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# An apporach of cytological study of canine tumour in routine clinical practice

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**Abstract**
An attempt was made to explore with the objectives to apply cytological methods in the diagnosis of canine tumours in routine clinical practice set up. All canine cases suspected for neoplasm, which attended the Small Animal Clinics of Madras Veterinary College Teaching Hospital were included in this study. Complete animal particulars, clinical history and particulars of lesions were collected. Samples for cytology by employing suitable technique were collected and studied. A total of 40 tumour suspected cases were examined. Fine needle aspiration was collected from all 40 cases. Out of which 31cases of neoplastic, 4 cases of inflammatory and 5 cases of non-diagnostic conditions were found.

**Keywords:** Canine, tumours, FNAC, cytology

## Introduction

Cytology is the study of cellular sample from fluid or tissue prepared directly on glass slides and stained for microscopic evaluation. Cytology is less invasive than tissue biopsies and in some cases can provide a rapid diagnosis that would otherwise take several days if done by histopathology.

Today, the word wide tendency is to look for quick and inexpensive method of tumour diagnosis as the routinely used histological method require invasive sampling and long hours for processing of the tissue samples, instead of this cytology is quick, inexpensive, less painful, less invasive and easily repeatable technique requiring minimal of sophisticated instrument.The success of cytological diagnosis depends upon collection of representative cells, preparation of the specimen and interpretation of smears. These procedures are interdependent (Roszel, 1981; Lumsden & baker, 2000). The role of cytology as a diagnostic tool in veterinary medicine is constantly developing and expanding. Although cytology is viewed as a screening tool, many reactions can be classified as inflammatory, hyperplastic, or neoplastic.

## Materials and methods

The tumour suspected tissue samples included in this study were collected from canine patients which attended the small animal OP ward, Madras Veterinary College Teaching Hospital, Chennai 600 007, during February to June 2013. Tumour samples were also collected during necropsy from the Department of Veterinary Pathology, MVC, Chennai.Particulars of animal like breed, sex and age ware recorded. Specific pathological data such as history, clinical manifestation, location, shape, size of growth were also recorded.

## Preparation of smears

### Fine needle aspiration biopsy

The technique described by Meinkoth and Cowell (2002) was followed After cleaning the area over the mass by alcohol swab, standard aspiration method of FNAB was practiced. The mass was stabilized with one hand while the needle (21 to 25 G) with syringe (3 to 20 mL) attached, was introduced into the center of the mass. Adequate negative pressure was applied by withdrawing the plunger to about three fourth of the volume of the syringe. If the mass is sufficiently large and patient is sufficiently restrain, negative pressure can be maintained while the needle is moved back and forth repeatedly, passing through approximately two third of the diameter of the mass. Several different areas of the mass can be sampled with separate collection attempts.

Care should be taken not to allow the needle to exit the mass while negative pressure is being applied. After that the negative pressure was relaxed and the needle was removed from the mass. The needle was removed from the syringe and air was drawn in to the syringe, the needle was replaced on to the syringe and some of the tissue in the barrel and hub of the needle was expelled on to the middle of a glass microscope slide by rapidly depressing the plunger.

### Impression smears

The incised or excised tissue was held firmly. The tissue was cut with a sharp blade to expose a flat, fresh cut surface. The surface was blotted with blotting paper to make it free of blood and tissue fluid. The blotted tissue was gently pressed on a new slide to get impression smears (Meinkoth and Cowell, 2002).

### Fixation

All smears are fixed by air drying rapidly after making smears on glass slide.

### Leishman – giemsa staining

The Leishman – Giemsa (LG) stain was prepared as follows-

Leishman powder (Merck) - 150 mg
Giemsa powder (Merck) - 30 mg
Methanol (acetone free) - 100 mL

1. The dry fixed smears were covered with LG stain for one minute.
2. The stain was diluted with double the quantity of distilled water and allowed for 20 min.
3. The smears ware washed under tap water and air dried.

**Interpretation of cytology smears**

**Screening of smears**

The smears were screened under the microscope for the cellularity of the smears, staining characteristic of the nucleus, cytoplasm and nucleoli.

**Criteria in evaluating the cytological smears for malignancy**

Neoplasms were cytologically classified as benign or malignant according to the following criteria of malignancy by Tyler *et al.* (1999) -

- Anisocytosis: variations in the cell sizes.
- Pleomorphism: variations in the shapes of cells of the same type.
- Hypercellularity: increase in the cell exfoliation because of weakened connections between cells.
- Macrokaryosis: increase in nucleus sizes.
- Anisokaryosis: variations in the nucleus sizes.
- Multinucleation: increase in the number of nuclei.
- Increased nuclear/cytoplasmic ratio.
- Nuclear molding: deformation of the nuclei.
- Increased mitotic figures and abnormal mitoses.
- Coarse chromatin pattern: coarse placement of nuclear chromatin.
- Macronucleoli: increase in the sizes of the nucleoli.
- Angular nucleoli: presence of angular nucleoli.
- Anisonucleosis: changes in the sizes of nucleoli.

