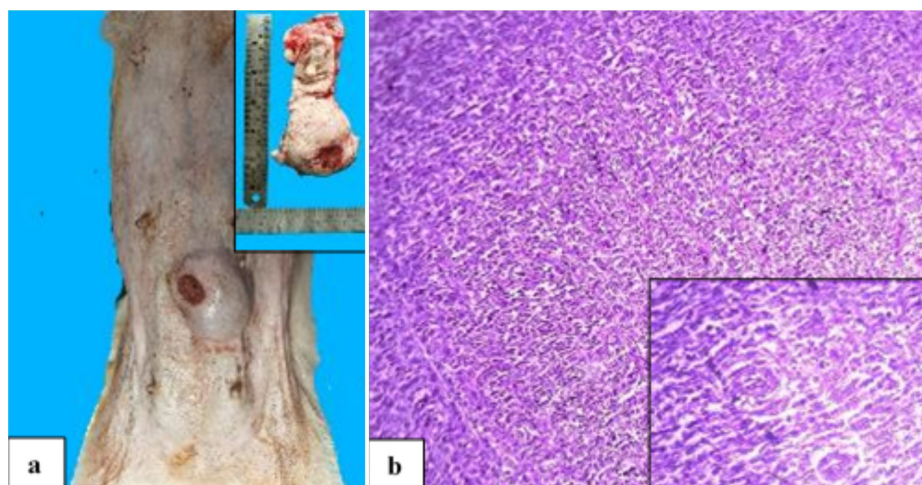


Fig.4. Capillary hemangioma **a.** Mass located in between caudal abdominal and inguinal mammary gland. Inset: Ulcerated solid hard mass with size of 8cm×6cm. **b.** Prominent neovascularization with compact arrangement of cells.H&Ex100. Inset: Capillaries are lined by endothelial cells which were elongated with oval nucleus and prominent nucleoli. H&Ex400

The carcinomatous cells showed anisokaryosis and anisocytosis with few vacuolations in some glands (Fig. 3c and 3d).

Capillary hemangioma appeared ulcerated, solid hard mass and located in between left caudal abdominal and inguinal mammary gland with a size 8cm×6cm (Fig. 4a). Histopathology revealed section showing compactly arranged microvasculature formed by neovascularization. Infiltration of inflammatory cells in between the capillaries was noticed amidst congested blood vessels. The capillaries were lined by endothelial cells which were elongated with oval nucleus and prominent nucleoli (Fig.4b and 4c).

