

Histological and Immunohistochemical Features of Pulmonary Metastatic Oral Melanoma in a Labrador Dog

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ABSTRACT

Melanoma is one of the malignant tumours which originate from melanocytes and is most commonly diagnosed as malignant tumours in the oral cavity of canines. One 13 years old, female Labrador presented for necropsy revealed left lung with white and grey mass of about 5 cm diameter with central black areas, occupying apical lobe and the cut surface was dark brownish to black in colour. The tumorous mass was collected in 10% Neutral buffered formalin and paraffin embedded sections were stained with H&E, Masson's trichrome stain, and Argyrophilic Nucleolar Organizer Region (AgNOR) stain. Microscopically, there was a multilobulated, neoplastic mass consisting of polygonal to spherical-shaped neoplastic cells containing round to oval vesicular nuclei. The melanoma was confirmed by cytoplasmic expression of Melan-A, Proliferative cell Nuclear Antigen (PCNA) and the sections also showed positive expression of both cytokeratin and vimentin indicating that the tumour was a mixed type, i.e., consisting of both mesenchymal and epithelial cells.

Keywords: Dog, Immunohistochemistry, Melanoma, Metastasis; Pulmonary.

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INTRODUCTION

Melanoma is a malignant tumour that originates from melanocytes. It has been reported in human beings as well as in many domesticated animal species (Reddy *et al.*, 1998), and wild terrestrial and marine animals. Melanomas are the most commonly diagnosed malignant tumours in the oral cavity of canines (Goldschmidt, 1985; Faramade *et al.*, 2017). Gingiva is the most common site for canine oral malignant melanoma (OMM) but other parts like palatine, labile or buccal mucosa also act as the sites of origin (Delverdier *et al.*, 1991). It is generally an aggressive tumour, often locally invasive, and frequently metastasizes to regional lymph nodes and lungs but metastasis to other organs like the brain, heart, spleen, and liver is not common (Goldschmidt and Hendrick, 2002). Canine OMM accounts for about 7% of all malignant tumours of canine, 11.5% to 17.1% of all oral tumours (Mikiewicz *et al.*, 2019), and 33% to 35.8% of all malignant oral tumours (Sarowitz *et al.*, 2017). OMM is reported in old age group animals mainly ranging from 7 to 14 years age (Esplin, 2008). Most common breeds affected by OMM include Cocker Spaniels, Golden Retrievers, Dachshunds, mixed-breed dogs (Gillard *et al.*, 2014) but histologically well-differentiated melanocytic neoplasms (HWDNM) also reported in Golden Retrievers, Labrador, Doberman Pinscher, Irish Setters, Cocker Spaniels, Beagles, etc. (Esplin, 2008). The diagnosis of melanoma is difficult mainly in tumors without appreciable melanin. Histological appearance resembles carcinoma, lymphoma, sarcoma, and osteogenic tumours. Therefore, immunohistochemistry with numerous melanoma specific markers is mostly used for confirmatory diagnosis in human and veterinary pathology (Wick, 2008). This case report is on the occurrence of oral melanoma with pulmonary metastasis in a Labrador dog.

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CASE HISTORY AND IMMUNOHISTOCHEMISTRY

A 13 years old, female, Labrador dog was presented for a necropsy to the Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animal was depressed and anorectic before death. Externally there was no discharge from natural orifices. However, visible mucous membranes were pale and a blackish mass of about 7×5×3 cm dimension was noticed on the anterior part of the hard palate.

The animal was examined thoroughly for the presence of any abnormal changes in different organs and the internal

body cavities. The left lung showed a white and grey mass of about 5 cm in diameter with central black areas, occupying the apical lobe (Fig. 1) and the cut surface was dark brownish to black in colour. Multiple, pinpoint to small spot calcified areas were scattered all over the lung parenchyma. The growth mass along with lung tissue was cut into pieces and collected in 10% Neutral buffered formalin (NBF) for histopathological examination. The fixed tissue was trimmed after 36 h, processed by paraffin embedding technique, and sections were stained with H&E and Masson's trichrome stain (Bancroft and Gamble, 2008). The degree of malignancy was assessed by using an Argyrophilic Nucleolar Organizer Region (AgNOR) stain. The immunohistochemical study was also performed for the confirmatory diagnosis by using markers like Melan A, Vimentin, Cytokeratin, and Ki-67 according to standard protocol.

