Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 6 Issue 6 June 2022

Research Article

Canine Mammary Tumor Fine Needle Aspirate Cytology and Identification of Tumor Types

Komal Basra and Ratan K Choudhary*

Animal Stem Cells Lab, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India

***Corresponding Author:** Ratan K Choudhary, Animal Stem Cells Lab, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Received: April 11, 2022

Published: May 27, 2022

Abstract

Fine needle aspiration cytology (FNAC) is a technique that can be used to diagnose canine mammary gland tumors early and easy. This technique may allow a preliminary test for diagnosing and differentiating mammary tumor types in canines. We hypothesize that cells of FNA from canine mammary tumors have tumor-specific cells, and cytomorphological investigation could distinguish between simple vs. complex and benign vs. malignant tumors. In this study, we planned to collect fine-needle aspirates from canine mammary tumors (CMT) and study morphological features of the cells present in FNA to distinguish types of tumors. Additionally, using image analysis software, a comprehensive investigation of quantitative cytomorphological data like cell shape, cell size, nucleus size, size of vacuolar cytoplasm, variable number of the nucleolus, and others will be analyzed. This technique can potentially act as a first-hand diagnostic tool for identifying mammary tumors in canines. Setting up this technique in the veterinary hospital is easy, quick, non-invasive, and cheaper. We can locate simple vs. complex tumors or benign vs. malignant tumors using FNAC without undergoing invasive mammary tissue biopsy.

Keywords: Fine Needle Aspiration; Canine; Mammary Tumor; Cytomorphology

Introduction

Canines are the earliest domesticated animals. They are symbolic of devotion and alertness and frequently serve as guardians and protectors. Dogs are generally easily susceptible to diseases like cancer, rabies, parvovirus, kennel cough, ringworm, diabetes, heartworm, etc. The occurrence of tumors or, more precisely, mammary gland tumors in dogs is widespread. According to data, the occurrence rate of canine mammary tumors (CMTs) per year is approximately 198 cases in every 100,000 dogs [1].

In general, a higher rate of mammary gland tumors is seen in breeds like point setters and retrievers (Moulton 1990). Around 6-7 years of dogs is considered the "cancer age," and the maximum rate of risk of cancer occurrence is at the age of 9-11 years [2,3] The overall mortality due to CMT reported around 6% [4]. Mammary gland tumors are most common in intact bitches or bitches with ovarian residues and rare in male dogs [5,6]. It has been noticed that the rate of CMTs has reduced due to their early neutering. They are relatively prevalent in healthy female dogs. Mammary tumors may stem from myoepithelial or luminal epithelial cells that form ducts and acini or connective tissue surrounding mammary ducts and acini [7]. The development of CMTs occurs due to the influence of gonadal hormones, obesity at a young age, and intake of red meat or a homemade diet [8,9].

Estrogens and progesterone are essential for proper mammary gland growth but have also been linked to tumor formation. Estrogens regulate the transcription of various nuclear proto-oncogenes and promoters of started cells. Both benign and malignant mammary gland cancers exhibit estrogen receptors (ERs). The ER may constitute a sensible therapeutic target in canine mammary gland cancers, similar to breast cancer in people because it is implicated in the first malignant transformation [10-12].

Fine needle aspiration (FNA) is a biopsy in which tissue and fluid samples are taken from solid or cystic breast lesions using a tiny needle. It's one of several techniques used to detect whether the lumps present in the gland are tumorous or not [13]. Hence, this technique could detect canine mammary gland tumors based on cytology known as fine needle aspiration cytology (FNAC). FNAC is being used commonly used for early diagnosis of human neoplasm and also in dogs to differentiate mammary lesions and correlate their relationship with histological grade of CMT [14]. Aim of this study was to differentiated the simple, complex and mixed mammary tumors of canine described by Robinson grading system [15] without histological study. The Robinson's grading system corresponds well with the histopathological grades and is considered a useful pre-operative diagnostic tool to human patient [16].

Material and Methods

Ethical statement

The Approval of 61st Institutional Animal Ethics Committee (IAEC) of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana with Registration no. 497/GO/Re/SL/02/CPCSEA was provided to collect FNAC on surgical cases. Samples were provided by the Teaching Veterinary Clinical Complex, GADVASU as a part of therapeutic intervention**.**

Animals

A total of 10 excised tissues of mammary glands affected with tumors obtained after the surgery have been used. Further, aspirates were collected from all ten tissues, and one healthy aspirate has also composed for comparison purposes. These aspirates were utilized for making smears and then stained afterward with hematoxylin and eosin. The dogs from which aspirates were collected were usually Pomeranian, Beagle, German-Shepherd, and Labrador-Retriever between the age of 6 to11 years.

