CYTOLOGICAL AND HISTOPATHOLOGICAL STUDIES OF MESENCHYMAL AND MELANOTIC NEOPLASMS IN DOGS

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Abstract: The incidence of neoplasia has assumed an increasing trend in domestic animals especially in canines due to their longevity of life. Neoplasms of mesenchymal tissue origin are found to occur with high incidence in dogs. The current work was undertaken with the objectives of studying the cytological and histopathological features of non round cell and non epithelial neoplasms and comparing them as diagnostic tools. The mesenchymal and melanotic tumors diagnosed in the study included fibrosarcoma (#5), malignant melanoma (#5), lipoma (#3), chondrosarcoma (#2), osteosarcoma (#1) and malignant schwannoma (#1). Cytologically varying degrees of cellular anaplasia, pleomorphism and mitotic activity were recorded. Microscopically the interlacing bundles of spindle to stellate shaped cells with elongated plumpy nuclei (fibrosarcoma), varying number of brownish to blackish colored granules (malignant melanoma) presence of mature uniform sized adipocytes with eccentrically placed nuclei (Lipoma), hyperchromatic nuclei, prominent nucleoli and occasional binucleated cells (chondrosarcoma), presence of bony spicules with osteoid material (Osteosarcoma) and bands of densely packed spindle shaped cells with oval or round hyperchromatic nuclei (malignant Schwannoma) were observed. Cytology in complementary with histological characterization resulted in better diagnosis of mesenchymal neoplasms.

Key words: Dogs, Malignant melanoma, Mesenchymal neoplasms

INTRODUCTION

Neoplasia, a disturbance of growth has remained as a fatal problem of human and animals for centuries. The incidence of neoplasia has assumed an increasing trend in domestic animals especially canines due to their longevity of life. Neoplasms of mesenchymal tissue origin also referred to as soft tissue or connective tissue tumors are found to occur with high incidence in cats (Second most) and dogs (Third most) [1-3]. Virtually, they may be located in any organ, with differential frequency of occurrence. The cluster of mesenchymal neoplasms mainly includes both benign and malignant forms of tumors arising from fibrous tissue, adipose tissue, striated muscle, smooth muscle, vascular tissue, mast cell, histiocytes, mesothelium, synovium or peripheral nerves [1-6].
Usefulness of cytological techniques in veterinary medicine has been acknowledged periodically but there has been little exploitation of it as a routine aid to diagnosis. Diagnostic cytology saves time in the differential diagnosis of cancer and early initiation of treatment. Therefore, the current work was undertaken with the objectives of studying cytology as a tool for diagnosing mesenchymal neoplasms. To characterize the masses based on histopathological features and to compare the cytological and histopathological findings.

MATERIALS AND METHODS

Sample collection: The current investigation was carried out in the Department of Veterinary Pathology, Veterinary College, Bangalore. Sixty-seven dogs presented to clinical complex, Department of Surgery, Veterinary College, Bangalore and other nearby private clinics with the history of neoplasms or growths formed the source for the present study. In addition, samples were also collected from cases with tumor necropsied at the Department of Veterinary Pathology, Veterinary College, Bangalore. Particulars of animals such as breed, age, sex, color, clinical manifestations exhibited by the animals, location, shape, size, color, consistency of growths were recorded.

Preparation of cells for cytological examination: Tissue samples for cytological examination were collected by various methods from the growths depending upon the type and location of the neoplasms. For nodular superficial growths, fine needle aspiration cytology (FNAC) and fine needle aspiration biopsy (FNAB) were used. For ulcerated neoplastic growths impression smears and scraping smears were used. From surgically excised neoplastic growths scraping smears were prepared. The smears were stained using standard Giemsa, new methylene blue, toluidine blue, and papanicolaou standard staining techniques [7].

Interpretation of cytological smears: The smears prepared for cytological examination were first checked for the cellularity and adequate staining quality and were classified into mesenchymal and nonmesenchymal (discrete round cell tumors and epithelial tumors) based on the predominant cell type. Further, they were differentiated either as benign or malignant based on the cytological criteria of malignancy. Criteria for malignancy were adopted [8,9] covering cellular, cytoplasmic and nuclear details as mentioned below:-

Cellular criteria: Cellularity of specimen – low/high; Type of cells present monomorphic/polymorphic/round, round to caudate/spindle; Degree of cellular pleomorphism or anisocytosis – low/high; Cellular arrangement – individual/clusters/sheets.