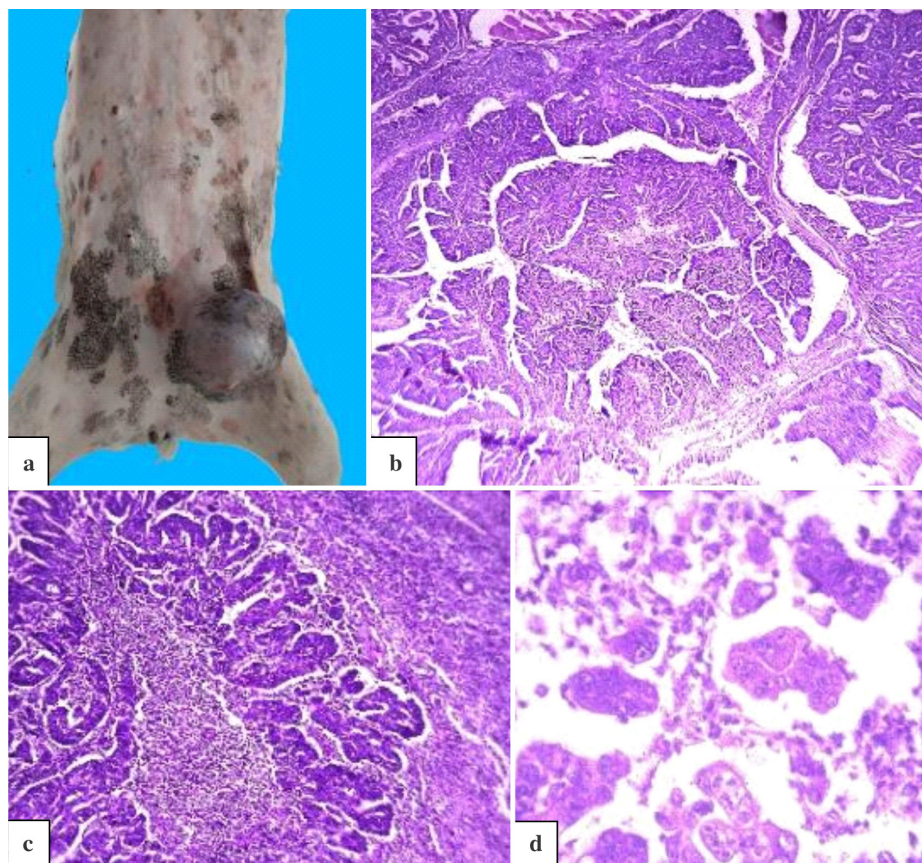


Fig.5. Simple tubulo-papillary carcinoma **a.** Mass located at cranial thoracic and left inguinal mammary gland. Inset: Simple tubulo-papillary carcinoma: Solid mass solid with few cystic spaces of sizes 10cm×8cm and a cystic mass of 11cm×12cm. **b.** Diffuse infiltration of glandular structures with several papillary projections into lumen.H&Ex40. **c.** Hyperplastic and hyperchromatic alveolar epithelial cells in single or multiple layers along the alveolar ducts exhibiting pleomorphism H&Ex100. **d.** Few giant cells with multiple nuclei and prominent nucleoli. H&Ex400.

The simple tubulo-papillary carcinoma was found in the cranial thoracic and left inguinal mammary gland. Grossly, mass appeared solid with few cystic spaces with sizes 10cm×8cm and a cystic mass of 11cm×12cm (Fig.5a). Histology showed diffuse infiltration of glandular structures with several papillary projections into lumen (Fig. 5b). Neoplastic cells were oval to round shaped with scanty cytoplasm arranged in multiple layers with prominent nucleus and multiple nucleoli. Areas of cholesterol deposition with cleft formation was evident.

Microacinar type mammary tumor was in left side between caudal abdominal and inguinal mammary gland. Mass was 12cm×7cm, hard, solid, and pedunculated. On incision, mass was solid white with cystic area towards periphery filled with serous fluid surrounded by necrotic borders (Fig.6a). Histological examination revealed formation of multiple islands of neoplastic cell infiltration

separated by fibrous vascular bands (Fig.6b). The islands revealed formation of acini by epithelial cells. The cells showed pleomorphism with eosinophilic cytoplasm and round to oval nuclei (Fig.6c). Van Gieson staining of section

