

Staining Characteristics of Mast Cell Tumor in a Hedgehog

Jong-Won Lee, Mi-Na Han, Gon-Hyung Kim, Dong-Woo Chang and Ki-Jeong Na¹

Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, South Korea

(Received: April 04, 2016 / Accepted: June 24, 2016)

Abstract : A 2-year old female African hedgehog (*Atelerix albiventris*) weighing 645 grams was presented with a well circumscribed mass in the subcutaneous of the left shoulder. It measured 2 × 2 cm in size and had a hard texture. There were no clinical signs. In a fine-needle aspiration (FNA) cytological evaluation, mast cells were identified with Wright-Giemsa stain. Mast cell tumor was confirmed and characterized by surgical sampling and histopathological evaluation. Four stains (hematoxylin-eosin, toluidine blue, periodic acid-Schiff, and Safranin O) were examined in order to find an applicable stain for a mast cell tumor histopathological evaluation in hedgehog. This case showed that periodic acid-Schiff and toluidine blue staining were the most useful for assessment of a mast cell tumor in a hedgehog.

Key words : hedgehog, mast cell tumor, fine needle aspiration, periodic acid-Schiff, toluidine blue.

Introduction

The hedgehog has recently become popular as an exotic pet in Korea. Neoplastic disease is common in captive hedgehogs (7). Prevalence of neoplasia in the hedgehog ranges from 29% to 51.5% (10). Tumor incidence is unrelated to gender, but age should be considered a risk factor. Up to 85% of tumors are malignant, and its prognosis is poor (10,11). Mast cells, which are normally found within the connective tissue of an animal, develop within the bone marrow of animals. They produce histamine, protease, chemotactic factor, cytokines, and metabolites of arachidonic acid which play an important role in immunological inflammatory and immediate-type allergic reactions (2,3,4,5,14).

Diagnosis of a mast cell tumor (MCT) is easy in routinely processed tissues compared to others. Fine needle aspiration (FNA) combined cytological evaluation is also effective in diagnosis of a MCT (12). MCTs are not always well differentiated malignant and vary in appearance ranging from a wart-like nodule to an ulcerated mass to a small lump. Diagnosis of a MCT can usually be obtained by performing a FNA or preferably with histological stain. FNA of dermal and subcutaneous masses is a simple technique and should be performed prior to surgery. A preoperative diagnosis of MCTs can generally influence the type and extent of surgical intervention (6,8,13). Cytological evaluation with FNAs samples for MCTs is a generally acceptable method in clinics (8). Cytology of MCTs reveals a discrete round-cell population with moderate amounts of cytoplasm containing purplish red cytoplasmic granules of variable numbers and size (1,6,9,13). Mast cell granules can be stained with routine histological stains (Wright-Giemsa, Leishman, etc.) as well as rapid modified

Romanowsky-type stains (e.g. Diff-Quik) used in most practices. Although convenient for diagnosis, FNA cytology does not provide a histological grade and excisional samples should be submitted for histological grading and margin assessment (15). However, poorly differentiated MCTs can be confused with other round cell tumors. This can result in misdiagnosis and a lack of aggressive therapy (12). Histological staining methods are used to evaluate histological grading and margin assessment in canine mast cell tumors (15). To the best of our knowledge, evaluations of hedgehog mast cell tumor staining methods are limited. The purpose of this case study was to evaluate the stains for a mast cell tumor histopathological finding in hedgehog.

Case

A 2-year old female African hedgehog (*Atelerix albiventris*) weighing 645 gram was presented with a circumscribed mass in the subcutaneous of the left shoulder. The mass, which was noticed one month before visiting a veterinary hospital, was 2 × 2 cm size and had a hard texture (Fig 1). FNA was performed in the subcutaneous mass. In the cytological evaluation, round cells with basophilic granules were observed on the FNA biopsy prepared Wright-Giemsa stained slide, which was diagnosed as a mast cell tumor in hedgehog (Fig 2). The tumor mass was surgically removed under 2% isoflurane anesthesia. The sample was fixed in 10% buffered formalin and then paraffin-embedded. For comparison of the difference between various staining methods for the mast cell tumor in the hedgehog, it was cut to a serial section of approximately 5 μm. The sliced tissues were stained by hematoxylin-eosin (HE), toluidine blue, periodic acid-Schiff (PAS), and Safranin O.