If three or more of the above tumour malignancy criteria were present, the tumour formations were cytologically classified as malignant, while all other cases were classified as benign.

**Results and discussion**

Out of 40 collected samples 31 were cytologically diagnosed as tumours, four were inflammatory conditions and five were non-diagnostic.The non-diagnostic cases were attributed to the following reasons-

1. Scanty biopsy material
2. Blood contamination of aspirates during sampling
3. Non availability of animal for further examination

All tumour cases were classified according to the cell type as-

1. Round cell tumours
2. Epithelial cell tumours and
3. Mesenchymal cell tumours

**Round cell tumours**

**Histiocytoma**

Out of 31 cases, one was diagnosed as histiocytoma. Grossly, the nodular mass was round, pink color measuring about 2- 3 cm in diameter (Plate-1a) in a eleven year old male Doberman dog. The mass was rubbery to hard in consistency and noticed on the lateral side of the digit of right forelimb.

The FNAC smear showed moderate cellularity. The cells were round to oval in shape with distinct outline (Plate-1b) as reported by Duncan and Prass (1979). Cytoplasm was pale blue in color as described by Hall and Mac Willam (1988). Nuclei were oval with indentation and chromatin was fine. Trinucleated cells were also present. Nucleoli were prominent.

**Mast cell tumour**

Four out of 31 cases were diagnosed as mast cell tumours. The light pink color growth (Plate-2a ) in a seven year old male non-descript dog, measured about one cm in diameter and was found on the left thoracic region. A 12 year female Labrador dog revealed round mass of about three cm in diameter on the right forelimb. The other two tumours were noticed in male non- descript dogs, eight and eleven year old, at the right forelimb digits and right thigh region respectively. The masses were oval to round in shape measuring about five to seven cm in diameter.

FNAC smear showed high cellularity and round individual cells. Variable amount of cytoplasmic granules were present inside and outside of the cell (Plate-2b & 2c) as stated by Duncan and Prass (1979). Chromatin was coarse. Cells showed marked anisokaryosis and presence of prominent nucleoli.

**Transmissible venereal tumour**

Five out of 31 cases were diagnosed as TVT. Three of them were noticed in three to seven year old non-descript females dogs. The masses were oval to round in shape, about three to five cm in diameter noticed on the vaginal opening. In a five year old male Labrador dog the masses were found on prepuce and base of penis and were cauliflower in shape. A three year old female Spitz revealed circular mass measuring about 4 cm in diameter at the vaginal opening (Plate-3a). All masses were highly fragile and showed bleeding tendency.

Cytologically, the smears were highly cellular and contained round to oval cells with centrally placed round to oval nuclei as reported by Barton (1978). Pale blue or moderately basophilic cytoplasm (Plate 3b) was filled with multiple discrete vacuoles ( Plate 3c) with distinct cytoplasmic borders. Anisocytosis, anisokaryosis, coarse chromatin and mitotic figure were also present. In one case neutrophilia was observed. These findings are earlier reported by Hall and Mac Williams (1988).

**Lymphoma**

Six out of 31 cases were diagnosed as lymphoma. Three cases were present in non-descript dogs and one each case in German shepherd, Labrador and Doberman breeds of dogs. Age of animals ranged from seven to twelve years. Four animals were male and two were females. In all dogs, generalized lymph node swelling (Plate 4a) was present with marked swelling of prescapular, popliteal and mandibular lymph nodes.

Cytologically, all smears revealed highly cellular with small to medium sized round neoplastic lymphoid cells containing scanty hyper basophilic cytoplasm and high N: C ratio. Nuclei were large round in shape with prominent nucleoli and mitotic figures were seen (Plate 4c). Lympho glandular bodies were also present (Plate 4b) as reported by Alleman and Bain (1984).

**Epithelial tumours**

**Squamous cell carcinoma**

One out of 31 cases was diagnosed squamous cell carcinoma. The mass was present in an eleven year old male Spitz at the tip of the tongue. Grossly the mass was light pink colored, round in shape and about three cm in diameter (Plate 5a).

Cytologically, smears showed clusters of variable sized neoplastic squamous cells with basophilic cytoplasm (plate 5c). Nuclei were large and round with coarse chromatin. Irregular shaped prominent nucleoli were also found.
Individual cells with blunted cytoplasmic tail and characteristic tadpole cells were also seen (Plate 5b). This was in accordance with the study of Thrall (2000). Keratin debris was not seen as described by Barton (1987).