Nuclear criteria: Anisokaryosis – variable; Nuclear to cytoplasmic (N:C) ratio – variable/high; mitotic activity – low/moderate/high with abnormal mitotic figures; nucleoli – indistinct/single/multiple etc

Nucleolar pleomorphism: moderate/high; chromatin pattern–hypochromatic/hyperchromatic/coarse/clumped/uneven margination at the nuclear membrane; nuclear molding – present/absent; multinucleation–present/absent

Histopathological examination: Tissue samples collected either by biopsy, after surgery or at postmortem from cases of canine neoplasms were fixed immediately in 10% neutral buffered formalin.

Representative samples from the neoplastic growths were processed by routine paraffin embedding technique. Sections of four to five micron thickness were cut using Erma microtome with disposable blades. These sections were then stained with routine hematoxyline and eosin (H & E) method. The following special stains were used as and when required to identify the type of neoplasms as recomomded by Luna [7] {a) van Giesons for collagen fibers, b) Masson’s trichrome for connective tissue, myoepithelium and keratin, c) Masson Fontana for melanin pigments, and d) Toluidine blue for mast cell granules}. The experiment was carried out from the materials obtained by clinical/surgical/post mortem procedures.
RESULTS

A total of 67 tumor suspected cases were studied. The age of susceptibility of the animals in the present study varied from 4 to 15 years with an average age of 8.76 years. Maximum number of cases were recorded in pure and crossbreds followed by nondescript dogs.

Methods of collection: For cytological examination, the cells from tumors were collected by fine-needle aspiration, scraping and impression smear methods. Among these, the scraping smear, which was adopted for collection of cells from ulcerated, easily accessible superficial tumors as well as in a few biopsy specimens, yielded a large number of cells for examination.

Staining characteristics: Among all the stains used, Giemsa and papanicolaou staining techniques yielded consistent results. Melanin pigments appeared bluish to blackish in Giemsa and new methylene blue, light brownish to dark brown in toluidine blue and papanicolaou. The tumors were initially classified based on the predominant cell type as (a) mesenchymal tumors (b) non-mesenchymal-round cell and epithelial tumors.

Overall, 17 cases (25.37%), mesenchymal/melanotic tumors were observed that included fibrosarcoma (#5), malignant melanoma (#5), lipoma (#3), chondrosarcoma (#2), osteosarcoma (#1), malignant Schwannoma (#1).

Fibrosarcoma: The cytological smears showed a moderate number of pleomorphic plumpy cells occurring either individually or in aggregates in smears. The cells were round to oval shaped with cytoplasmic extensions from both the ends. The nuclei showed marked anisokaryosis and contained coarse chromatin with multiple aggregations. Occasional binucleated and multinucleated cells were observed. Pinkish collagenous material in the intercellular spaces as shown in figure 1 was also observed.

Histopathology of the fibrosarcomas revealed interlacing bundles of spindle to stellate shaped cells with elongated plumpy nuclei. Cytoplasmic processes were observed trailing away from the nucleus. High degree of cellular anaplasia, pleomorphism and varying degree of mitotic activity were the other features noticed (Fig. 2). Van Gieson stained sections of fibrosarcoma revealed spindle shaped cells with hyperchromatic nuclei and collagen, which appeared red in color.

Malignant melanoma: Cytologically, the smears contained a large number of cells arranged individually or in clusters. The cells showed a high degree of anaplasia with pleomorphism and anisokaryosis. The nuclei revealed coarse chromatin and a prominent nucleolus. The nucleus to cytoplasmic ratio was high and occasional cells revealed presence of varying number of brownish to blackish colored granules which varied in their size. A few mitotic figures and binucleated cells were also observed. The granules were brownish in papanicolaou and H & E and deep blue to black in Giemsa and toluidine blue (Fig. 3).

Microscopically, all the melanomas were confirmed as malignant with varying grades of malignancy and were characterized by high cellularity, high cellular anaplasia and numerous mitotic figures. All five cases were diagnosed as melanotic melanomas as the cytoplasm showed brown to black colored melanin pigment, at times obscuring the nuclear details. The histological diagnosis was confirmed by silver stain (Masson Fontana), which revealed granular black colored melanin pigments in proliferating neoplastic cells (Fig. 4). Histopathologically melanomas were further sub-classified based on the predominant cellular type as epitheloid (#1), spindle and dendritic (#1) and mixed type (#3).