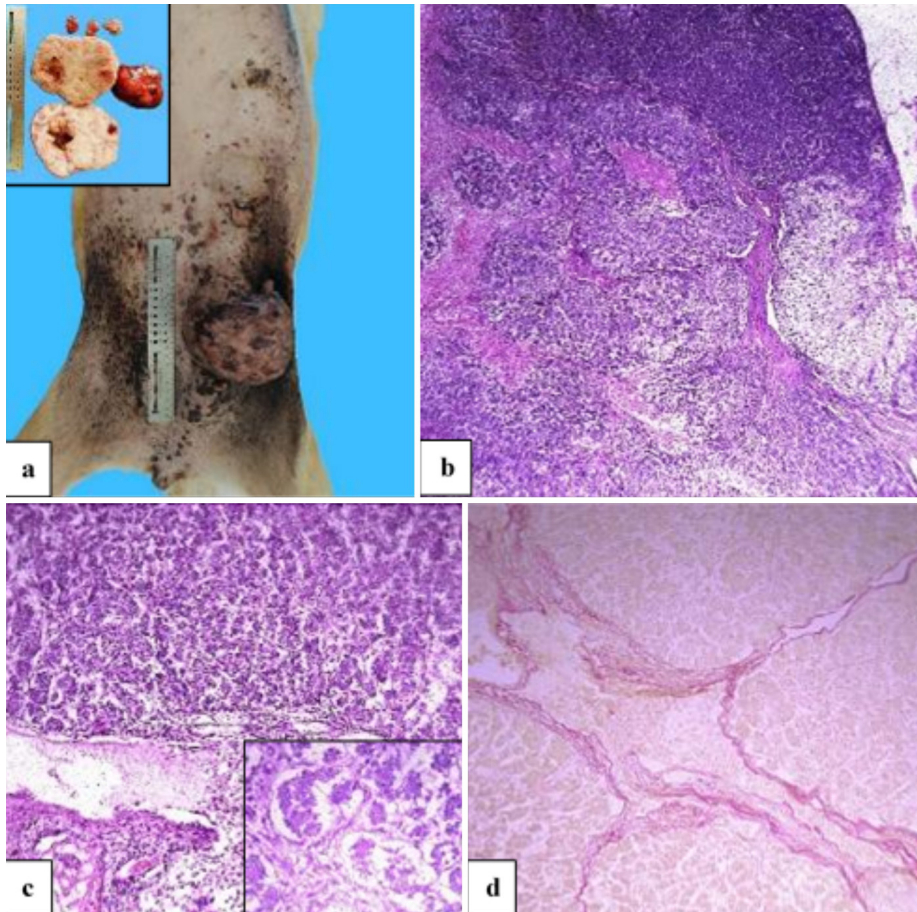


Fig.6. Microacinar adenocarcinoma **a.** Mass located on left side between caudal abdominal and inguinal mammary gland. Inset: Cut section showed solid white with cystic area towards periphery filled with serous fluid surrounded by necrotic borders. **b.** Multiple islands of neoplastic cells separated by fibrous vascular bands. H&Ex40. **c.** Acinar pattern with epithelial cells showing pleomorphism. H&Ex100. Inset: Few cells showing vacuolation with multiple nuclei and prominent nucleoli. **d.** Collagen stained red with glandular epithelium showing yellow stain. VanGiesonx100

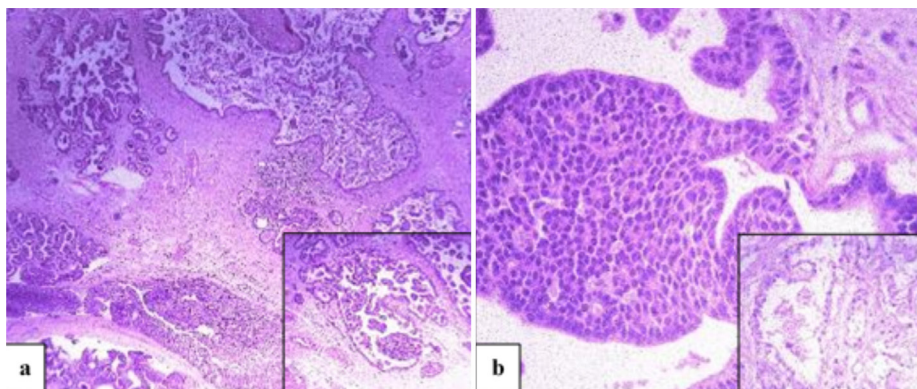


Fig.7. Mixed cystic-papillary carcinoma **a.** Papillary projections into lumen of glands surrounded by fibrous tissue. H&Ex40. Inset: Epithelial cells proliferation into long papillary projections. H&Ex100. **b.** Cells were proliferating from one pole of gland into lumen with round to elongated nucleus and eosinophilic cytoplasm. H&Ex400. Inset: Cells with foamy eosinophilic cytoplasm and round nucleus with prominent nucleoli and distinct borders. H&Ex400

showed positive staining for collagen (red) and glandular epithelium (yellow) (Fig. 6d).

