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CYTOLOGICAL AND HISTOPATHOLOGICAL STUDY ON CUTANEOUS ROUND CELL TUMOURS IN CANINES*

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ABSTRACT

Out of 65 cases of cutaneous tumours studied in canines, 14 were round cell tumours. The transmissible venereal tumour (TVT) constituted seven, mast cell tumours four, histiocytomas two and histiocytic sarcoma one. Cytologically, the tumours yielded high cellularity. The TVT had punctate vacuoles in the cytoplasm. Mast cell tumours showed cytoplasmic granules. Histiocytoma revealed multinucleated cells. The histopathological study confirmed the cytological diagnosis. Thus, cytology can be used as a rapid and inexpensive technique for diagnosis of neoplasms. An algorithm was developed for cytological diagnosis of different round cell tumours in canines.

Key words: Canine, cytology, round cell tumours

INTRODUCTION

Skin tumours are said to be the commonest tumours encountered in canines. Though diagnosis by histopathology is routinely done, little emphasis is placed on cytology, which is a very rapid diagnostic technique. This paper deals with the use of rapid diagnostic technique, cytology and compares it with that of histopathology for detection of round cell tumours in canines.

MATERIALS AND METHODS

Sixty-five tissue samples from tumour suspected cases were collected from canine cases, which attended the Department of Clinics, Madras Veterinary College and a private clinic at Chennai. Animal particulars, history, radiographic data, haematological values and gross pathological changes were collected. Fine needle aspiration biopsy (FNAB) was taken using 23-25 G needle and 2-5 ml syringe. Impression smears were made from tumour masses. Swabs were made from vaginal passage lesions[8]. Smears made were either wet fixed with 95 per cent ethanol[2] or absolute isopropanol for 20 min or rapidly air-dried. Wet fixed smears were stained with Harris haematoxylin and eosin[3]. Air-dried smears were stained with Wright's (W), Wright-Giemsa (WG), May-Grunwald-Giemsa (MGG), Toluidine blue and Leishman-Giemsa -LG (Himedia, India) stains. The Wright's and May-Grunwald stains were diluted with distilled water in a ratio of 1:1 and slides were stained for 3min. The Giemsa working solution was made by diluting it with distilled water in the ratio of 1:10 and stained for 20 min. The Leishman (150 mg) - Giemsa (30 mg) stain was made with acetone free methanol (100 ml). The smears were flooded with the stain for 1 min and then diluted with double the quantity of distilled water and allowed to stain for 20 min. Then, the smears were evaluated cytologically[2,12]. Tissue samples were simultaneously collected in 10 per cent buffered formalin, paraffin embedded and processed to obtain 4-6μ thick sections for histopathological studies[3].

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RESULTS AND DISCUSSION

Out of 65 cases, 14 were found to be round cell tumours.

Transmissible venereal tumours: Seven out of 14 cases were transmissible venereal tumours (TVT). Six were encountered in non-descript animals out of which only one was a female. The remaining one case was 4 years old, Spitz bitch. The non-descript animals were 3 to 13 years of age and the mass occurred mainly in the prepuce and at the base of the penis. In the female animal, it was in the vaginal passage. All the masses were grossly irregular, cauliflower like, 1 to 4 cm in diameter, reddish in colour, soft in consistency and had bleeding nature. The multiple nodular masses in the external genitalia observed in this study were in accordance with the earlier observations[9].

Romanowsky stains combined with Giemsa stained the cytoplasm of neoplastic cells greyish, while HandE stained it baso-eosinophilic. The cellularity of cytological smear was high with round individual cells arranged in a sheet like pattern. Anisocytosis and anisokaryosis were observed. The nucleus was round to oval in shape and centrally placed. The nucleoli were basophilic and one to three in number. Chromatin was coarse to reticulate. Cytoplasm showed punctate vacuoles and delineated outline. Mitotic figures were present. Neutrophils and keratinised squamous cells were encountered in a case (Fig. 1). These findings are in accordance with earlier reports[1,6,7,10]. Histopathology revealed sheet of round individual cells containing round vesicular nuclei. Single distinct centrally placed nucleolus with dispersed

chromatin was noticed. Stroma was scanty. Neutrophilic infiltration and mitotic figures were seen. These observations were in agreement with the findings of MacLachlan and Kennedy[9].