**Carcinoma**
Two out of 31 cases were diagnosed as carcinoma. The firm, spherical, grey white nodule (Plate 6a) of about three cm in diameter was noticed in a five year old, female German shepherd dog at the stifle joint. In an eleven year old, female German shepherd a seven cm oval mass was observed over right side of neck region.
Cytologically, the cells were variable sized, spherical in shape, occurred in clusters and showed basophilic cytoplasm (Plate 6b & c). Nuclei were round in shape with prominent nucleoli and fine to coarse chromatin.

**Mammary tumour**
Five out of 31 cases were diagnosed as mammary adenocarcinoma. The multilobulated and encapsulated round, grey white colored nodule (Plate 7a) was present at left abdominal mammary gland of nine year old female Spitz dog. Two cases were of eight and ten year old female Labrador dogs. The masses were round to oval in shape and about nine cm in diameter. Remaining two cases involved are ten year old German shepherd and another seven year old non-descript dogs with nine to twelve cm size growth at the left abdominal and right inguinal glands.
Cytologically, smears showed the presence of medium to large sized clusters of neoplastic cells. Cells were round to spherical in shape with basophilic cytoplasm and arranged in glandular pattern (Plate-7c). Anisocytosis and anisokaryosis were also present. Nuclei were round to oval in shape with prominent single to multiple nucleoli (Plate-7b). These findings are in accordance with Allen *et al.* (1986) and Allison and Maddux (2008).

**Seminoma**
One out of 31 cases diagnosed as seminoma. The mass was oval in shape and about ten cm in diameter noticed at the caudal aspect of testis on seven year old, non-descript, male dog.
Neoplastic cells were spherical in shape variable sized with lightly stained cytoplasm (Plate 8a & b) as reported by Zinkle (2008). Multinucleated cells were also seen. Nuclei were round to oval in shape with finely reticulated chromatin and prominent nucleoli.

**Lipoma**
Four out of 31 cases were diagnosed as lipoma. The round, encapsulated movable nodular mass (Plate 9a) of about five cm in diameter was seen in a seven years old male Labrador dog at right side of thorax region.
Two other cases involved in twelve and eight year old female and male non-descript dogs respectively. In female dog, the mass was about eight cm in diameter, oval in shape and located at inguinal region while in a male dog it was circular in shape, about six cm in diameter and located on the ventral neck region. In a ten year old male German shepherd, oval mass with ten cm in diameter was noticed on the ventral thoracic region.
Cytologically, smears revealed the presence of variable sized, round neoplastic lipocytes with pyknotic nuclei and clear cytoplasm (Plate 9b) as described by Tyler *et al.* (2008).

**Fibromatous epulis**
One out of 31 cases was diagnoced as epulis. The red color round mass about one cm in diameter located at the gum region of inner side of lower jaw (Plate 10a) in a twelve year old Dachshund male dog.
Cytological evaluation revealed the presence of a few spindle shaped cells with basophilic cytoplasm as stated by Tyler *et al.* (1993). Nuclei were round to oval in shape with coarse chromatin and showed prominent nucleoli (Plate-10b).

**Osteosarcoma**
One out of 31 cases was diagnosed as osteosarcoma. The hard, encapsulated and round mass (Plate 11a) about 5 cm in diameter was present in a four year old male Doberman dog on the distal part of radius and ulna at carpal joint of right fore limb.
Clusters of round to oval osteoblastic cells with bright pink stained intercellular matrix (Plate 11b & c) were seen as reported by Fielder and Mahaffey (2008). The cells were round to fusiform in shape with anisokaryosis, anisocytosis, large nucleoli and clear micro vacuoles in the cytoplasm. Radio graphically the growth showed characteristic sunburst appearance.

**Conclusion**
The present work was undertaken with the objectives to apply cytological methods in the diagnosis of canine tumours in routine clinical practice set up. A total 40 tumours suspected cases were encountered. Out of which 4 cases were inflammatory and 5 cases were of non-diagnostic conditions. Fine needle aspiration was collected from all 40 cases. Out of 40 cases studied 31 were neoplastic conditions which included 16 round cell tumours, 9 epithelial tumours and 5 mesenchymal tumours. The 16 cases of round cell tumours included 1 histiocytoma, 5 mast cell tumours, 4 TVTs and 6 lymphoma cases. The 9 epithelial tumours included 1 squamous cell carcinoma, 2 carcinoma, 5 mammary tumours and 1 seminoma case. The 5 mesenchymal tumours included 3 lipoma, 1 fibromatous epulis and 1 osteosarcoma case.
FNAC was found inexpensive and less invasive technique for collection of tumour samples. LG stained smears showed excellent diagnostic efficacy by staining cytoplasm, extracellular material and nucleus.

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