In mixed cystic-papillary carcinoma, firm nodular mass was located on left cranial thoracic mammary gland. Histology revealed multiple glands with papillary projections into lumen with fibrous tissue proliferation in between the glands. Cystic spaces of glands with long papillary projections due to epithelial cell proliferation into spaces was noticed. Few areas showed glandular epithelial proliferation into solid structures (Fig.7a). The cells were proliferating from one pole of gland into lumen with round to elongated nucleus and eosinophilic cytoplasm. Few cystic spaces showed cells with foamy eosinophilic cytoplasm and round nucleus with prominent nucleoli and distinct borders characteristic of mammary foam cells (Fig.7b).

Fibrosarcoma of mammary gland was located at left caudal abdominal mammary gland with size of 11cm×5cm, which was hard solid with scarcely nodular appearance. Mass upon incision, showed viscous surface with greyish white areas and mild haemorrhagic areas (Fig. 8 a). Histopathology revealed high cellularity of fusiform shaped fibroblast cells proliferating into distinct interwoven pattern with varying size and shape. Fibroblasts showed spindle shape with small quantity of eosinophilic cytoplasm and whispy borders. Nucleus were oval to elongate which contained finely stippled chromatin and prominent nucleoli (Fig.8b).

Out of 10 tumor cases, two cases of mixed mammary tumors were recorded. Cystic chondro lipofibroma case with mass was located at caudal abdominal

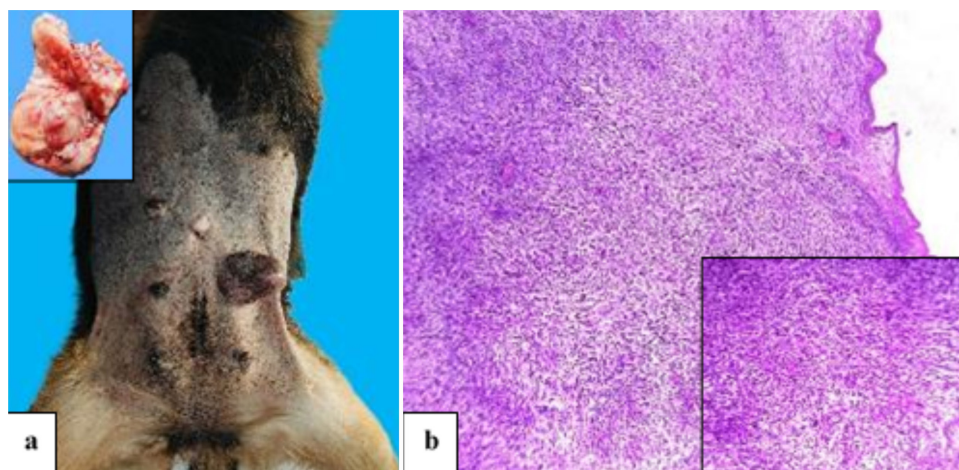


Fig.8. Fibrosarcoma **a.** Mass was located at left caudal abdominal mammary gland. Inset: Cut surface showing greyish white areas with mild haemorrhages. **b.** High cellularity of fusiform shaped fibroblast cells proliferating into distinct interwoven pattern. H&Ex40. Inset: Spindle shaped fibroblasts with small quantity of eosinophilic cytoplasm and wispy borders. H&Ex100