Mast cell tumours: Four cases out of 65 were found to be mast cell tumours. Grossly, both single and multiple growths were encountered. The growth in a 10-year-old, non-descript male dog, was 10 cm in diameter, with flat, and raised ulcerated surface on the right lateral thorax. In a cross-bred male dog aged 10 years, the tumour, around 3 cm in diameter, was located on the posterior aspect of the scrotum and had bleeding tendency with ulceration. In a non-descript dog aged 12 years, there was an irregular swelling around the penis. All the masses were hard in consistency. The multiple growths found in a 3 years old, Rajapalayam male dog, were 0.5 to 2 cm diameter, pedunculated with erythematous vesicular eruptions, soft in consistency and spread over the medial aspect of thigh, ventral abdomen and scrotum. All the tumours occurred in males. The single form, which was large and ulcerated and the multiple form characterised by erythematous vesicular eruptions, which were encountered in this study, have been recorded[7].

The cytological smears showed high cellularity with round individual cells. Cells were uniform in size but areas of anisocytosis were also observed. The cytological smears stained with Romanowsky stain and combinations with Giemsa revealed purplish staining granules in the cytoplasm. These granules were not seen when stained with HandE. The staining of granules with MGG was sharper than with WG. Toluidine blue staining showed purplish intracytoplasmic granules. The granules were seen aggregated within the cells, sometimes masking the nuclei also in the extracellular area. The granules appeared in clusters or diffusely distributed in the cytoplasm. Nucleus was round in shape. Anisokaryosis, binucleation and trinucleation were also seen. The nucleus was eccentric. The nucleolus was indistinct but seen in some cells as round, pale basophilic bodies varying from one to three in number. The chromatin was coarse. Large blast like cells without granules were also seen. Mitotic figures and neutrophils were observed (Fig.2). These were in conformity with the cytological features described earlier for mast cell tumour[6,7,10,11]. The authors mentioned that nucleoli were indistinct. But in this study round basophilic single to multiple nucleoli were distinctly observed. Further, anisocytosis, eccentric nuclei with clustering of granules and trinucleated cells were also noticed.

Histopathologically, roughly spherical to oval cells were observed. Anisokaryosis was noticed. The nuclei were spherical and vesicular with one to three distinct nucleoli. The cytoplasm was sparse and eosinophilic. Some cells had faintly eosinophilic to baso-eosinophilic cytoplasmic inclusions. Stromal tissue was moderate and highly vascular. Mitotic figures were noticed. These findings confirmed the diagnosis of mast cell tumour[7].

Histiocytomas: Two out of 65 cases were histiocytomas. Grossly, the tumorous mass in a Cocker Spaniel, 7 years old, male dog was 2 cm in diameter, flat, in form of a button like nodule with reddish ulcerated surface, rubbery in consistency with whitish cut surface, weighing 5 g. The growth was found on the left anterior lateral part of neck. The mass was present for the past 2 months (Fig. 3). The other case in a non-descript male dog, aged 9 months was similar to previous descriptions except that it was 2.5 cm in diameter and was reported to occur since 15 days. These concurred with the earlier report[6].

The FNAB smears showed moderate cellularity. The cells were round with distinct outline. Cytoplasm was pale grey in colour when stained with W, WG and MGG. The centrally placed nuclei varied from round or oval to bean shape. Nuclear indentation, nuclear moulding, binucleation and multinucleation were noticed. Nuclei were also placed eccentric. The nucleoli were indistinct. Chromatin was coarse and etched. Presence of mitotic figures and neutrophils were noticed (Fig. 4). Cytological features observed in this study agreed with those of others[1,6,7,10] except that the chromatin was found to be fine and granular in earlier studies[1,8]. There were also multinucleated cells and mitotic figures, which were not described previously. These cytological features might be due to active proliferation of the tumour cells. Histopathologically, the tumour showed sheet of densely packed round cells. The nuclei were round to oval and slightly bean shaped. Some cells had folded nuclei. The chromatin was condensed along the nuclear membrane. The cytoplasm was moderate and eosinophilic. The stroma was scanty (Fig. 5). These observations pointed towards histiocytoma[7].