Epitheloid type revealed round cells with vesicular nuclei and the growth appeared as a carcinoma. Spindle/dendritic form was characterized by densely packed heavily pigmented spindle or dendritic cells arranged in band like pattern with high cellular pleomorphism and presence of occasional giant cells. Mixed types of malignant melanomas showed presence of both epitheloid and spindle shaped cells. In all the cases the pigments stained brownish black in H. & E. In silver stained sections, the cytoplasmic melanin pigments stained black in color.

Lipoma: Cytologically the smears revealed adipocytes with abundant clear cytoplasm and a
small condensed basophilic nucleus placed eccentrically. The cells were well appreciated in toluidine blue and New methylene blue stained smears (Fig. 5). Histologically, the lipomas were characterized by presence of mature almost uniform sized fat cells with flattened eccentrically placed nuclei. Bands of dense connective tissue were also observed between the clusters of cells.

**Chondrosarcoma:** Cytologically, the chondrosarcomas revealed presence of pleomorphic individually occurring cells with high nucleus to cytoplasmic ratio. The nucleus was eccentrically placed and contained coarse chromatin and a prominent nucleolus. Many binucleated cells and cells under division were also observed. Cytoplasm revealed fine vacuolations and magenta colored fibrillar material indicative of chondroitin was observed intercellularly (Fig. 6).

Microscopically, it was characterized by highly cellular pleomorphic cells with hyperchromatic nuclei and prominent nucleoli. Differentiation into cartilaginous mass was observed at multiple places with chondroid tissue formation. Masson’s trichrome stained section revealed typical chondrocytes with bluish colored intercellular substance. Chondrosarcoma involving mammary gland was found originating from fibrous connective tissue of mammary gland on metaplasia, whereas chondrosarcoma from nasal bridge was from the cartilage of nasal passage.

**Osteoblastic osteosarcoma:** Cytologically, the smears contained a large number of pleomorphic cells showing anisokaryosis. The nuclei revealed stippled chromatin and prominent nucleolus. The cytoplasm was moderately abundant and basophilic with indistinct borders. Numerous binucleated and multinucleated cells were also observed. Eosinophilic matrix (osteoid) produced by the neoplastic cells in the intercellular spaces was an additional feature (Fig. 7).

Histopathologically, the sections revealed highly cellular proliferating tissue consisting of mostly immature osteoblasts with pleomorphic and hyperchromatic nuclei and prominent well defined nucleoli. Frequent mitotic figures and a large number of multinucleated giant cells were observed. The tumor was also characterized by presence of bony spicules, thin layers of osteoid material in the midst of proliferating cells and multiple areas of necrosis.

**Malignant schwannoma:** Malignant schwannoma cytologically revealed abundant number of round to oval shaped cells with cytoplasmic extensions on either side of cells. The nucleus contained granular chromatin with prominent nucleolus (Fig. 8).

Microscopically, malignant schwannoma revealed bands of densely packed spindle shaped cells with oval or round hyperchromatic nuclei. These cells often formed interlacing network and there was no collagen or reticulin formation. There were numerous mitotic figures, with high cellular anaplasia and pleomorphism.

**DISCUSSION**

A total of 67 neoplastic growths were screened by cytological and histopathological procedures. The results revealed 17 cases (25.37%) of mesenchymal/melanotic neoplasms.

**Fibrosarcoma:** Cytologically, smears from fibrosarcoma cases yielded malignant mesenchymal cells occurring either as individual cells or in aggregates.

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**Legend for Figures:**

- **Fig. 1:** Cytological smear showing spindle shaped cells with elongated nucleus containing coarse chromatin in multiple aggregates and cytoplasmic extension from both the ends in fibrosarcoma. (Giemsa X 125).
- **Fig. 2:** Section of fibrosarcoma showing interlacing bundles of spindle shaped cells with elongated nuclei. (H&E X 12.5).
- **Fig. 3:** Smear of malignant melanoma showing pleomorphic cells, anisokaryosis, nuclei with coarse chromatin and prominent nucleoli. Note one cell containing bluish black colored granules in the cytoplasm. (Giemsa X 125).
- **Fig. 4:** Section of malignant melanoma spindle cell type showing whorl arrangement of cells with abundant black colored melanin pigments (Masson Fontana X 12.5).
- **Fig. 5:** Cytological smear showing adipocytes with abundant clear cytoplasm and small condensed nuclei in lipoma. (Toluidine blue X 50).
- **Fig. 6:** Cytological smear of chondrosarcoma showing anisocytosis and anisokaryosis (Papanicolaou X 125).
- **Fig. 7:** Smear showing pleomorphic cells, multinucleated cells, anisokaryosis, inter and intracellular eosinophilic granular material in osteoblastic osteosarcoma (Giemsa X 125).
- **Fig. 8:** Cytological smear of malignant Schwannoma showing cells with round to oval shaped nucleus containing granular chromatin with prominent nucleolus (Papanicolaou X 125).
aggregates. The cells revealed anisocytosis and anisokaryosis. Occasional binucleated and multinucleated cells and pinkish intercellular collagenous material were noticed. These observations were in accordance with those described by [10,11]. Further, Raskin [10] indicated that the plumpy elongated cells with cytoplasmic extensions and pink collagen material intercellularly contribute in identification of fibrosarcomas. Histopathologically recorded broad interlacing bundles of spindle cells with malignant features and excess of collagen which were noticed in the present study as well [10-13].

