

Point calling diagnosis of melanocytic neoplasm in canine through cytological examination

S. Senapati¹, K. D. Mahesh A¹, R. Hoque¹, M. Namasudra¹, R. N. Hansda¹,
S. Pradhan¹ and S. Mondal^{1*}

¹Department of Veterinary Pathology, West Bengal University of Animal and Fishery Sciences, Kolkata- 700 037, West Bengal, India

Abstract

A 10-year-old male Labrador dog was reported with a history of growth inside the upper gums involving the local lymph node. Non aspiration FNC was performed at Belgachia Veterinary Clinics, and smears were coloured using Leishman stain and observed under oil immersion. Discrete round to polygonal mononuclear cells having round to oval nucleus with irregularly clumped chromatin were appreciated in the cell population. Cytoplasm was moderate in amount and filled with fine, black melanin granules. Growth was diagnosed as a melanocytic tumour.

Keywords: Cytology, Discrete cells, Leishman stain, Melanin granules

Highlights

- Melanocyte as round cell could be clearly identified by cytology.
- The presence of melanin granules in cytoplasm of round cells is a confirmatory clue in cytology.
- The least amount of effort is spent for identifying the cell and granule.

The use of cytological samples to diagnose abnormalities in a wide range of tissues has become common. The best method for sampling of any type of tissue, cutaneous lumps, or proliferative development involves FNAC (Fine-Needle Aspiration Cytology). FNB (Fine Needle Biopsy) can be conducted with or without aspiration using a simple syringe and needle. FNB allows for little contamination when collecting cells from a lesion (Valenciano and Cowell, 2020). Melanoma is a type of cancer that arises from pigment-producing cells (melanocytes). Since the pigment in these cells gives the skin its colour, these tumours are frequently dark in colour. Melanocytic tumours are most common in the skin and mouth, with oral melanocytic neoplasms accounting for 30–40% of all canine oral neoplasms (Smedley *et al.*, 2022). The terminology melanocytoma refers to benign cutaneous melanocytic neoplasms, while malignant melanoma refers

to all malignant melanocytic neoplasms, independent of the site. Malignant melanomas are rapidly growing, locally invasive tumours that often spread early in the disease's course. The gums and lips are the most common sites for canine oral melanocytic tumours. Gingival tumours are oval and immovable, formed by the architecture of the jaw. Although the surface may be black, pigmented tumours may be camouflaged by white or pink mucosa, and a red granulation tissue reaction secondary to ulceration may cover melanin pigmentation (Meuten, 2017). Unless necrosis and subsequent infection have softened the tumour, its consistency is hard. On the sliced surface, some tumours are uniformly black, but more typically, there are foci with less pigment, which are brown, grey or white.

A 10-year-old male dog was brought into the Department of Veterinary Clinical Complex, Belgachia, WBUAFS, Kolkata with a history

*Corresponding Author, E-mail: vetsamiran@gmail.com

of growth inside upper gums involving the local lymph node (Fig. 1 and 2) since last one month. There was a rapid growth of the swelling, as reported to the clinics by the owner. The growth was darkly pigmented, firm and hard. The animal was having excessive salivation and difficulty in prehension. FNB was performed (Non aspiration technique) using a 21G needle and 10 mL disposable needle, and smears were made using squash preparation. Air dried smears were stained by Leishman stain using standard protocol and observed under oil immersion. Haematological parameters revealed neutrophilia (81%) and anaemia (Hb-9.2 g/dL).

On cytological examination, round cells were noted individually with irregularity in shape and size. Discrete cells were found to be in single, occasional clumps of a few cells, loose clusters, or 3-dimensional groups with a peritheliomatous pattern (Fig. 3D) which make up the cellular population. Polygonal cells, moderate-to-abundant granular cytoplasm, ambiguous cytoplasmic boundaries, and slightly or moderately hyperchromatic large nuclei with granular and clumped chromatin were also seen in the material (Fig. 3C). Eccentric nuclei, thicker and uneven nuclear membranes, basophilic cytoplasm, clearly defined boundaries, large and generally a single nucleolus, and frequent nuclear pseudo inclusions are all characteristics of plasmacytoid cells (Fig. 3A). Small cells have sparse basophilic cytoplasm and nuclei that are spherical or oval and arranged eccentrically (Fig. 3B). In most of the differentiated cells, cytoplasm contained fine, dark black pigmented granules (Fig. 3A, 3B and 3C). There was a high nuclear to cytoplasmic ratio with a large nucleus. Most cells have a moderate amount of cytoplasmic melanin granules. No mitotic figures were observed. It was diagnosed as melanocytic tumour based on cytologic characteristics. Melanocytic tumours were excellent mimics, with characteristics similar to distinct cells, epithelial cells, and mesenchymal cells. All of these morphological features could

