Int.J.Curr.Microbiol.App.Sci (2018) *7*(6): 1400-1403

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 06 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.706.166

Cytological, Histopathological and Immunohistohemical Study of Canine Melanoma

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Keywords

Dog, melanoma, cytology, histopathology, immunohistochemistry, S100, Melan A

Article Info

Accepted:
15 May 2018
Available Online:
10 June 2018

A B S T R A C T

An eight years old female Labrador dog was presented with a history of diffuse swelling in the submandibular region. The mass was firm with no pain on palpation. Fine needle aspiration cytology (FNAC) revealed spindle shaped to pleomorphic neoplastic cells with brown to black pigments. The mass was then surgically excised. Histopathology section revealed cells with melanin pigment with round to oval nuclei. Immunohistochemical study showed positive expression for vimentin, S100 and Melan A and no expression for cytokeratin. Based on above findings the mass was confirmed as melanoma.

Introduction

Melanocytic neoplasms have been described in men and most domesticated animal species including dogs, cats, horses and also in wild terrestrial and marine mammals (Sweet *et al.*, 2012). They account for 0.8 -2 per cent of all canine cutaneous tumours, seen in dogs with heavily pigmented skin (Williams *et al.*, 2003). They occur more commonly in the skin and oral cavity. Histologically they are composed of three cell types, epithelioid, spindloid and mixed. In depth study on various attributes of spontaneously occurring even individual cases of canine melanoma is important, as canine melanoma is considered as the best model to study the pathophysiology of cancer in humans. As dogs live in association with humans and have the same environmental exposure to ultravilolet radiation of sun, carcinogens etc., they can be ideally used in comparative pathology trials for various chemotherapeutic interventions for cancer in humans. The present study was undertaken to study the pathology and application of specific markers in diagnosis of cutaneous melanoma in a Labrador dog.

The peak incidence of melanoma in dogs is reported to be between the ages of 5 and 11 years (Goldschmidt and Hendrick, 2002). Spangler *et al.*, (2006) in a survey of 384

melanocytic canine tumors have reported that oral, cutaneous, lips/feet, and eye sites comprised 19%, 59%, 19%, and 3% respectively.

Benign skin melanomas are usually solitary, firm and freely movable while malignant melanoma tend to be fast growing and often ulcerated (Goldschmidt, 1994).

Smedley *et al.,* (2011) has reported that among the cutaneous melanocytic neoplasms, digital neoplasms have a worse prognosis than from other sites in the skin.

Materials and Methods

An eight years old, male, black, Labrador was brought to Small Animal Clinic - Out Patient unit -Surgery unit of Madras Veterinary College Teaching Hospital with a history of swelling in the submandibular region.

Clinical examination revealed black coloured, firm mass, measuuring about 4 cm in diameter in the submandibular region and did not evince pain on palpation.

Fine needle aspiration cytology (FNAC) was performed. Radiographical exxamination revealed no metastatic foci. Following this, surgery was done to excise the mass. The cut surface was blackish. Excised mass was collected in 10 per cent neutral buffered formalin for histopathological examination.

Cytological smear was air dried and stained with Leishman and Giemsa cocktail stain as described by Garbyal *et al.,* (2006). In histopathological processing, paraffin embedded tissue sections were cut into 4-6 microns thickness and stained with Haematoxylin and Eosin (H&E). For immunohistochemistry, sections were stained for Cytokeratin, Vimentin, S100 and Melan A as per the procedures given by the immunnohistochemistry kit manual.

Results and Discussion

Grossly the mass was examined for the ABCDEs in melanoma like the asymmetry, border irregularity, colour variegation, diameter of the mass and evolution. Accordingly, the excised mass was symmetric, smooth borders with no ulceration, intense black in colour, measuring approximately 4 cm in diameter.

Cytologically, spindle shaped to pleomorphic neoplastic cells containing brownish black intracytoplasmic pigments were seen.