RESULTS AND DISCUSSION

In this case, the oral melanoma was reported in a 13 years old Labrador dog. In general the oral melanoma occurs in the age range of 10.5 to 12 years (Head *et al.*, 2002). Microscopically, there was discrete distribution of numerous round cells with dark colour pigments and presence of haemorrhage in the tumorous mass collected from hard palate (Fig. 2). The lungs tissue with tumorous mass showed presence of multilobulated areas, polygonal to spherical shaped neoplastic cells (Fig. 3) containing round to oval vesicular nuclei. The nuclei showed moderate hyperchromatic with anisokaryosis. A moderate amount of cytoplasm along with fine grey to black pigment was observed. The neoplastic cells were of mixed populations with spindle shaped mesenchymal cells and epitheloid type cells (Fig. 4). The presence of the variable amount of melanin surrounded by stroma was also observed. Masson's trichrome stain was taken by proliferated stroma (Fig. 5). The findings of the present case coincide with histological features described by Goldschmidt and Goldschmidt (2017). Regarding staging, this malignant metastatic tumor showed T3N0M1 as the tumorous growth

of about 5 cm, no involvement of lymph node and with distant metastasis. This tumour can be classified as stage IV oral melanoma with pulmonary metastasis according to the template for the staging of tumours in dogs set by World Health Organization (Owen, 1980). But in this case there was no involvement of lymph nodes which may be due to spread of tumour cells through blood or body's tissues. There were 1 or 2 AgNORs (black colour) presenting the melanocytic nevi per cell. Most parts of the tissue section showed the presence of AgNORs, but the distribution pattern was variable (Fig. 6). 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. So AgNOR method is difficult to use for differential diagnosis between benign pigmented lesions and malignant melanoma. Still, an AgNOR count of two or more than two per cell favours a diagnosis of malignant melanoma (Nagatani *et al.*, 1991). The lungs showed infiltration of mononuclear cells in the interstitial spaces as well as in peribronchiolar spaces indicating broncho interstitial pneumonia (Fig. 7). There was presence of cholesterol clefts along with caseating granuloma and mineralization. Lungs also showed congestion, haemorrhages, and oedema. The melanoma was confirmed by cytoplasmic expression of the Melan-A marker (Fig. 8). Cytoplasmic expression of both cytokeratin (Fig. 9) and vimentin (Fig. 10) indicated that the tumour was a mixed type, *i.e.*, consisting of both mesenchymal and epithelial cells. The proliferation of cells was confirmed by the cell's nuclear part expression by PCNA markers (Fig. 11). The confirmation of melanoma was done by immunohistochemistry markers like vimentin and Melan A (Ramos-Vara and Miller, 2011). Based on the gross, histopathology, and immunohistochemical reactivity, the case is diagnosed as malignant oral melanoma with pulmonary metastasis. As around 60% of cases showed the transformation from benign to malignant during the first decade of life. So, it is recommended for prophylactic excision as early as possible (Tannous *et al.*, 2005).



Fig. 1: Lungs- White and grey mass of about 5 cm size

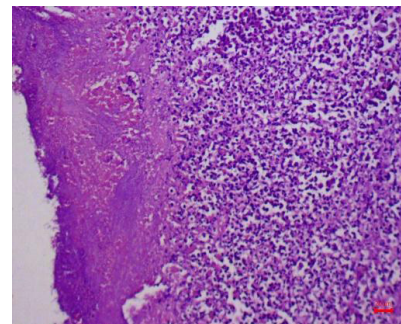


Fig. 2: Tumour mass (Hard Palate)-Distribution of numerous round cells with dark colour pigments and presence of haemorrhage (H&E, Scale bar=20 µm)

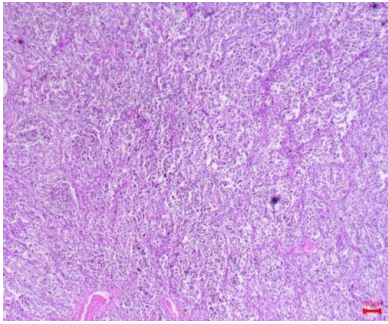


Fig. 3: Lungs- Melanoma: Multilobulated, neoplastic mass consisting of polygonal to spherical shaped neoplastic cells (H&E, Scale bar =100µm)

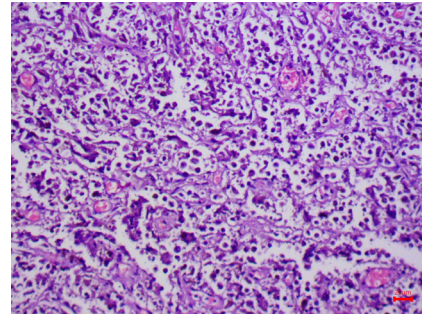


Fig. 4: Lungs- Melanoma: Neoplastic cells, round to oval vesicular, hyperchromatic nuclei, fine grey to black pigment (H&E, Scale bar=20 µm)