Aspirate collection

The excised tissue is collected from the mammary gland of the affected animal. A 22-gauge needle attached to a 3-5 ml syringe is used to collect aspirates. The needle is inserted into the tissue where lumps are felt, and then a backward and forth motion is applied to the syringe. The needle is inserted in all the directions to ensure a proper collection of aspirates from all the directions. After the collection, aspirates are pushed out onto the glass slides. This process is repeated again and again for aspirates collection from different areas of the mammary tissue. Then aspirate smears are made and kept for air-drying for further staining.

Staining

The air-dried slides are fixed with the help of absolute ethanol. Then the slides are air-dried for the next staining procedure. Then the slides are dipped in Hematoxylin and eosin for 10 and 5 minutes, respectively. The slides are then washed under running tap water for 2-3 minutes, after staining with Hematoxylin and eosin. Then after air-drying the stained slides, they are mounted with coverslips using DPX mountant (HiMedia Laboratories, Mumbai, India).

Cytological examination

Cells of the smears were examined of those slides which were high quality- smear thickness, distinct cell types with less to no RBCs and inflammatory cells, cell morphology, proper staining. Firstly, cytological smears were scanned at 10x objective per 10 fields followed by 40X, and 100X objective piece using a brightfield microscope. Cellularity, presence of clusters and background features were evaluated according to the scoring presented under the Robinson grading system. Size of the nuclei were calculated using imageJ and at least twenty nuclei per samples were analyzed.

Results and Discussion

Cell dissociation

The cells differ from each other in terms of mainly if they are present in clusters (Grade 1) or they are a mixture of single cells and groups (Grade 2), or they are primarily single cells (Grade 3). The cells of samples 1 and 10 showed cluster formation, whereas samples 2, 3, 4, 5, 8 and 9 had cells present in clusters and singles. The cells of sample 6 were all scattered. Hence, they were present in a singular form.

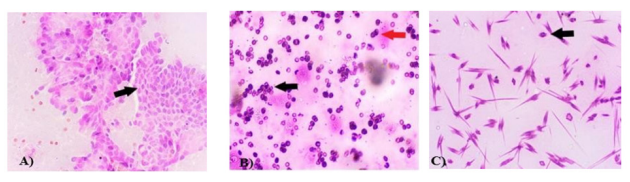

Figure 1: Representative pictures of H-E-stained smears of CMT fine-needle tissue aspirates in canine. A) Cells in clusters (black arrow) B) Cells in clusters (black arrow) and singles (red arrow) C) Single cells (black arrow).

Cell uniformity

The uniformity of cells differs from each other in 3 ways, whether they are monomorphic (Grade 1), mildly pleomorphic (Grade 2), or pleomorphic (Grade 3).

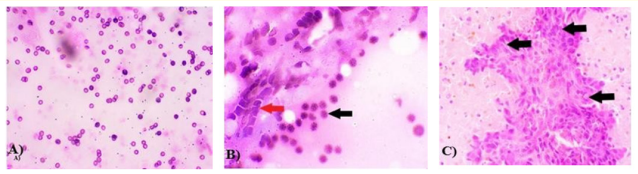

Figure 2: Representative pictures of H-E-stained smears of CMT fine-needle tissue aspirates in canine. A) Monomorphic cells B) Mildly pleomorphic cells (circular cells with a black arrow and exaggerated cells with a red arrow) C) Pleomorphic cells (black arrow).

The cells of samples 1, 7 and 10 were all similar in terms of shape and size, so they showed monomorphism, whereas the cells of samples 2, 3, 4, 5, 8 and 9 differed from each other slightly, so they showed mild pleomorphism. The cells of sample 6 varied greatly from each other hence showing complete pleomorphism.

Nuclear margin

The appearance of nuclear margin is graded in terms of whether it is smooth (Grade 1), slightly irregular (Grade 2), and presence of buds, clefts, or tufts (Grade 3). The cells of samples 1,7 and 10 had smooth nuclear margins, whereas the cells of samples 2, 3, 4, 5 and 9 had slightly irregular nuclear margins. The cells of samples 6 and 8 showed distortions or buds, clefts, and tufts in the nuclear margin.

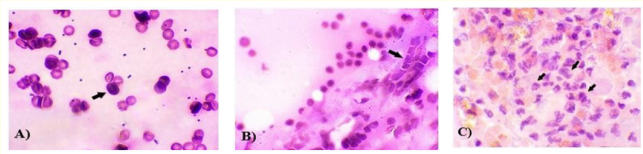

Figure 3: Representative pictures of H-E-stained smears of CMT fine-needle tissue aspirates in canine. A) Smooth nuclear margin B) Irregular nuclear margin C) Distorted nuclear margin.