Histopathological examination revealed heterogeneous cells, some with melanin pigment and rest of the cells devoid of pigmentation. Nuclei were round to oval with one to few basophilic nucleoli. A few mitotic figures and mononuclear cell infiltration were also seen. Immunohistochemical staining to identify the origin of tumour revealed negativity for cytokeratin and positive expression for vimentin. Immunostaining with melanoma specific markers of S100 and Melan A revealed positive expression for both these markers. While S100 has been reported to be highly sensitive in detecting melanomas especially amelanotic melanomas which evade from routine diagnosis as they show no pigmentation, it lacks specificity, as they can stain even the cells of neural origin to give false positive results (Choi and Kusewitt, 2003).

Koenig (2001) has reported that Melan A was positive in 90 per cent of pigmented tumours but failed to stain amelanotic tumours. In contrast, Ramos-Vara *et al.,* (2000) has reported that Melan A is a specific and sensitive marker for melanomas.

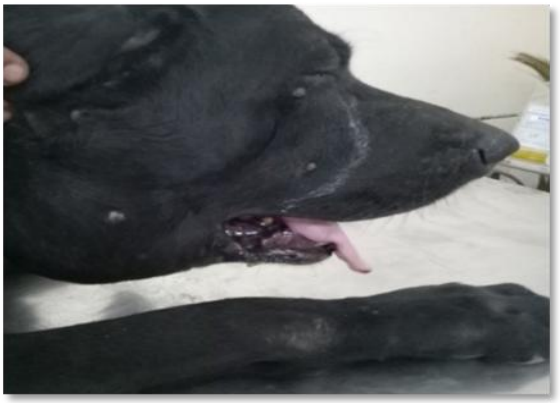

Fig.1 Dog - Melanoma- Mass in the submandibular region

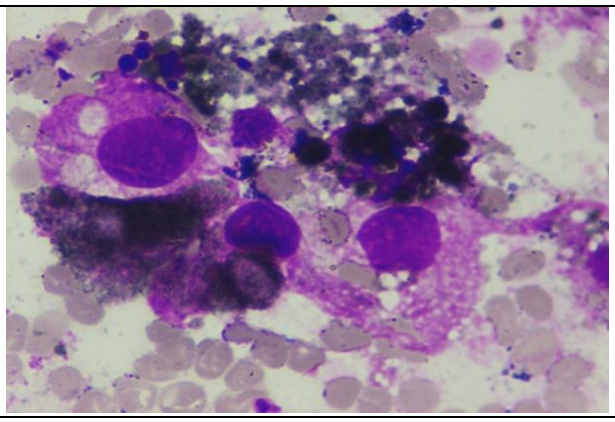

Fig.2 Dog - Melanoma – Cytology- LG 100x Pleomorphic cells with black brown pigments