**Malignant melanoma:** Cytologically, melanomas revealed large number of round to oval shaped cells either occurring individually or in clusters, with cellular pleomorphism and anisokaryosis. The nuclei were round, central to eccentrically placed with occasional prominent nucleoli. In some cells, the cytoplasmic pigments obscured the nucleus [9,14,15]. In addition, the cytoplasmic granules stained bluish black in Giemsa and toluidine blue, brownish to black colored in papanicolaou and H & E stained smears [8,14]. In New methylene blue stained smears the melanin granules appeared greenish black, a finding also well supported by (6). Histopathologically, all the cases were melanotic and malignant showing high cellularity, anaplasia and numerous mitotic figures. The types of malignant melanomas included epitheloid, spindle and dendritic and mixed type histologically as also shown by other workers [14,16]. In addition the silver stained sections of all the cases of melanomas showed brown to jet black cytoplasmic pigments. Campagne et al. [17] reported that melanoma diagnosis in dogs is challenging due to variability of histological appearance and markers of malignancy such as receptor for activated C-kinase 1 (RACK1) can be used for the definitive diagnosis.

**Chondrosarcoma:** The smears showed moderate cellularity containing pleomorphic individually arranged cells with eccentrically placed nucleus, increased nuclear to cytoplasmic ratio, binucleated forms, mitotic figures, cytoplasmic vacuolations and magenta colored intercellular matrix. These findings were similar to that feline chondrosarcomas [20]. Histopathologically, pleomorphic cells with plumpy single nucleus, hyperchromasia, prominent nucleoli, frequent mitotic figures and cartilage formation with presence of cartilaginous intercellular substance were recorded. The tumor had a lobular appearance with surrounding fibrous capsule consisting of compact collagen fibrils. These findings tallied well with previous observations [4,19,21].

**Osteoblastic osteosarcoma:** One case of osteoblastic osteosarcoma was recorded in the present study. It was in association with spirocerca lupi granuloma of esophagus. The tumor mass was hard, round shaped and grayish white in color, which coincided with the descriptions of osteosarcoma, described by Nikander [22]. Cytologically the presence of individualized cells, binucleate as well as multinucleate giant forms with stippled chromatin were in accordance with earlier workers [10,11]. In addition, the eosinophilic colored material that was observed intracellularly and extracellularly indicated osteoid material and helped in identification of tumor along with occurrence of many multinucleated cells, as also reported by Raskin (10). Histopathologically, osteosarcoma was characterized by proliferating osteoblastic cells, osteoid matrix and bony spicule formation, a feature also recorded others [22,23].

**Malignant Schwannoma:** Single case of malignant Schwannoma located at left axial region was encountered in the investigation. It was grayish white, hard in consistency and well encapsulated as also reported in previvious [24,25]. Round to oval shaped cells with cytoplasmic extensions in occasional cells were noticed. The nuclei were plumpy, elongated, contained granular chromatin and prominent nucleoli. The diagnosis of malignant Schwanoma based on cytology alone was difficult as the cells closely resembled fibroblasts. Further, there is a difficulty in preparation of appropriate smears, marked difference between the cytological and

**Lipoma:** Cytologic smears were composed of adipocytes with condensed eccentrically placed basophilic well appreciated in new methylene blue and toluidine blue stained smears [18]. Histopathologically, lipomas were composed of mature adipocytes with eccentrically placed nucleus and thin connective tissue stroma in between the cells. These findings were in tune with other reseaches [5,19].
histological features and/or immunohistochemical examination for confirmation of malignant Schwannoma.

REFERENCES