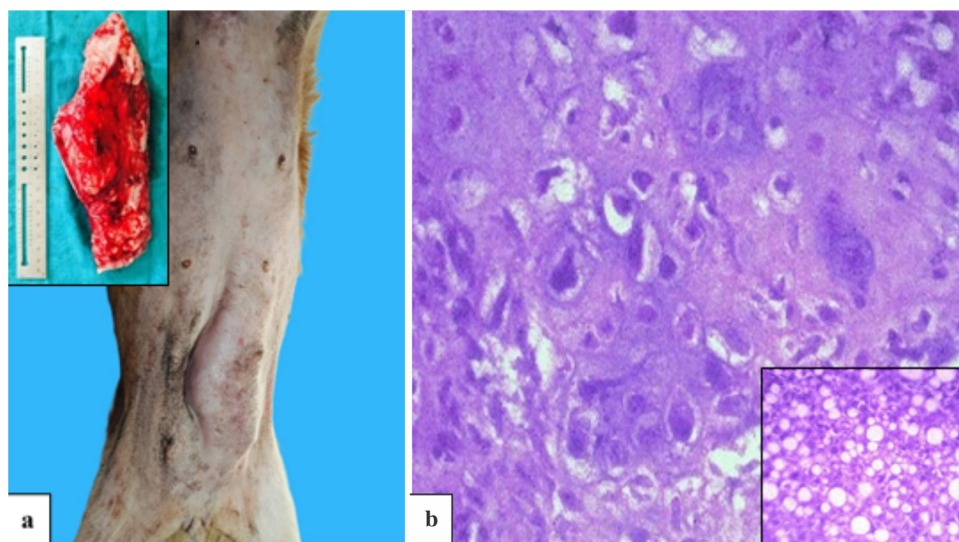


Fig.9. Cystic chondro lipofibroma **a.** Mass located at caudal abdominal mammary gland with hyperaemic areas. Inset: Solid hard mass measuring 15cm x 9cm. **b.** Matrix of chondroid tissue, adipocytes with cystic spaces of glandular tissue and proliferation of glandular epithelium. H&Ex40. Inset: Adipocytes with large cytoplasmic vacuolation and nucleus pushed to periphery. H&Ex400

mammary gland with size measuring 15cm x 9cm and appeared hard with fluctuation and hyperaemic areas (Fig.9a). Cut section of mass appeared cystic with mild grittiness and brown color slimy fluid. Histopathological examination of mass revealed multiple matrices of chondroid tissue, adipocytes with cystic spaces of glandular tissue and proliferation of glandular epithelium into multiple layers in few areas (Fig.9b). Adipocytes appeared with large cytoplasmic vacuolation and nucleus pushed to periphery. Some fibroblasts proliferated in between the adipocytes (Fig.9b).

Other tumor was cystic hemangio chondro fibrolipoma, mass was located in between caudal thoracic and cranial abdominal mammary gland (Fig.10a) with soft fluctuating and mild firm consistency with size of 13cm x 10cm. On cut section mass showed solid white areas with focal haemorrhagic areas and surface appeared viscous nature with blood tinge (Fig.10b). Histopathology revealed solid infiltration

with high cellularity and few areas showing numerous blood pockets with fibrous tissue proliferation around it (Fig.10c). Fusiform shaped fibroblasts proliferated with adipocytes which had large vacuoles with nucleus pushed to periphery (Fig.10d). Blood pockets were characterised numerous spaces filled with erythrocytes and lined by endothelial cells. Chondroid matrix was evident in few areas with bluish stroma having cells with multiple nucleus and vacuolated space around nucleus (Fig.10e). Special staining of tissue section with Van Gieson stain revealed positive for collagen staining red, muscle and glandular epithelium with erythrocytes staining yellow (Fig.10f).

Immunohistochemistry: In this study, a panel of four monoclonal antibodies was used to demonstrate various tissue markers in sequential paraffin-embedded tissue sections from clinical cases of CMTs. The mammary gland tumor showed positive reaction to the vimentin marker by staining cytoplasm of the neoplastic mesenchymal cells appearing brown color (Fig.11a and 11b).

