

Pulmonary metastasis of mammary tumor in a Labrador Retriever

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Abstract

Mammary tumors, known as the most frequent malignant neoplasm of intact female dogs, constitute an important problem in veterinary medicine. A 10-year-old female, intact Labrador Retriever was brought to the Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai for an autopsy. External examination revealed one growth of around 7×4 cm in the left caudal mammary gland. After opening the carcass, multiple greyish-pink colour nodular growth of around 0.5×0.4 cm were observed on the surface of the lung parenchyma. The mammary growth mass and lung tissue with growth were collected in 10% NBF for fixation and further processing, and sections were stained with H&E, Masson's trichrome stain and AgNOR stain. Histopathological examination revealed the presence of proliferating glandular epithelial cells arranged predominantly in a tubular fashion indicating tubular adenocarcinoma. Also, the proliferated cells showed positive nuclear expression of the PCNA marker.

Key words: Canine, Immunohistochemistry, Mammary gland, Metastasis, Pulmonary

Highlights

- Distant metastasis of the canine mammary tumor to the lungs was observed.
- Gross and histopathological diagnoses were performed.
- Immunohistochemistry with different markers were studied.

Mammary tumor constitutes a vital problem in both medical and veterinary science as it ranks as the second most prevalent tumor in canines and the first most prevalent tumor among human beings. The incidence of this is also observed in other animals like cats etc. (Nunes *et al.*, 2019). The origin of this tumor may be from the glandular and non-glandular epithelium of mammary glands (Misdorp, 2002). According to the origin of tissues, these tumors are of two types i.e. benign and malignant. Based on the histological diagnosis, around 41-53% of mammary tumors in bitches are considered malignant (Rezaie *et al.*, 2009). Primarily older animals with a mean age group of about 10 years are affected by mammary tumors. Most of the

affected animals are intact females or females who have undergone ovariohysterectomy in the latter part of life. But these tumors are rare in males and young animals of either sex (Nelson and Couto, 2003). Performing early ovariohysterectomy is strongly protective against the development of this tumor. Bitches neutered before the first oestrous cycle are at no greater risk for the development of this tumor. However, after 2.5 years of age and the second oestrous cycle, ovariohysterectomy has no protective values (Misdorp, 2002; Nelson and Couto, 2003). Canine mammary carcinoma is often used to metastasize to lymph nodes and lungs (Salas *et al.*, 2002). Mesenchymal structures of carcinoma can metastasize to the lungs with the

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help of a haematogenous vessel, whereas epithelial structures can metastasize to both lymph nodes and lungs by using lymphatic vessels. But sometimes, metastasis of both structures of carcinoma is also reported (Goldschmidt *et al.*, 2017). To recognition of the tumor type correctly, there is a need for proper histological and immunohistochemical diagnosis. This study was conducted to describe the occurrence of pulmonary metastasis of canine mammary tumour in a Labrador dog and diagnosed by histopathological and immunohistochemical methods.

A 10-year-old female, intact Labrador Retriever was presented to the Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India for necropsy. External gross examination revealed growth of about 7×4 cm in the left caudal mammary gland. Upon incision, multiple pink-coloured growths were seen. Internal gross examination revealed multiple, greyish pink color nodular growths of around 0.5×0.4 cm on the surface of the lung parenchyma (Fig. 1A). The nodular growth also spread into the deeper structure. The mammary growth mass and lung tissue with growth were cut into pieces and collected in 10% NBF (Neutral buffered formalin) for histopathological examination. The fixed tissue was trimmed after 36 hours, processed routinely by paraffin embedding technique, and sections were stained with H&E, Masson's trichrome stain (Bancroft and Gamble, 2008) and AgNOR stain. The immunohistochemical study was also performed for the markers like Vimentin, Cytokeratin and PCNA according to standard protocol.

Histopathological examination revealed the presence of the proliferating glandular epithelial cells arranged predominantly in a tubular fashion indicating tubular adenocarcinoma (Fig. 1B). There were significant nuclear and cellular pleomorphisms with the presence of a high number of mitotic figures (Fig. 1C). The pleomorphic cells were separated by proliferated connective stroma, and the

proliferation was confirmed by Masson's trichrome staining (Fig. 2A). Regarding staging, this malignant metastatic tumor showed T1N0M1 as the tumorous growth was less than 2 cm in size, no involvement of lymph node and with distant metastasis. There were 1 or 2 AgNORs (black colour) present in the neoplastic cell. Most of the tissue sections showed the presence of AgNORs, but the distribution pattern was variable (Fig. 2B). The cytokeratin expression was highly positive in the cytoplasm of epithelial cells (Fig. 3A). Also, the proliferated cells showed positive nuclear expression of the PCNA marker (Fig. 3B).

