RESEARCH ARTICLE
JIVA 18 (2) August 2020

Received : 10-7-2020
Accepted : 31-7-2020

CYTOLOGICAL DIAGNOSIS OF RECURRENT MULTIPLE CUTANEOUS MAST CELL TUMOUR IN A DOG

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ABSTRACT

An eight year old male Pomeranian dog was brought to District Veterinary Centre, Kannur, Kerala with complaints of multiple cutaneous masses with ulcerations, erythema and pruritis in the lower abdomen. Haematological examination revealed mild anaemia, leucocytosis, neutrophilia, monocytosis and eosinophilia. Wet film examination and blood smear examination revealed presence of numerous microfilarial organisms. Fine needle aspiration cytology of the cutaneous masses revealed discrete round cells displaying variable degrees of granulation, moderate anisokaryosis and anisocytosis. Based on the cytological findings, a straight forward diagnosis of high grade mastocytoma was made by the presence of metachromatic granules in the cytoplasm of cells and in the background.

Keywords: Mastocytoma, Canine, Cytology, Giemsa stain, Microfilariae

INTRODUCTION

In dogs, cutaneous masses occur due to a multitude of underlying causes like cysts, tumour, parasites, hypersensitivity, infectious causes, granulomatous and non-granulomatous conditions (Balima and Vairamuthu, 2020). In dogs, the skin was reported to be the commonly affected organ for both neoplastic and non-neoplastic tumours like hemangiosarcomas, histiocytomas, melanocytomas, mast cell tumors, lipomas, hemangiopericytomas, papillomas, fibrosarcomas, hemangiomas and squamous cell carcinomas (Murphy, 2006; Chikweto *et al.* 2011). Mast cell tumours (MCTs) are the common cutaneous neoplasms in dogs with variable biological behaviour, accounting for up to 7-21% of all canine skin tumours (Endicott *et al.*, 2007). MCTs arise from malignantly transformed cells, which can affect dogs of any age especially middle-aged to older dogs. Any breed may be affected with MCTs, however

certain breeds like Labrador Retrievers, Golden Retrievers, Boxers, Beagles and Pugs have been reported with increased risk (Endicott *et al.,* 2007; Garrett, 2014). Apparent sex predisposition has not been reported (Gieger *et al.,* 2005). The behaviour of cutaneous MCTs is often unpredictable and their gross appearance may range from raised to superficial; very deep to fixed; soft, fluctuant to firm or slow-growing to rapidly enlarging ulcerated masses (Endicott *et al.,* 2007). Hence, cutaneous MCTs may frequently mimic that of other tumours. Certain paraneoplastic syndromes produced in MCTs include vomiting, gastric and duodenal ulceration, delayed healing, impaired blood coagulation and anaphylactoid reactions as a result of histamine and heparin released from mast cell granules (van Pelt *et al.,* 1986). MCTs show variable biological behavior in canines either as solitary nodules that can be treated and managed surgically or as non-curable systemic metastatic and fatal disease (Gopal *et al.,* 2017). Fine needle aspirate cytology (FNAC), a non-invasive, rapid and low cost method, may provide prognostic cytologic information in canine cutaneous and subcutaneous MCTs. It may be an effective tool for planning treatment utilizing the histologic grade, stage and other clinical picture (Weeden, 2017). Cytoplasmic granules of mast cells are more easily recognizable in cytology than histology, because of the larger cell size and the use of metachromatic stains like Giemsa (Scarpa *et al.,* 2016).

Two histologic grading systems in common use for evaluation of canine cutaneous MCTs are the Patnaik system and Kiupel's system. The Patnaik's system is a 3-tiered grading system in which features like cellularity, cell morphology, mitotic index, extent of tissue involvement, and stromal reaction are assessed to grade MCTs of I to III, from well differentiated to poorly differentiated, respectively (Patnaik *et al.,* 1984). The main lacunae of the Patnaik system are that many MCTs fall into grade II, which undermines the overall usefulness of this scheme due to the variability in the behavior of the grade II tumors (Camus *et al.,* 2016). The Kiupel's system is a 2-tiered grading scheme which relies on cell morphology likes mitotic figures, multinucleated cells, bizarre nuclear features and karyomegaly, to grade as low- and high-grade MCTs (Kiupel *et al.,* 2011). This method enables less variability in interpretation between pathologists as it eliminates the difficulty to predict grade II category (Camus *et al.,* 2016). The present paper describes a recurrent case of multiple cutaneous mastocytoma in a male Pomeranian dog.