The papillary projections of mammary gland epithelium in tubular papillary type tumor showed strong positive reaction with brown precipitate in the epithelial surfaces with cytokeratin (Fig.11c). The cystic papillary type of mammary tumor showed strong positive reaction to the cytokeratin marker staining the cytoplasm of the neoplastic epithelium (Figs.11d). The mixed mammary tumor showed

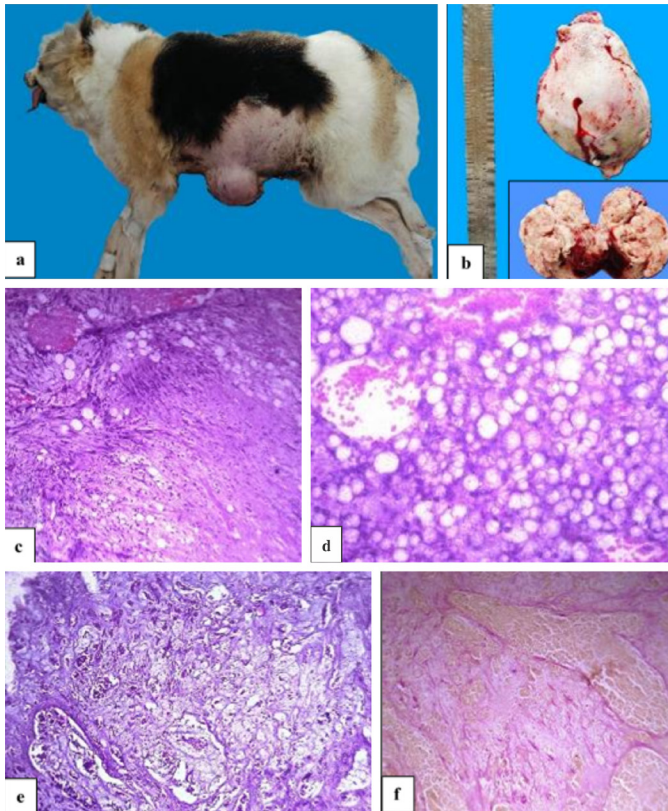


Fig.10. Cystic hemangio chondro fibrolipoma a. Mass located in between caudal thoracic and cranial abdominal mammary gland. b. Soft fluctuating mass with mild firm consistency and of 13cm×10cm size. Inset: Cut section showing solid white areas with focal haemorrhagic. c. Solid infiltration with high cellularity and few areas showing numerous blood pockets along with fibrous tissue proliferation. H&Ex40. d. Spindle shaped fibroblasts proliferation in between adipocytes. H&Ex100. e. Adipocytes having large vacuoles with nucleus pushed to periphery. H&Ex400. f. Chondroid matrix evident in few areas of bluish stroma having cells with multiple nucleus and vacuolated space around nucleus. H&Ex100. g. Positive staining for collagen appeared red; muscle and glandular epithelium along with erythrocytes staining yellow. VanGiesonx100

strong immunopositive reaction to the cytokeratin marker staining the epithelial cells cytoplasm.

ER and PR expressions revealed papillary adenoma of mammary gland showing mild positive immunostaining for both estrogen (Fig. 11e) and progesterone receptors as nucleus of glandular epithelium have taken brown color (Fig. 11f).

DISCUSSION

Based on the findings of the current study and those of previous researchers, it can be concluded that pet owners tend to prefer specific breeds such as Labrador, German Shepherd, and Pomeranian, among others, influenced by the popularity of these breeds in their respective regions. Consequently, the population of these breeds varies across different geographical areas, reflecting the breed-specific occurrence of various disorders, including

