

CLINICOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND METASTATIC POTENTIAL ASSESSMENT OF LIPID-RICH (SECRETORY) CARCINOMA OF MAMMARY GLAND IN A DOG

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Received: 29.05.2022; Accepted: 19.07.2022

SUMMARY

A 5-years-old intact female Sptiz dog had a single spherical and multinodular, greyish white mass, measuring about 7 cm, on the left cranial abdominal mammary gland. Haematological examination revealed anaemia with neutrophilia while biochemical estimation revealed elevation of Blood Urea Nitrogen (BUN), creatinine, Alanine transaminase (ALT), Alkaline phosphatase (ALP), Calcium and Phosphorus. Cytological examination revealed the presence of numerous clusters of epithelial cells, showing anisokaryosis and vacuolated background. Histopathologically, the neoplastic cells were round to oval with distinct cell borders and arranged as predominantly as lobules and were separated by fibrovascular connective tissue. The cytoplasm contained multiple small to large solitary vacuoles, which pushed the nuclei to the periphery of the cell (Signet ring cell). Immunohistochemically, the cytokeratin was expressed in the cytoplasm of the neoplastic cells with moderate intensity. Immuno positive for vimentin noticed within the interlobular connective tissue and 60-70 % of the neoplastic cells were showed moderate immune reactivity to Matrix metalloproteinase-2 (MMP-2). Based on the cytological, histopathological, and immunohistochemical examination, the case was diagnosed as special type of lipid- rich carcinoma which is more prone to metastasis. Post survival study confirmed the death of animal within three months after surgical excision of tumour.

Keywords: Dog, Lipid-Rich Carcinoma, Mammary Tumour, Cytokeratin, Vimentin

How to cite: Kumar, V., Ramesh, S., Thangathurai, R., Parthiban, M., Ramprabhu, R., Thangapandiyam, M. and Rao, G.V.S. (2023). Clinicopathological, immunohistochemical and metastatic potential assessment of lipid-rich (secretory) carcinoma of mammary gland in a dog. *Haryana Vet.* 62(SI): 119-121.

After skin tumours mammary gland tumour is the second most common type of tumour affecting dogs accounting for 25-42% of canine neoplasms and of various special types of tumours arising from mammary gland includes squamous cell carcinoma, haemangioma, chondroma and osteosarcoma etc. Amongst these, lipid rich carcinoma is a rare type of tumour, which appears mostly in younger intact female dogs. It is more aggressive, poorly differentiated, lacks estrogen receptors with more chances for metastasis to regional lymph node and other organs (Espino *et al.*, 2003). It is a very unusual type of infiltrating duct mammary cancer in dogs, and its pathological characteristics are still unknown (Misdorp, 2002). Anaemia is one the most common haematologic findings in human cancer. About 30 to 50% of patients are anaemic at the time of initiating cancer therapy in solid tumours (Spivak *et al.*, 2009). Elevation of ALT, ALP and total protein occur in few mammary tumour cases in dogs (Kumar *et al.*, 2018). Based on histopathological characteristic features, this tumour is classified under simple carcinoma of mammary gland. The neoplastic cells are round to oval and vesicular in origin from glandular epithelium which is resembling sebaceous gland tumour has been reported in human being (Pena *et al.*, 2003). Cytokeratin and vimentin are the most important tumour markers used for differentiation of epithelial tumours in canine (Kumar *et al.*, 2020; Thangapandiyam *et al.*, 2022).

Immunohistochemical expression of cytokeratin was negative and vimentin positive, which takes interlobular connective tissue (Meuten, 2017). The incidence of lipid rich carcinoma in dogs very low and diagnosis is challenging due to minimal immunohistochemical expression. The present paper describes and recorded about the clinico pathological and metastatic potential of lipid- rich carcinoma in a Sptiz intact female dog.

A five-years-old intact female Sptiz dog was presented to Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli with the history of single spherical and multinodular, greyish white mass, measuring about 7 cm, on the left cranial abdominal mammary gland (Fig. 1). Fine needle aspiration cytology was performed following the standard procedure. Smears prepared from tumour were stained with Leishman-Giemsa stain. Haematological and biochemical studies were carried out by automated cell counter and automated bio chemical analyzer using standard diagnostic kits (Agappe Diagnostic Ltd). Following surgery, tissue samples were collected in 10% formalin followed by routine paraffin embedded tissue sections were prepared and stained with haematoxylin and eosin (H&E) stain for histopathological studies. Immunohistochemical staining was performed using primary antibodies *viz.* cytokeratin and vimentin, oestrogen receptor (ER), Progesterone receptor (PR) and Matrix metallo proteinase (MMP-2) and as secondary antibody with super sensitive labelled poly