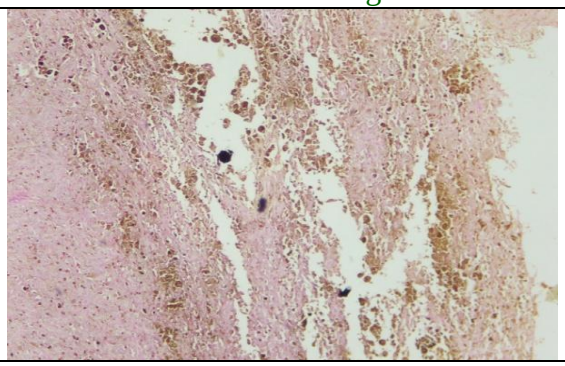

Fig.3 Dog-Melanoma - pleomorphic cells with melanin - H&E 4x

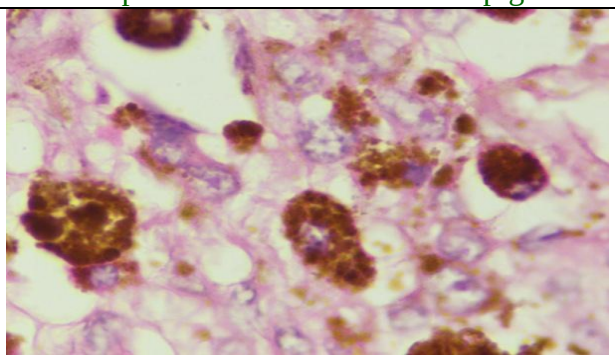

Fig.4 Dog-Melanoma - pleomorphic cells with melanin - H&E 100x

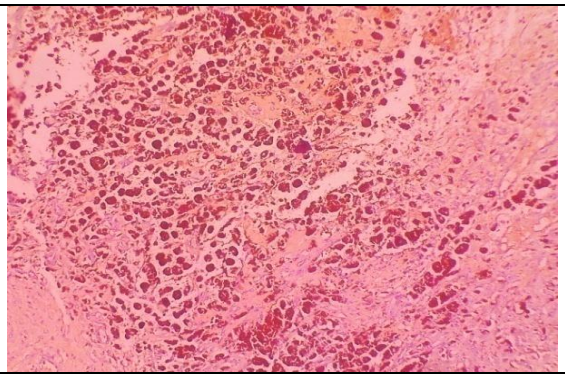

Fig.5 Dog – Melanoma – Immunohistochemistry – S100 – Positive expression – 4x

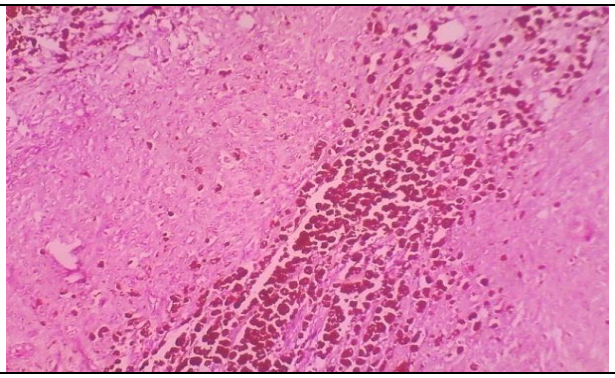

Fig.6 Dog – Melanoma – Immunohistochemistry – Melan A– Positive expression – 4x

Hence employing and interpreting the expression of both the markers Melan A and S100 and correlating with the findings of cytology and histopathology, the tumour was confirmed as melanoma. In the present case, the tumor was identified in an eight years old male dog. Earlier Goldschmidt and Shofer, 1992, have reported the higher frequency of melanoma in male dogs. Aronsohn and Carpenter, 1990 have reported higher frequency of melanomas in dogs more than 10 years. Mitotic index (MI) is a parameter that is of clinical significance in assessing the prognosis of canine melanocytic neoplasms occurring in skin and oral cavity. Bostock, 1979 has also stated that dogs with MI less than 3 per 10 hpf (high power field) had better survival times than those tumours with a

mitotic index more than 3 per 10 hpf. Accordingly, microscopic examination of the present case revealed only few mitotic figures and less of cell atypia indicating that it is less aggressive. Further the tumour was located in the haired skin of submandibular region. Goldsmith and Hendrick, 2002 have earlier reported that in dogs, tumours arising from the haired skin are benign and those arising from mucocutaneous junctions were malignant. Hence the tumour being located in haired submandiblar region with less mitotic figures was considered benign and in concurrence the dog showed no recurrence during the follow up for tumour free survival time.

Acknowledgement

The authors are thankful to the Dean, Madras Veterinary College, TANUVAS for providing the necessary facilities to carry out the work.

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How to cite this article:

Subapriya S., S. Vairamuthu, N. Pazhanivel, Ravi Sundar George, K. Vijayarani and Mohammed Shafiuzama. 2018. Cytological, Histopathological and Immunohistohemical Study of Canine Melanoma. *Int.J.Curr.Microbiol.App.Sci.* 7(06): 1400-1403
doi: https://doi.org/10.20546/ijcmas.2018.706.166