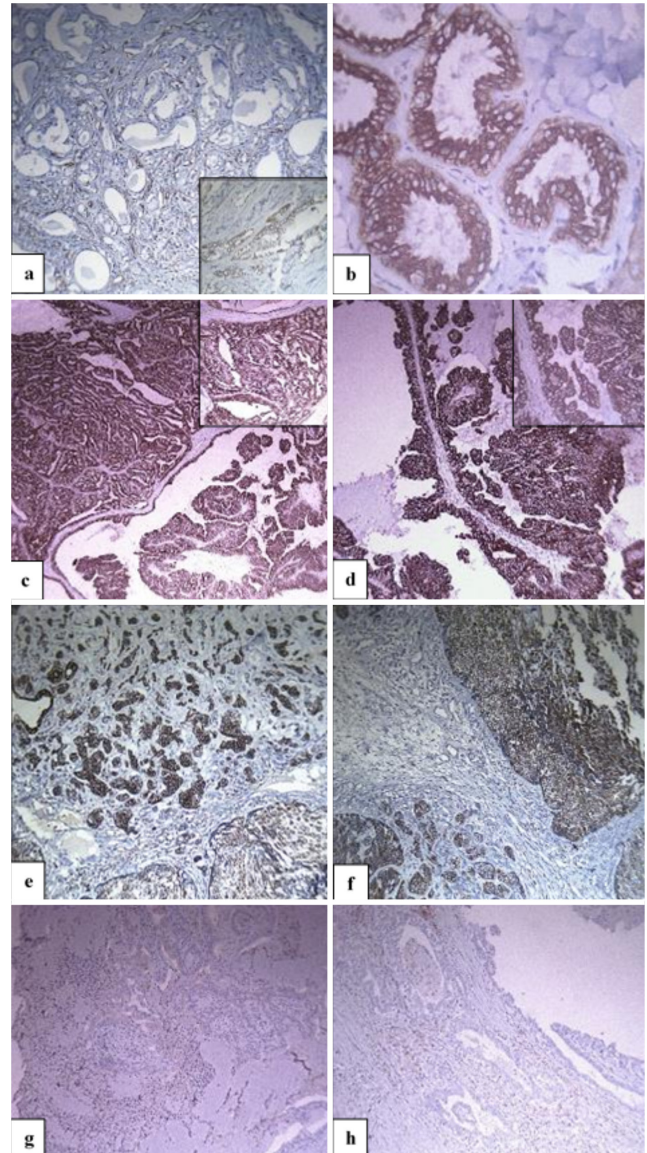


Fig.11. Immunohistochemistry. a. **Vimentin:** Strong positive reactivity of cells with marker in cytoplasm. IHCx100. Inset: Strong positive reactivity of mesenchymal cells with marker in cytoplasm. IHCx400; b. **Cytokeratin:** Epithelial cells showed strong positivity in the cytoplasm as a brown precipitate. IHCx400; c. **Cytokeratin:** Papillary projections of mammary gland epithelium in tubular papillary type showed strong positive reaction with brown precipitate in the epithelial surfaces. IHCx40. Inset: Epithelial cells of papillary projections showing high positivity to marker in cytoplasm. IHCx10; d. **Cytokeratin:** Cystic papillary type showed strong positive reaction to the cytokeratin marker staining the cytoplasm of the neoplastic epithelium. IHCx100. Inset: Cystic papillary type showed strong positive reaction to the cytokeratin marker staining the cytoplasm of the neoplastic epithelium. IHCx400; e. **Cytokeratin:** Mixed mammary tumor showed strong immunopositive reaction to the marker staining the cytoplasm of glandular epithelial cells. IHCx40; f. **Cytokeratin:** Mixed mammary tumor showed strong immunopositive reaction to the marker staining the epithelial cells cytoplasm. IHCx40; g. **Estrogen receptor:** Papillary adenoma of mammary gland showed moderate positive nuclear immunostaining. IHCx100; h. **Progesterone receptor:** Papillary adenoma of mammary gland showed moderate focal positive immunostaining. IHCx100.

mammary tumors. While the precise reasons for the natural predispositions observed in certain breeds remain unclear, the inheritance of abnormal genes or oncogenes in purebred dogs can be partially attributed to the selective breeding of specific genetic traits over time to achieve the desired morphological characteristics of a breed⁵.