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Fig. 1. Dog-Mammary growth- Left cranial abdominal-Multinodular-Cystic-Lipid rich carcinoma

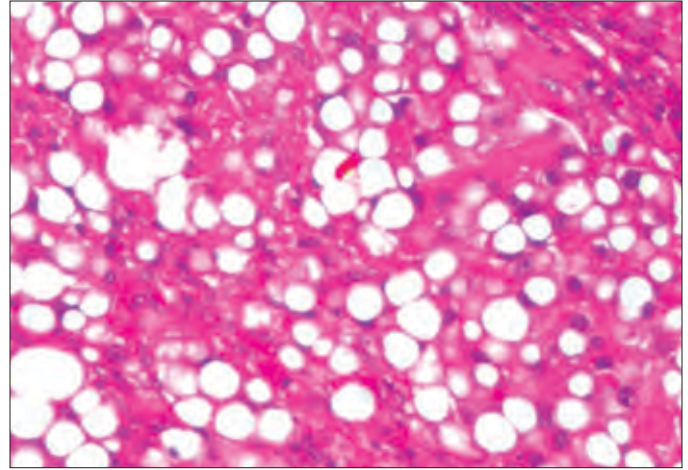


Fig. 2. Lipid rich carcinoma-Numerous varying sized clear vacuoles in cytoplasm and Background- H&E x 200

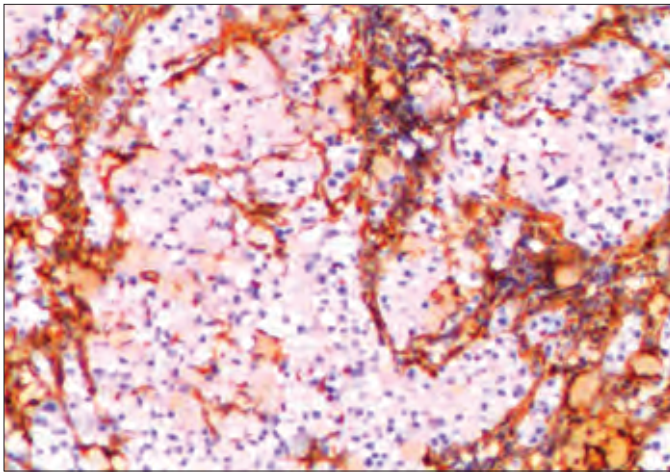


Fig. 3. Lipid rich carcinoma-Vimentin-Brown coloured-Inter lobular tissue- IHC x 100

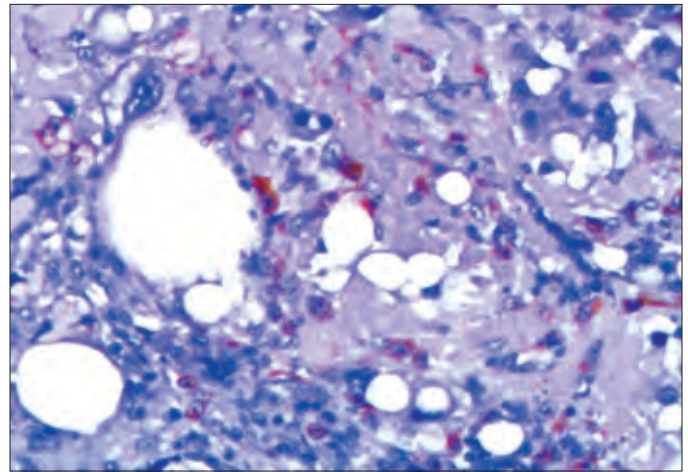


Fig. 4. Lipid rich carcinoma-MMP-2-Brown coloured-60-70%-Positive-IHC x 200

horse radish peroxidase (Anti-rabbit IgG raised in goat) used as per the recommendations of the manufacturer (Pathansitu, USA).

On clinical examination, the dog was emaciated, dehydrated, pale mucous membrane, enlarged prescapular lymph nodes. Radiological examination of the lung revealed moderate milliary metastases. Haematological examination showed the Hb-3.4 g/dL (Normal:12-18 g/dL), PCV-14.5% (Normal: 37-55%), RBCs- $1.71 \times 10^6/\mu\text{L}$ (Normal: $5.5-8.5 \times 10^6/\mu\text{L}$), WBC-21000/ μL (Normal: 5500-16900/ μL), platelets-95000 (Normal: 1.75-5 lakhs) MCV-35 fL (Normal: 60-72 fL) and MCHC-23.4 g/dL (Normal: 31-37 g/dL). Differential leucocyte count revealed neutrophils-85% (Normal: 65-75%), lymphocyte-09% (Normal: 20-25%), eosinophils-2% (Normal: 2-5%) and monocytes-4% (Normal: 5%). The present haematological findings confirmed microcytic hypochromic anaemia based on MCV value. The MCV value was lower than the normal limit. The differential leucocytes count showed increased numbers of neutrophils, which might be due secondary bacterial infection followed