The canine mammary tumor is mostly related to hormonal imbalance i.e. sexual steroid hormones during an early stage of mammary carcinogenesis (Perez-Martinez *et al.*, 2000). Mainly middle-aged (9 to 11 years) intact female dogs were affected with mammary tumors beginning at approximately 6 years of age. In the present case, the animal was also 10 years old, which was in accordance with previous studies (Nelson and Couto, 2003). Among canine mammary tumors, approximately 50% are malignant, mainly metastasize to the lungs and brain, and a few cases metastasize to other organs (Benavente *et al.*, 2016) and around 26% in dogs spayed after a second oestrous (Henry, 2007). Only early ovariohysterectomy is strongly protective against mammary tumor development (Misdorp, 2002; Nelson and Couto, 2003). Metastasis of mammary tumors is commonly observed in lungs and regional lymph nodes and sometimes in distant lymph nodes, adrenal glands, pancreas, and central nervous system (Campos *et al.*, 2017; Goldschmidt *et al.*, 2017; Canadas *et al.*, 2019). But in this case, metastasis was only observed in the lungs. (Dantas *et al.*, 2012; Tunc *et al.*, 2022). Though there is a slight correlation between AgNOR count and the thickness of primary lesions but the correlation between AgNOR count and pathological staging was uncertain. Still, an AgNOR count of two or more per cell favours a diagnosis of malignancy of the tumor (Nagatani

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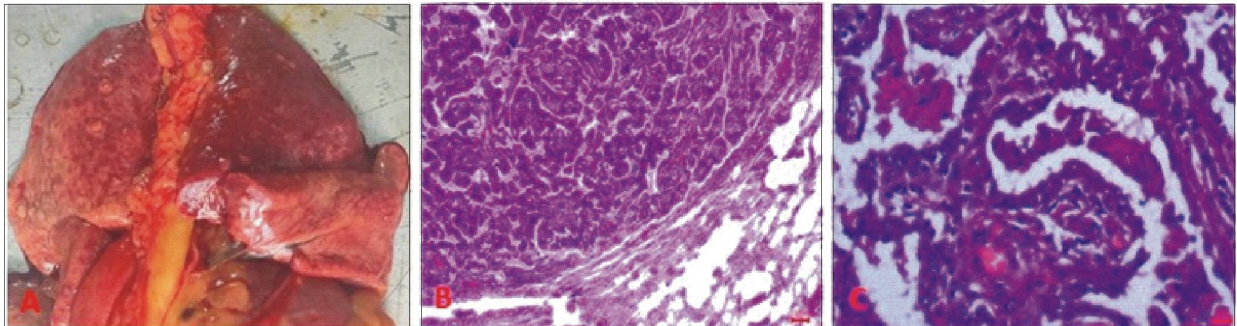


Fig. 1. A: Lungs- Multiple variable size nodular growth in lungs surface; B: Lungs- Adenocarcinoma (H&E, bar=100 µm); C: Lungs- Adenocarcinoma, nuclear and cellular pleomorphisms with the presence of mitotic figures (H&E, bar=10 µm)