Histiocytic sarcoma: One out of 65 cases was a histiocytic sarcoma of the spleen. Grossly, there were multiple nodular masses of 0.5 - 2 cm in diameter in the spleen of a 11 years old, Labrador bitch. The mass had a haemorrhagic creamy cut surface. The cytological smears had high cellularity. Cells were mostly round in shape and exhibited anisocytosis. Nuclei were round to oval, bean shaped, often showing indentation and folding. Nucleus : cytoplasmic (N:C) ratio was decreased. Giant cells and multinucleated cells were noticed. Chromatin was reticulate and nucleolus was indistinct. Some cells showed binucleation and prominent nucleoli (Fig. 6). Histopathology revealed pleomorphic mononuclear cells and multinucleated giant cells. There was population of discrete neoplastic cells with very less stroma. Cytoplasm was sparse to moderate and eosinophilic. Nuclear indentation was noticed. There were multiple nucleoli. Erythrophagocytosis by the multinucleated cells and neoplastic histiocytes were noticed (Fig. 7). The presence of nodules on the spleen with a slight creamy cut surface encountered in this study has earlier been reported[4]. Cytological observations made in this study have also been recorded earlier in histiocytic sarcoma of the spleen[5]. Histopathological findings concurred with those of

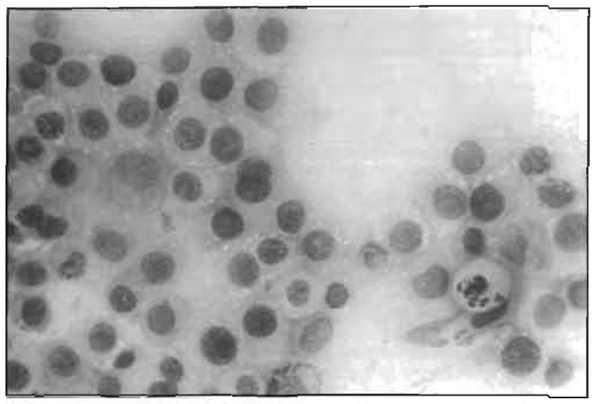

Fig. 1: TVT cytology: Cytoplasm with punctate vacuoles and atypical mitosis. HE x 800.

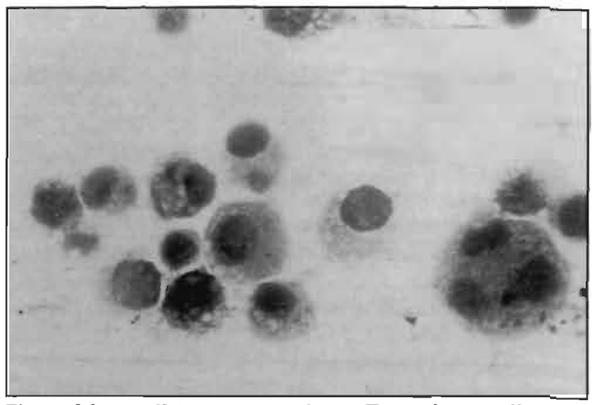

Fig. 2: Mast cell tumour cytology: Trinucleate cells with purplish cytoplasmic granules. MGG x 1000

Fig. 3: Cutaneous histiocytoma – Button like nodular mass

earlier workers[4,11].

The histopathological study confirmed the cytological diagnosis. Thus the cytology can serve as a rapid, inexpensive and non-invasive technique in the diagnosis of round cell tumours in canines aiding the

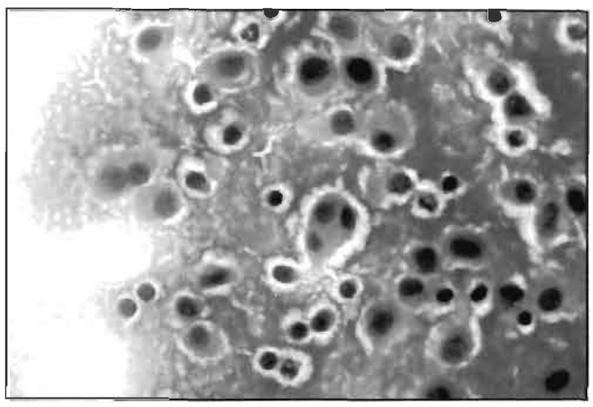

Fig. 4: Cutaneous histiocytoma cytology: Round Cells with binucleation and trinucleation, mitotic figures, basoeosinophilic cytoplasm and neutrophils. HEx800