The age-related incidence of canine mammary tumors (CMT) can also be explained by hormonal fluctuations, particularly the exposure to progesterone and estrogen⁶. Dogs that are spayed before their first heat cycle have a significantly lower risk of developing mammary cancers compared to intact (unspayed) females⁶. With each heat cycle a dog experiences, the risk of CMT decreases further⁶. The results of the current study indicated a rising frequency of mammary tumors from the cranial to the caudal region majorly involving the caudal and followed by inguinal mammary gland. In several previous studies, the majority of CMT cases were reported in two pairs (inguinal and caudal abdominal). This may be attributed to the fact that these areas contain the largest glands with a greater amount of mammary tissue, making them more vulnerable to various physiological changes that could result in neoplasms⁶.

Clinical signs were cancer anorexia and cachexia along with pigmented plaques which are observed by several researchers⁷. Haematology reported anaemia with neutrophilic leucocytosis, lymphocytosis, monocytosis and thrombocytopenia due to lack of erythropoietin, release of cytokines which suppress megakaryopoiesis, necrosis and inflammation^{8,9}. Serum evaluation revealed decreased levels of ALT, AST; hyperproteinemia with hypergammaglobulinemia, hypoglycemia and hypocalcemia due to severe damage of liver leading to increase due to antigenic stimulation leading to production of gammaglobulins or decrease in production of proteins and deviated gluconeogenesis^{7,10-12}.

Cytology is a fast and cost-effective method for diagnosing tumors. This technique aids in the early detection of mammary gland tumors in dogs. Cytological classification and prognostic methods are well established in human breast cancers, achieving a correct cytological diagnosis rate exceeding 90% when compared to diagnoses in veterinary medicine¹³. Cytological examination is utilized to distinguish between benign and malignant tumors, which can be regarded as adequate for the cytological diagnosis of benign and malignant tumors^{7,13}.

Histopathologically 70% malignant and 30% benign were reported, revealing simplex cystic papillary is most common, followed by mixed type carcinoma, tubulopapillary carcinoma, microacinar, chondrosarcoma, fibrosarcoma, capillary hemangioma

and mixed mammary tumors such as cystic chondro lipofibroma and hemangio chondro cystic fibrolipoma¹⁴⁻¹⁹. The participation of multiple mammary glands in dogs is relatively common, as noted in this study and others²⁰.

Immunohistochemical analyses of papillary type tumors demonstrated moderate positive reactivity for vimentin and strong positive reactivity with cytokeratin, as evidenced by the staining of the cytoplasm in neoplastic epithelial cells. The vimentin marker also exhibited positive immunostaining in the cytoplasm of mammary gland epithelial cells, indicating positive reactivity. Estrogen Receptor (ER) and Progesterone Receptor (PR) play crucial roles in the normal development and functioning of the mammary gland. However, their involvement in the development and progression of mammary gland tumors has recently captured the attention of the scientific community, aiming to unravel the complex behavioral patterns of this neoplasm²¹. Additionally, estrogen and progesterone receptors showed positive staining in the immunohistochemical examination^{13,21}.

Paraneoplastic syndrome reported during this study were mainly anaemia, neutrophilic leucocytosis, lymphocytosis, monocytosis and thrombocytopenia along with hypoproteinemia, hypoalbuminemia, hypoglycemia, hypocalcemia, inflated levels of AST, ALT and elevated levels of ALP and BUN in some cases^{7,10,11}.

CONCLUSION

Mammary cancer ranks among the most commonly diagnosed malignant tumors in canines, and it is the most prevalent tumor found in non-spayed female dogs¹. CMT is a significant health issue in dogs and because of its traits, such as size similarities, swift growth, and comparable clinical stages to human breast cancer, it is regarded as a compelling option for comparative studies.⁶ Our research concludes that paraneoplastic syndrome can be used as an early indicator for early diagnosis of canine mammary tumors. Haematology and serum biochemistry give information regarding the status of the condition and prognosis. Histopathology add the best tool for the diagnosis of different canine mammary tumors. Immunohistochemistry is a potent method for classifying the CMTs²². The findings of this study indicate that canine mammary tumors can be utilized as models for research comparable to human breast cancers, promoting further studies in the fields of medicine, pharmacology, and molecular pathways.

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