MATERIALS AND METHODS

A male Pomeranian dog aged about 8 years with past history of surgical resection of a tumour mass on lower abdominal area was presented again with history of remission at the lower abdominal area at the Out Patient Ward of District Veterinary Centre, Kannur, Kerala. The animal was physically examined for determining the nature of masses. Blood (4 ml) was collected from the saphenous vein for haematology and serum biochemistry. Peripheral blood was collected for wet film examination and for preparation of blood smears. Fine needle aspirates from different areas of the cutaneous masses were collected using 24G needle and 2ml syringe and multiple smears were made for fine needle aspiration cytology. The smears made were fixed with absolute ethanol, air dried immediately, stained with Giemsa stain and observed microscopically for cytological evaluation.

RESULTS AND DISCUSSION

On physical examination, multiple (n = 3) cutaneous masses of around 2 x 2 cm size were observed around the lower abdominal area with erythema, ulcerations and pruritis (Fig. 1). Haematology revealed mild anaemia, leucocytosis, neutrophilia, monocytosis and eosinophilia, however, serum biochemistry analysis for kidney and liver function did not reveal any remarkable results (Table 1). Wet film examination revealed presence of numerous moving microfilariae with a grade of 4+ under low power (10X magnification). Blood smear examination revealed numerous unsheathed microfilaria and absence of haemoprotozoa.

Both the fine needle aspirate cytology and impression smear cytology revealed high cellularity with discrete cells (Fig. 2). Unsheathed microfilariae could also be detected in the smears. In certain areas, cells had variable degree of granularity with fine to large cytoplasmic granules that prevented the visualization of nucleus (Fig. 3A). The cells also had moderate anisokaryosis, anisocytosis, binucleation, mitotic figures (Fig. 3B) and occasional cytoplasmic vacuolation (Fig. 3C). Numerous purple granules could be seen packed within the cytoplasm pushing the nucleus to the periphery (Fig. 3B). The nuclei were round to spherical in shape, centrally placed with coarse chromatin and multiple nucleoli. Extracellular granules released from mast cells could also be detected in many areas (Fig. 4). Moderate number of non-degenerate neutrophils, lymphocytes, fewer macrophages and few bacterial cocci were also detected.