Hematoxylin and eosin

Round cells showed basophilic granules stained an intense

¹Corresponding author.
E-mail : sigol@cbnu.ac.kr



Fig 1. A circumscribed hard mass beneath the skin of the shoulder.

dark blue, and background matrix was stained pale blue. Mast cells varied from pale to intensely basophilic, with an occasional distinct granular pattern (Fig 3A).

Periodic acid-Schiff

The cytoplasm of mast cells was pink to light red. Other cells were not stained with PAS. The nuclei of all cells stained pale green. All connective tissue stained pale pink to red (Fig 3B).

Toluidine blue

Granules in mast cells were from blue to darker blue in the sections stained. Background matrix and nuclei of the mast cell were not stained (Fig 3C).

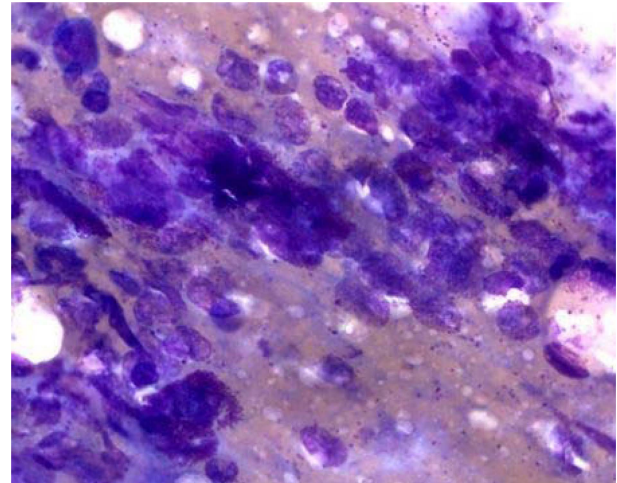


Fig 2. Cytology with FNA. Wright-Giemsa stain × 1000.

Safranin O

The granules showed brick red in mast cells. The connective tissue stained light green, as did the background, making those cells difficult to distinguish (Fig 3D).

Discussion

In this case, the tentative diagnosis was a mast cell tumor, based on FNA cytology. The mass was removed surgically under isoflurane anesthesia. The mast cells showed basophilic round granules in the cytoplasm with Wright-Giemsa stain on the FNA smear slide. FNA yields a correct diagnosis in 92% to 96% of histologically confirmed canine mast cell tumors (14). Although cytological evaluation for mast cell tumor