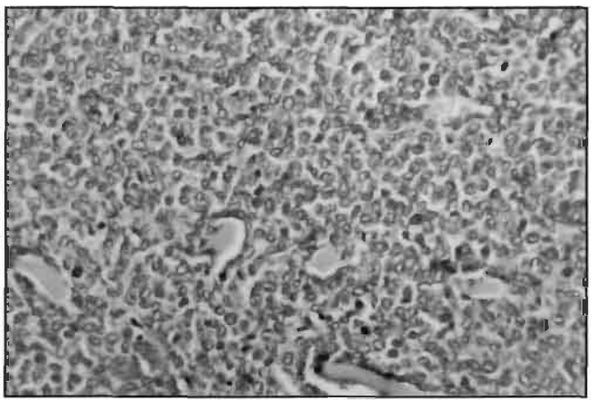

Fig. 5: Cutaneous histiocytoma: Section showing sheet of round cells with oval, bean shaped folded nuclei, eosinophilic cytoplasm, mitotic figures and scanty stroma. HE x 400

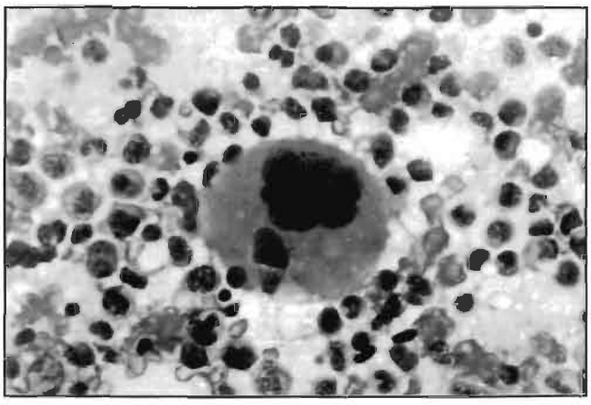

Fig. 6: Histiocytic sarcoma cytology: Multinucleated giant cell with irregular borders, pleomorphic round cells and red blood cells. HE x 800

clinicians to decide upon the line of treatment. Based on the cytological features of neoplastic cells obtained by FNAB, an algorithm was developed for diagnosis of round cell tumours (Fig.8).

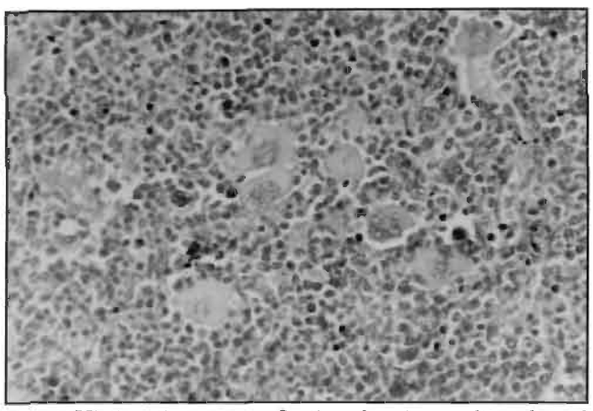

Fig. 7: Histiocytic sarcoma: Section showing multinucleated giant cells and histiocytes exhibiting erythrophagocytosis. HE x 800

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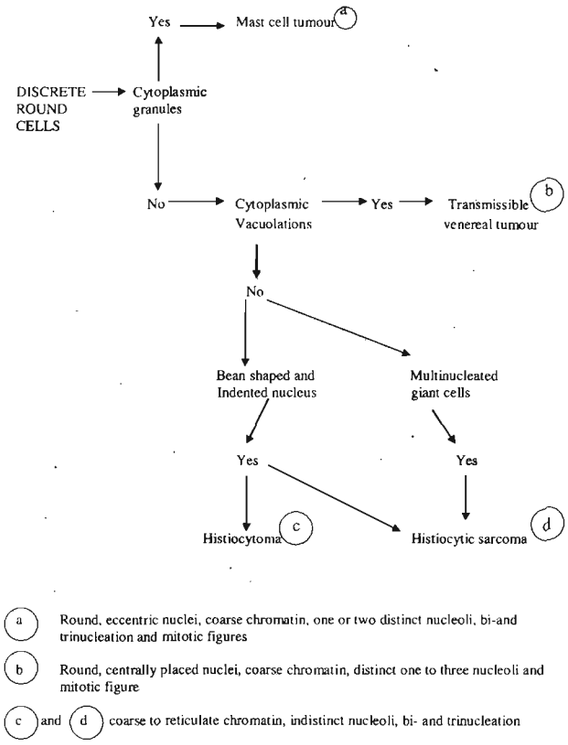

Fig. 8: An algorithm to aid in evaluating aspirates of round cell tumours

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