Based on the cytological examination, a diagnosis of high grade mast

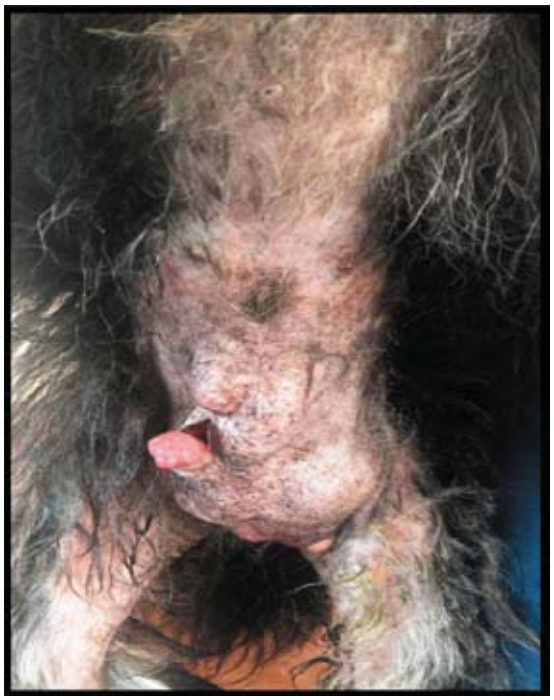

Fig. 1. Multiple cutaneous swellings around the penile area

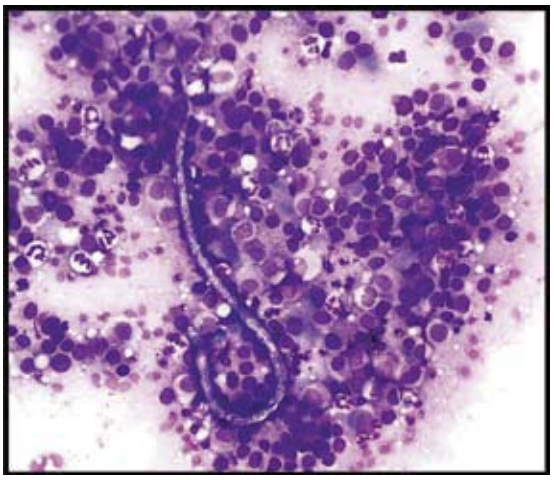

Fig. 2. Fine Needle Aspirate Cytology (FNAC) of the cutaneous mass revealing high cellularity with a predominant population of discrete round cells with a pale basophilic cytoplasm and purple intracytoplasmic granules. Each cell had central nuclei with dispersed chromatin and a single prominent nucleolus. Mild anisocytosis and anisokaryosis were present. These round cells were morphologically consistent with mast cells. A serendipitous finding of unsheathed microfilaria was also observed (Giemsa staining, 40X magnification).

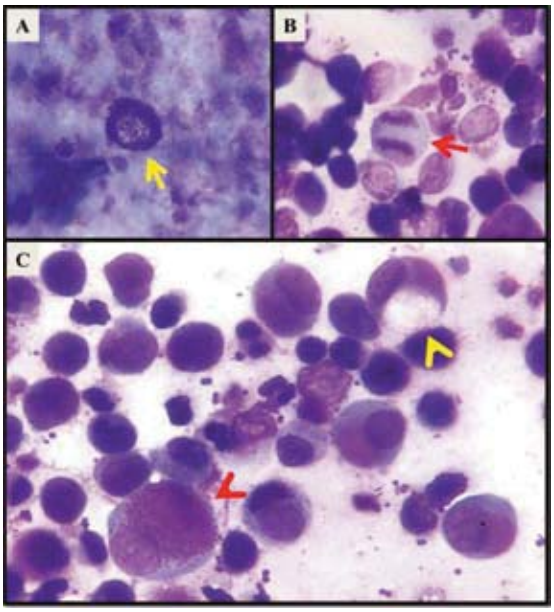

Fig. 3. A. Photomicrograph of a mast cell (Yellow arrow) on cytological smear showing abundant granules both in cytoplasm and background; B. Poorly granulated mast cell and a few mitotic figures (Red arrow); C. A large cytoplasmic vacuole (Yellow arrowhead) and coarsely stippled granules occupying the cytoplasm (Red arrowhead) and the nucleus at the periphery of the cell (Giemsa staining, 100X magnification).

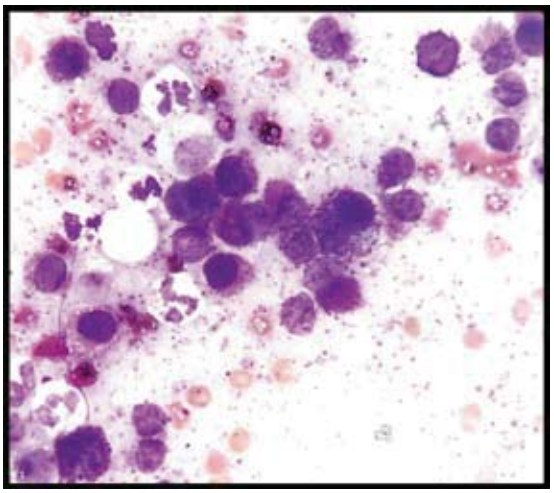

Fig. 4. Fine Needle Aspirate Cytology (FNAC) from the cutaneous masses revealed a highly cellular sample with many mast cells, displaying variable degrees of granulation, moderate anisokaryosis and anisocytosis (Giemsa staining, 100X magnification).

Table 1: Haematology and serum biochemistry values of the dog

Parameters	Observed values	Reference range	Key findings
Haemoglobin (g/dl)	**11.2**	12-18	**Anaemia**
PCV (%)	**32.0**	37-55	
RBC count (millions/cu.mm)	**5.32**	5.5-8.5	
MCV (fl)	66.53	60-77	
MCH (pg)	23.28	21-26	
MCHC (g/dl)	35	32-36	
WBC count (thousands/cu.mm)	**26.4**	6.0-17.0	**Leucocytosis**
Neutrophil (thousands/cu.mm)	**17.4**	3-11.5	**Neutrophilia**
Neutrophil (%)	65	58-85	
Lymphocytes (thousands/cu.mm)	4	1-4.8	
Lymphocytes (%)	15	8-21	
Monocyte (thousands/cu.mm)	**2.5**	0.15-1.350	**Monocytosis**
Monocyte (%)	10	2-10	
Eosinophil (thousands/cu.mm)	**2.5**	0-1.3	**Eosinophilia**
Eosinophil (%)	10	0-9	
Platelet (lakhs/cu.mm)	4.95	2-5	
MPV (fl)	7.4	6.7-11.1	
Creatinine (mg/dl)	0.74	0.5-1.5	
Urea (mg/dl)	20.13	10-28	
ALT (U/L)	22.74	10-109	
ALP (U/L)	123.61	1-114	