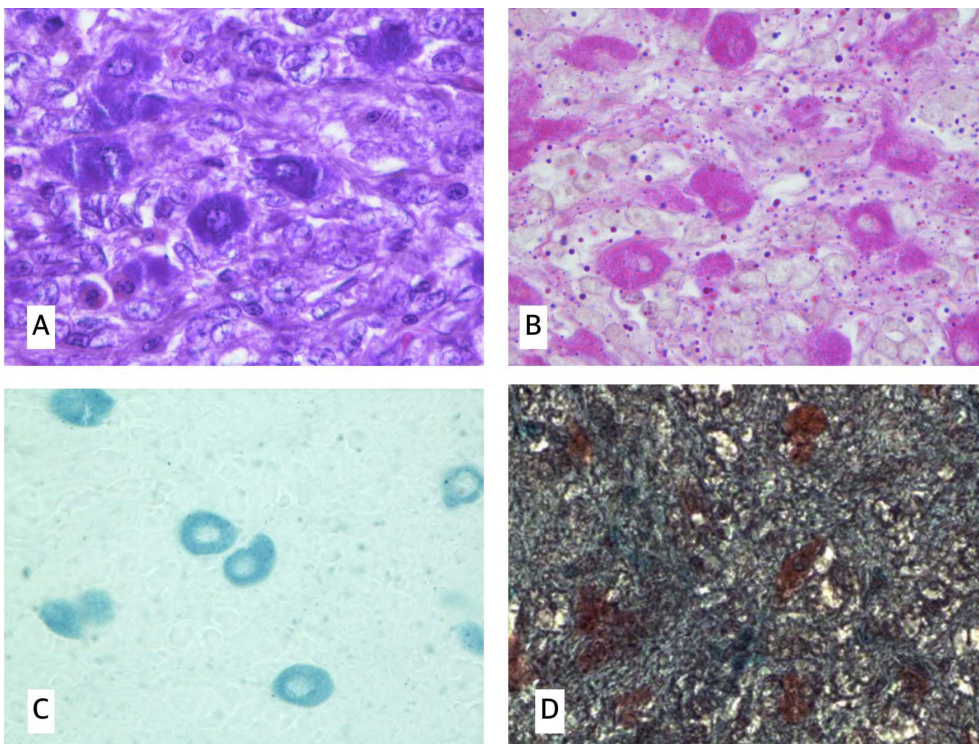


Fig 3. Compare of stain type in hedgehog’s mast cell tumor. A, hematoxylin-eosin; B, Periodic acid-Schiff; C, toluidine blue; D, Safranin O.

provides pathognomic information, surgical pathology is an inevitable procedure for proper treatment. MCTs have distinct cell membrane, a central round nucleus and variable numbers of cytoplasmic granules that stain light-grey blue with haematoxylin and eosin or purple with metachromic stains such as toluidine blue in canine (15).

In the histological stain, a reddish cell with many granules in HE (Fig 3A), red granules more than the nucleus in PAS (Fig 3B), blue or darker blue in toluidine blue (Fig 3C), and black nucleus and brick-colored granules in Safranin O stain (Fig 3D) were confirmed in a hedgehog mast cell tumor. PAS and toluidine blue stain showed more distinguishable figures than HE and Safranin O stain. This case was diagnosed as a well differentiated mast cell tumor based on histopathological evaluation. Cellular morphology depends on the degree of differentiation. In conclusion, FNA cytology and HE stain of histological sample combined results showed acceptable methods for evaluation of hedgehog's MCTs. In addition, these results suggested that PAS and TB staining methods were useful for histological evaluation of hedgehog MCTs.

Acknowledgement

This work was supported by the research grant of Chungbuk National University in 2013.

References

1. Bostock DE. Neoplasms of the skin and subcutaneous tissues in dogs and cats. *British Vet J* 1986; 142: 1-19.
2. Galli SJ, Maurer M, Lantz CS. Mast cells as sentinels of innate immunity. *Curr Opin Immunol* 1999; 11: 53-59.
3. Galli SJ, Nakae S. Mast cells to the defense. *Nature Immunol* 2003; 4: 1160-1162.
4. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nature Immunol* 2005; 6: 135-142.
5. Galli SJ, Wershil BK. The two faces of the mast cell. *Nature* 1996; 381: 21-22.
6. Govier SM. Principles of treatment for mast cell tumors. *Clin Tech Small Anim Pract* 2003; 18: 103-106.
7. Ko KR, Hong KH, Kim JH. Basal cell carcinoma in a domestic hedgehog. *J Vet Clin* 2015; 32: 548-550.
8. London CA, Galli SJ, Yuuki T, Hu ZQ, Helfand SC, Geissler EN. Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. *Exper Hematol* 1999; 27: 689-697.
9. Macy DW. Canine mast cell tumors. *Veterinary Clinics of North America: Small Ani Pract* 1985; 15: 783-803.
10. Raymond JT, Garner MM. Spontaneous tumors in captive African hedgehogs (*Atelerix albiventris*): A retrospective study. *J Comp Pathol* 2001; 124: 128-133.
11. Raymond JT, White MR, Janovitz EB. Malignant mast cell tumor in an African hedgehog (*Atelerix albiventris*). *J Wildlife Dis* 1997; 33: 140-142.
12. Simoes JPC, Schoning P. Canine mast cell tumors: a comparison of staining techniques. *J Vet Diagn Invest* 1994; 6: 458-465.
13. Tams TR, Macy DW. Canine mast cell tumors. *Compend Contin Educ Vet* 1981; 17: 869-878.
14. Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol* 2000; 12: 624-631.
15. Welle MM, Bley CR, Howard J, Rfenacht S. Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. *Vet Dermatol* 2008; 19: 321-339.