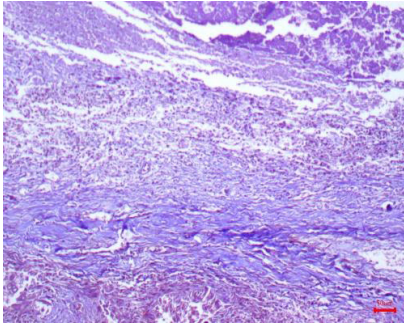


Fig. 5: Lungs-Proliferation of surrounding stroma (Blue) (Masson's trichrome, scale bar=20 µm)

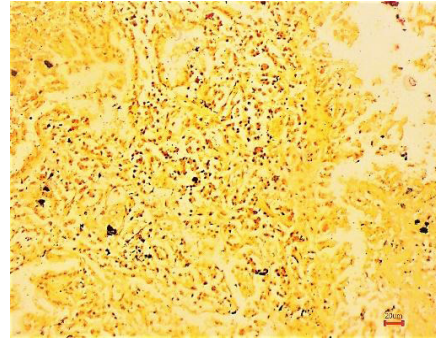


Fig. 6: Lungs- Distribution of AgNORs in neoplastic cells (Black) (AgNOR, Scale bar=50 µm)

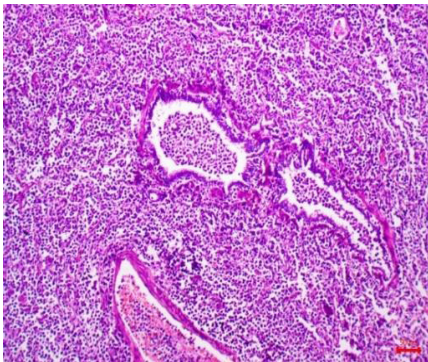


Fig. 7: Lungs- Bronchointerstitial pneumonia showing mononuclear cells infiltration with severe congestion and haemorrhage (H&E, bar=50 µm)

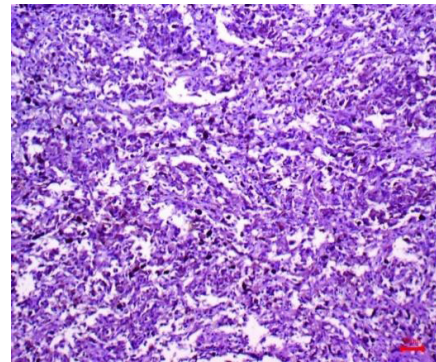


Fig. 8: Lungs- Melanoma, Positive Expression of Melan-A (IHC, Scale bar=50 µm)

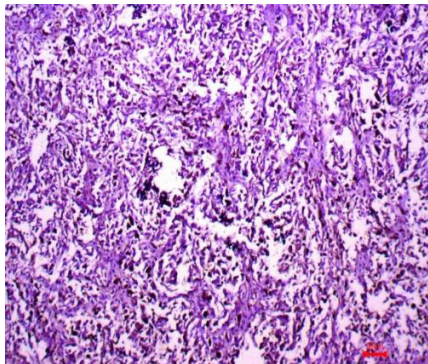


Fig. 9: Lungs- Melanoma, Positive expression of Cytokeratin (IHC, Scale bar=50 µm)

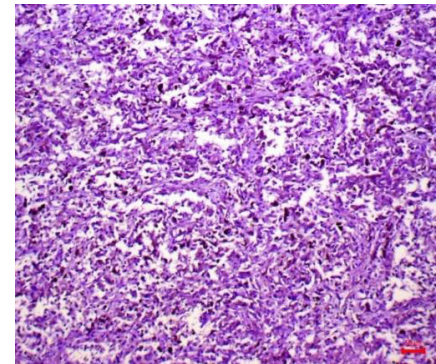


Fig. 10: Lungs- Melanoma, Positive expression of Vimentin (IHC, bar=50 µm)

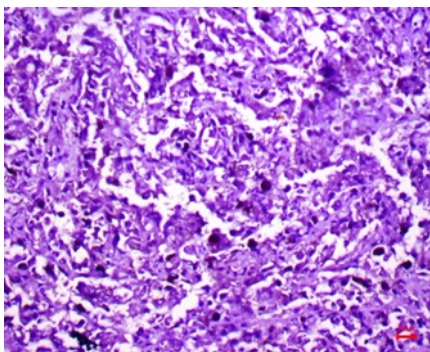


Fig. 11: Lungs- Melanoma, nuclear positivity reaction to PCNA, (Arrows) (IHC, bar=20 µm)

CONCLUSION

The present case of pulmonary metastatic oral melanoma was observed in a 13 years old female Labrador and confirmed by both histopathological and immunohistochemical technique.

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