sometimes be found in a single aspirate. Cytology had been demonstrated to have 100% sensitivity and specificity for the diagnosis of pigmented melanocytic neoplasms; however, when attempting to diagnose amelanotic melanocytic neoplasms, both sensitivity (67–100%) and specificity (85–100%) declined (Griffiths *et al.*, 1984; Ghisleni *et al.*, 2006). The pigment of melanocytic tumours made them easy to spot. Individual melanin granules were rod-shaped with a dark grey to black colour when Leishman stain was used. These granules appeared black when tightly packed within the cells. Melanoma cells could be strongly or sparsely pigmented, depending on the degree of tumour differentiation. Well-differentiated melanocytes exfoliate in sheets, tiny clusters, or individually, according to cytologic preparation. They feature minimal amounts of pale-grey to blue cytoplasm with melanin pigment that appeared rod-shaped or finely granular and stains blue-green to golden to black with conventional Romanowsky-type stains (Griffiths *et al.*, 1984; Ghisleni *et al.*, 2006). Nuclei were spherical to oval, delicately stippled, and contained one large, conspicuous nucleolus in most cases of cytologic atypia. Melanin-laden macrophages (melanophages) might also be available in varying degrees. Increased anisocytosis and anisokaryosis, multinucleated cells, multiple and variably sized and shaped nucleoli, variable chromatin patterns, and variable nuclear-to-cytoplasmic ratios were common in poorly differentiated neoplastic melanocytes with cytoplasm that might contain few to many, small to large, clear vacuoles (Griffiths *et al.*, 1984; Ghisleni *et al.*, 2006). As melanocytic neoplasms lacked or had very little melanin pigment, it could be difficult to determine cell origin; consequently, any tumour that was difficult to diagnose cytologically should be considered a melanocytic neoplasm. In the present case, the neutrophilia supported the melanocytic progression by modulating tumour microenvironment and its progression. Melanoma was a fairly common tumour in dogs