cell tumour was made and wide surgical excision was then performed to remove the cutaneous masses, along with other surrounding structures. Multiple cutaneous masses could be observed in this case of canine MCT with erythema, ulcerations and pruritis. It was previously described that MCTs have variable appearances which include intermittent shrinkage and swelling of the tumour mass, peritumoral erythema, edema and bruising attributed to the presence of histamine and heparin within mast cell granules (Gieger *et al.*, 2005). The haematological changes observed in this case include mild anemia, leukocytosis, neutrophilia, monocytosis and eosinophilia. Similar, cytopenia of blood cells has been reported by Endicott *et al.* (2007) in MCTs due to marrow infiltration or systemic and visceral mastocytosis. These changes may also be due to physiological inflammation and infection of the primary tumour, leucomoid response and stress leucogram (Brunch *et al.*, 1998). Both peripheral wet film examination and FNAC revealed the presence of unsheathed microfilaria in

the animal. A report by Mazaki-Tovi *et al.* (2016) suggests that infiltration of highly granulated mast cells and eosinophils in *Dirofilaria repens*- associated subcutaneous lesions may also be considered as differential diagnosis for mast cell tumour, especially in geographic areas endemic for the nematode. However in this case, the mast cells observed where non-uniform and variably granulated, hence *D.repens*-associated subcutaneous lesions was considered less likely even in view of the microfilariae in the lesion. Nevertheless, the lacunae of histopathological examination of the masses are a limitation in this study. However, Duncan and Prasse (1979) stated that cytology is more sensitive than histopathology in the diagnosis of MCT.

Multiple smears were made; all were stained and evaluated as mostly only one of the smears may be sufficiently cellular to obtain a proper cytological diagnosis. The cytology results are consistent with that of high grade mast cell tumour as the cells were poorly to moderately granulated. According to Scarpa *et al.* (2016), a straight forward diagnosis of MCT may be made by the presence of abundant well identifiable metachromatic granules in the cytoplasm of cells and in the background. In high grade tumours, there may be atypical distribution of granules with frequent arrangement in a half moon configuration at a cell pole or coarsely stippled making the nuclei and part of the cytoplasm visible. In the study, certain mast cells showed decreased cytoplasmic granulation, however it exhibited other features of malignancy like variation in cell and nuclear size, bi-nucleation, cytoplasmic vacuolation, nuclear pleomorphism and mitotic figures. Even though cytology is highly accurate at diagnosing malignancy, in certain cases, inflammation may mask neoplasia, making difficult proper diagnosis (Ghisleni *et al.,* 2006). Wide surgical removal of the mass was done in this case to prevent the recurrence of the MCT . Similarly, it was stated by Gieger *et al.* (2005) that wide surgical excision is indicated if a cutaneous MCT is diagnosed in a location which is amenable to surgery via cytology and with no negative prognostic indicators like peritumoral edema or bruising. Similarly, a case of MCT extending from the inguinal region to the caudal mammary gland had been managed surgically through wide margination and caudal epigastric flap in a bitch (Gokulakrishnan and Kirthika, 2018).

SUMMARY

A case of high grade mastocytoma in dogs and its cytological diagnosis is presented. Fine needle aspiration and cytological examination are valuable and

quickest tools for the diagnosis of MCTs in dogs. Cytological grading of the tumour along with the clinical staging, helps the practitioner to choose an appropriate treatment protocol and accurate long term prognosis.

ACKNOWLEDGEMENT

The authors are extremely thankful to Chief Veterinary Officer, District Veterinary Centre, Kannur and Director, Department of Animal Husbandry, Kerala state for providing the technical facilities to conduct this study. Authors are grateful to the pet parents for their non-objection for publishing this case for educational purpose.