by lymphocyte, monocytes and eosinophils. Blood pictures revealed anisocytosis, poikilocytosis, hypochromasia, nucleated RBCs and few immature neutrophils. The present findings were in accordance with that of (Lallo *et al.*, 2016) who also reported few mammary tumour cases showed anaemic changes due to increased erythrocytic destruction. Serum BUN-102 mg/dL (Normal: 10-28 mg/dL), Creatinine-1.8 mg/dL (Normal: 0.5-1.5 mg/dL), ALT-180 IU/L (Normal: 21-102 IU/L), ALP-243 IU/L (Normal: 20-156 IU/L), TP-4.2 g/dL (Normal: 5.4-7.1 g/dL), albumin-1.4 g/dL (Normal: 2.3-3.8 g/dL), calcium-12.5 mg/dL (Normal: 9.0-11.3 mg/dL), phosphorus-3.5 mg/dL (Normal: 2.6-6.2 mg/dL), total bilirubin-0.25 mg/dL (Normal: 0.15-0.5 mg/dL) and direct bilirubin-0.05 mg/dL (Normal: 0.06-0.12 mg/dL). The increase in BUN and Creatinine might be due to dehydration. While elevation of ALT, ALP and calcium might be attributed to paraneoplastic syndrome. These findings were in agreement with that of (Kumar *et al.*, 2018). Ultrasound and X-ray examination revealed military metastatic foci in lungs. Grossly, numerous cystic spaces

contained yellowish viscous fluid. Cytological examination showed polyhedral cells with high cellularity and marked anisocytosis and anisokaryosis. Neoplastic epithelial cells were arranged in clusters and separately. Small to large lipid vacuoles of various sizes were as reported earlier in cytoplasm and between the neoplastic cells (Myung *et al.*, 2014). The presence of lipid vacuoles in the cytoplasm of tumour cells might have aided in the spread of cancer to distant organs and clinical aggressiveness (Le *et al.*, 2009). Histopathologically, cystic spaces were separated by fibrovascular connective tissue, solid nests and cords were formed, round to oval neoplastic cells with defined cell boundaries and moderate amount of cytoplasm separated by fibrous stroma. Anisokaryosis and anisocytosis were moderate to severe with variable numbers of mitoses and minimal tubular formation. There were multiple small or large solitary lipid vacuoles in the cytoplasm (Fig. 2) that pushed nuclei to the periphery of the cell (Signet ring cell) and interlobular space, which was in concurred with Perez *et al.* (2005) who described that the tumour had tubular structure and lipid vacuoles in its cytoplasm, allowed tumour cells to survive in the blood and enhance tissue invasion. Lymphocytes, plasma cells, and eosinophils penetrated the tumour growths in a scattered form. Moritani *et al.* (2011) also reported lipid rich carcinoma contained lipid droplets in more than 90% of the neoplastic cells. The cytokeratin expression was showed moderate immuno-reactivity with 60% of cells were positive but negative for vimentin. In contrast vimentin (Fig. 3) showed positivity of interlobular connective tissues. In the present case, estrogen receptor alpha and progesterone receptors were negative. Our findings were in agreement with that of Espino *et al.* (2003) who also reported cytokeratin positive and vimentin negative in lipid rich carcinomas. MMPs play an important role in the mechanism of degrading the stromal connective tissue and basement membrane components of tumours. MMP-2 and MMP-9 have been associated with malignant tumour progression and metastasis in canine mammary carcinoma (Papparella *et al.*, 2002). In the present case, the MMP-2 expression was showed strong intra cytoplasmic and membranous with 60-70% of cells were positive (Fig. 4). Our findings were correlated with that of (Loukopoulos *et al.*, 2003) the reported higher MMP-2 levels could be observed in, malignant tumours than in inflammation and benign tumours.

In conclusion, a case of metastatic lipid rich carcinoma tumour was conformed in a Sptiz intact female dog based on cytological, histopathological and immunohistochemical findings. However, after surgical removal of tumours the dog died within 3 months (78 days).

ACKNOWLEDGMENT

The authors express their gratitude to The Director

of Clinics (Tamil Nadu Veterinary and Animal Sciences University) for providing the samples and for permitting the usage of facilities to conduct the work.

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