Cytology of melanocytic tumour in an adult dog



Fig. 1. Palpation of lump feels firm and hard to touch

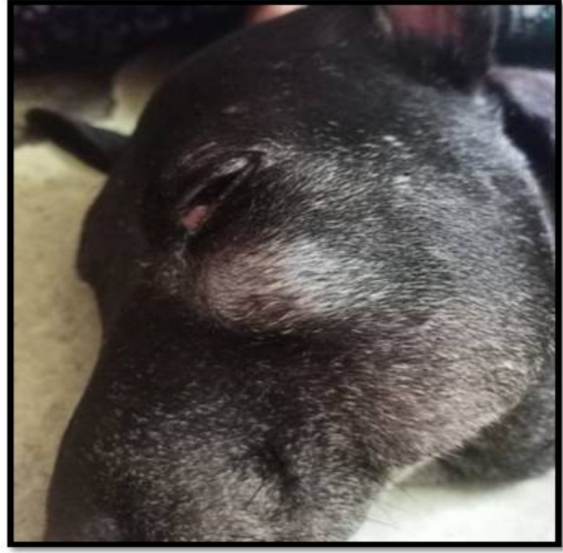


Fig. 2. Nodule is seen below the eye

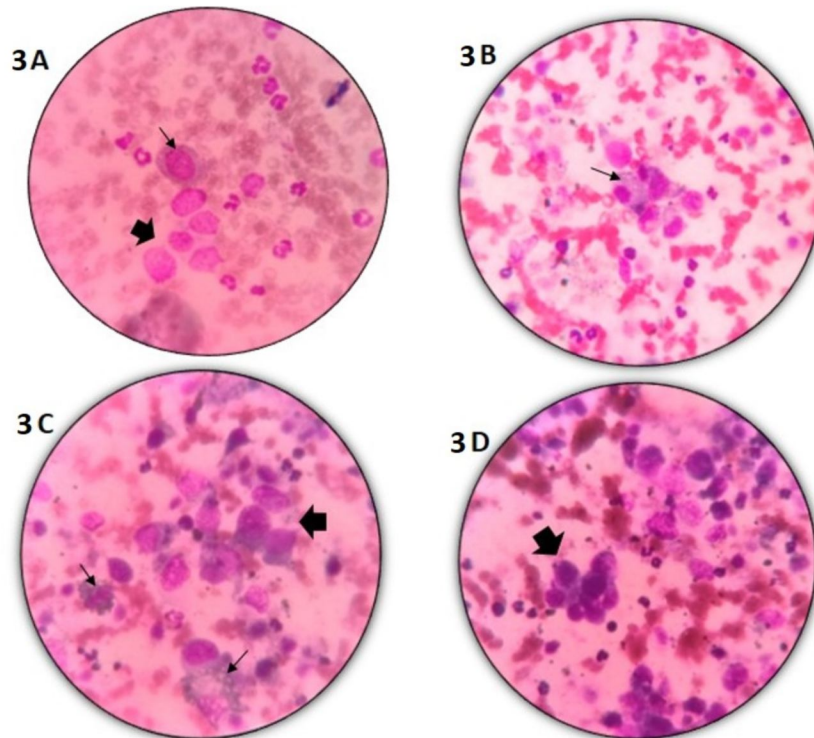


Fig. 3. Discrete round to polygonal mononuclear cells with eccentric round to oval nucleus having irregularly clumped chromatin (3C & 3D; black arrow). Cytoplasm is moderate in amount and filled with fine, black melanin granules (3A & 3B; arrow), (100x; Leishman stain)

but rather uncommon in cats. Cutaneous and oral melanomas were the most prevalent, with the majority of cutaneous melanomas behaving in a benign manner. Oral melanomas, on the other hand, were usually malignant and were the most commonly diagnosed oral malignancy in dogs.

Due to difficulty in oral nutrient intake, the dog might suffer from chronic anaemia, which was evident in this case. Malignant melanomas had also been found in the nailbed, footpad, eyes, GI system, and mucocutaneous junctions. Even in the least pigmented melanomas, meticulous microscopic examination of the samples often revealed a few melanin granules. It should be highlighted that simply identifying melanin granules does not imply a melanoma diagnosis. A pigmented basal cell tumour or a pigmented keratin-containing cyst was frequently misdiagnosed as melanoma by a beginner cytologist; normal melanocytes distributed melanin to epithelial cells of the

epidermis. Additionally, some might confuse melanin-laden macrophages (melanophages) as melanocytes.

In occupational safety, pointing and calling was a way of avoiding mistakes by pointing at crucial signs and audibly calling out their status. Here, the discrete round cells with neoplastic features presented a variable number of melanin pigments characterised by round to variable size and clumped to varying degrees in individual cells, which made sure to be a melanocytic tumour.

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

Author's contribution: SS: Carried out the sampling; KDMA: Slide preparation and staining; RH, MN: Draft preparation; SP: Diagnosis; RNH: Report writing and draft revision; SM: Participated in data analysis, draft and revision of the manuscript.

REFERENCES

- Ghisleni G, Roccabianca P, Ceruti R, Stefanello D, Bertazzolo W *et al.*, 2006. Correlation between fine-needle aspiration cytology and histopathology in the evaluation of cutaneous and subcutaneous masses from dogs and cats. *Vet Clin Pathol*, 35(1): 24-30, doi: 10.1111/j.1939-165x.2006.tb00084.x
- Griffiths GL, Lumsden JH and Valli VE, 1984. Fine needle aspiration cytology and histologic correlation in canine tumors. *Vet Clin Pathol*, 13(1): 13-17, doi: 10.1111/j.1939-165x.1984.tb00628.x
- Meuten DJ, 2017. Tumors in Domestic Animals. John Wiley & Sons, Inc., pp 516-524
- Smedley RC, Sebastian K and Kiupel M, 2022. Diagnosis and prognosis of canine melanocytic neoplasms. *Vet Sci*, 9(4): 175, doi: 10.3390/vetsci9040175
- Valenciano AC and Cowell RL, 2020. Diagnostic Cytology and Hematology of the Dog and Cat. 5th edn. Elsevier, pp 70-71