REFERENCES

Balima, N.S. and Vairamuthu, S. 2020. Cytological Evaluation and Report on a Case of Cutaneous Mast Cell Tumour in a Dog. *Int. J. Curr. Microbiol. App. Sci.* **9**(3): 2180-2184.

Brunch, S.E., Grauer, G.F., Hawkins, E.C., Johnson, C.A., Lappin, M.R., Taylor, S.M., Ware, W.A. and Willard, M.D. 1998. Leukopenia and leukocytosis. In: *Small Animal Internal Medicine*, 2nd edn., RW Nelson and Couto CG, eds., St Louis, Mosby. pp. 1178 – 1186.

Camus, M.S., Priest, H.L., Koehler, J.W., Driskell, E.A., Rakich, P.M., Ilha, M.R. and Krimer, P.M. 2016. Cytologic criteria for mast cell tumor grading in dogs with evaluation of clinical outcome. *Vet. Pathol.* **53**(6): 1117-1123.

Chikweto, A., McNeil, P., Bhaiyat, M.I., Stone, D. and Sharma, R.N. 2011. Neoplastic and nonneoplastic cutaneous tumors of dogs in Grenada, West Indies. *ISRN Vet. Sci.* **2011**: 416435.

Duncan, J.R. and Prasse, K.W. 1979. Cytology of canine cutaneous round cell tumors: mast cell tumor, histiocytoma, lymphosarcoma and transmissible venereal tumor. *Vet. Pathol.* **16**(6): 673-679.

Endicott, M.M., Charney, S.C., McKnight, J.A., Loar, A.S., Barger, A.M. and Bergman, P.J. 2007. Clinicopathological findings and results of bone marrow aspiration in dogs with cutaneous mast cell tumours: 157 cases (1999–2002). *Vet. Comp. Oncol.* **5**(1): 31-37.

Garrett, L.D. 2014. Canine mast cell tumors: diagnosis, treatment, and prognosis. *Veterinary Medicine: Research and Reports*, **5**: 49-58.

Ghisleni, G., Roccabianca, P., Ceruti, R., Stefanello, D., Bertazzolo, W., Bonfanti, U. and Caniatti, M. 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**: 24-30.

Gieger, T., Northrup, N. and Wall, M. 2005. Clinical management of mast cell tumors in dogs. *Compend. Contin. Educ. Pract. Vet*. **27**: 56-68.

Gokulakrishnan, M. and Kirthika, D. 2018. Surgical management of mast cell tumour through wide margination and caudal epigastric flap in a bitch. *Int. J. Recent Sci. Res.* **9**(5): 27039-27041.

Gopal, K., Balachandran, C., Vairamuthu, S., Pazhanivel, N. and George, R. 2017. Recurrent Mast Cell Tumour in a Non-Descript Dog. *Indian Vet. J.* **94**(03): 62-63.

Kiupel, M., Webster, J.D., Bailey, K.L., Best, S., DeLay, J., Detrisac, C.J., Fitzgerald, S.D., Gamble, D., Ginn, P.E., Goldschmidt, M.H. and Hendrick, M.J. 2011. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet. Pathol.* **48**(1): 147-155.

Mazaki-Tovi, M., Reich, M., Karnieli, A., Kuzi, S. and Aroch, I. 2016. Marked subcutaneous mast cell and eosinophilic infiltration associated with the presence of multiple Dirofilaria repens microfilariae in 4 dogs. *Vet. Clin. Pathol.* **45**(4): 703-709.

Murphy, S. 2006. Skin neoplasia in small animals. Common canine tumours. *In Practice*. **28**(7): 398-402.

Patnaik, A.K., Ehler, W.J. and MacEwen, E.G. 1984. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet. Pathol.* **21**(5): 469-474.

Scarpa, F., Sabattini, S. and Bettini, G. 2016. Cytological grading of canine cutaneous mast cell tumours. *Vet. Comp. Oncol.* **14**(3): 245-251.

Van Pelt, D.R., Fowler, J.D. and Leighton, F.A. 1986. Multiple cutaneous mast cell tumors in a dog: a case report and brief review. *Can. Vet. J.* **27**(7): 259.

Weeden, A. 2017. Can cytology provide prognostic information in canine cutaneous mast cell tumours? *Vet Script.* 52-54.