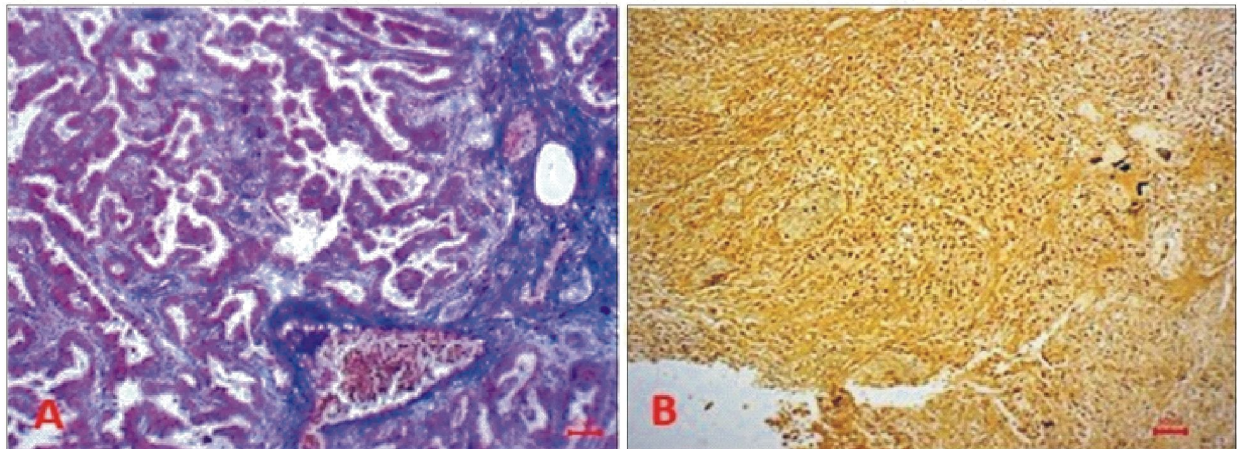


Fig. 2. A: Lungs- Adenocarcinoma, proliferating collagen fibre (blue colour) (MTC, bar= 50 µm); B: Lungs- Distribution of AgNORs in neoplastic in neoplastic cells (black colour) (AgNOR, bar= 50 µm)

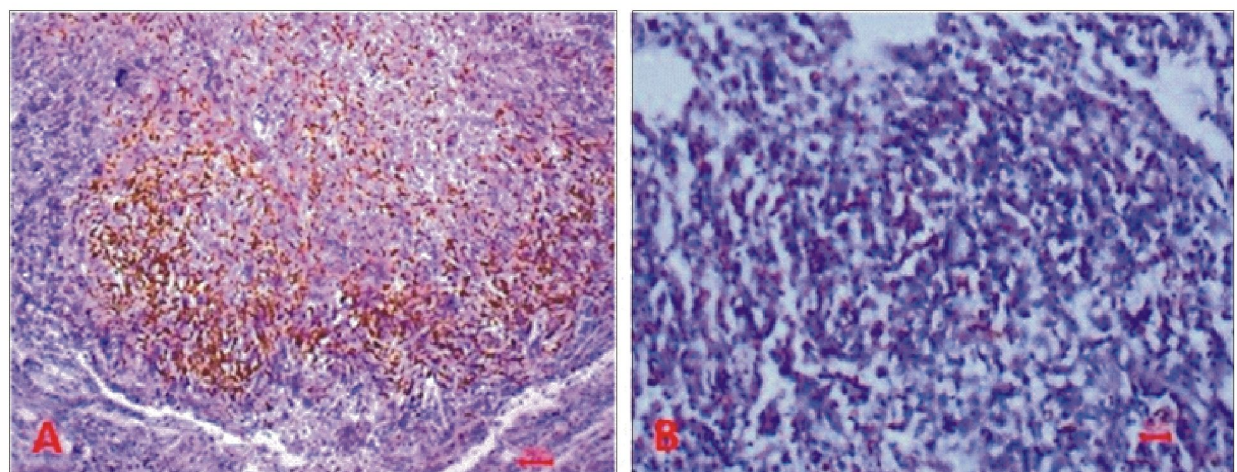


Fig. 3. A: Lungs- Adenocarcinoma, brown colour cytoplasmic positivity to cytokeratin (IHC, bar=50 µm); B: Lungs- Adenocarcinoma, brown colour nuclear positivity to PCNA (IHC, bar=20 µm)

et al., 1991). Once the tumor has undergone metastasis, then the prognosis for the patient is poor. The present case revealed the presence of an epithelial component confirmed by a positive immunohistochemistry reaction of cytokeratin, which is in accordance with other reports (Gudan *et al.*, 2011; Campos *et al.*, 2017). Some areas also showed a positive reaction to PCNA. Thus, the results showing adenocarcinoma with metastases were supported not only

histopathologically but also immunohistochemically.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: BB: Methodology and data curation; SV: Conceptualization; NP: Investigation; JP: Formal analysis; GVS: Editing and revision of the manuscript.

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