Chromatin pattern

When discussing chromatin patterns, the cells showed vesicular chromatin (Grade 1), granular chromatin (Grade 2), and clumped chromatin (Grade 3). The cells of samples 1, 3, 7, 9 and 10 showed a chromatin pattern, which was vesicular; sampled, 2, 4, 5 and 8 showed a granular chromatin pattern, and sample 6 showed clumped chromatin.

Nuclear size

Based on the Robinson Grading System, the cells vary from each based-on differences in nucleus size. The cells if 1-2 times larger than the size of RBC (Grade 1), 3-4 times larger than RBC (Grade 2), and if more than five times larger than RBC (Grade 3). The cells of samples 1, 4, 5, and 8 had a nucleus size around 1-2 times larger than the size of RBC, and samples 2, 3, 6, 7, 9 and10 had a nucleus size around 3-4 times larger than the size of RBC.

Collagen fibers

The presence of collagen fibers was seen only in sample # 2. Other samples lacked the presence of collagen fibers. Collagen fibers occur in the tumor microenvironment as there is an abundance of protein polymers, collagen itself. This increases the tissues stiffness and plays an act in tumor immunity regulation and helps in the promotion of metastasis [17,18]. A p53 pathway regulates the formation of collagen in the tumor [19].

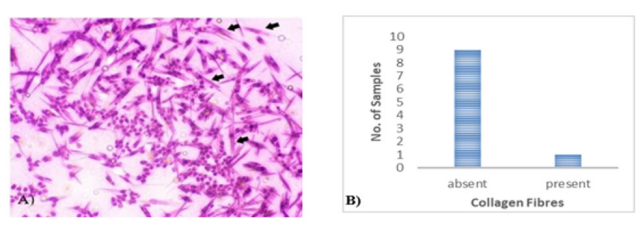

Figure 4: A) Presence of collagen fibers. B) Bar graph showing the number of samples with and without the collagen fibers present in a smear of fine-needle aspirates of CMT.

Cytoplasmic vacuolation

Cytoplasmic vacuolation was seen in all samples except sample 7. These vacuolations occur in order to limit the amount or degree of damage to the cell due to its excessive growth.

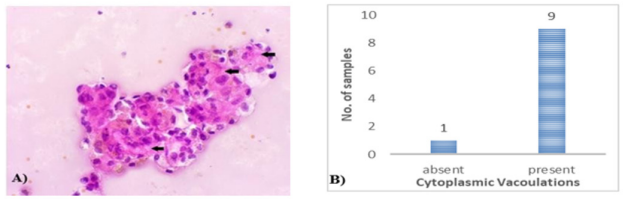

Figure 5: A) Presence of cytoplasmic vacuolation (40X) image. B) Bar graph showing the number of samples with and without the cytoplasmic vacuolation in a smear of fine-needle aspirates of CMT.

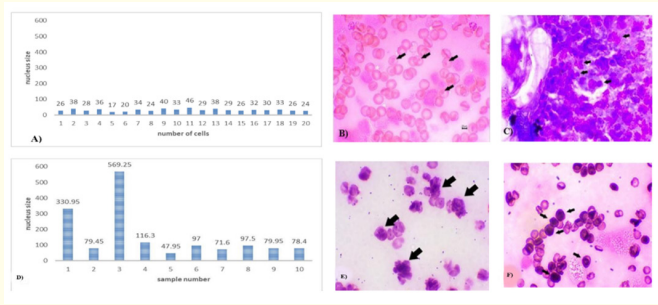

Figure 6: Comparison of nuclear size of cells of FNAC from healthy and CMT glands. A) Bar graph showing the variation between randomly counted nuclei of healthy canine mammary gland aspirate. B) Image showing nucleus size of hematoxylin and eosin-stained healthy canine mammary gland aspirate (black arrows). C) Giemsa-stained nucleus size of tumorous canine mammary gland aspirate sample 1 (black arrows). D) Bar graph showing sample-wise variation in the nuclei of randomly counted nuclei (~100 nuclei were counted from 3-4 representative photomicrographs from each sample). The figure at the top of each bar shows the cells' mean nuclear size (in pixel). Cares were taken not to count the inflammatory cells identified by their nuclear morphometry. E) Hematoxylin and Eosin-stained nucleus size of tumorous canine mammary gland aspirate sample 2 (black arrows). F) Hematoxylin and eosin-stained nucleus size of tumorous canine mammary gland aspirate sample 3 (black arrows).

Nucleus size

Based on calculations, a variation was seen in the nuclear sizes of all the samples. Some nuclei were small in size, whereas others were very much exaggerated. Some were distorted, whereas some were circular irrespective of the size.

Dividing cells

The presence of dividing cells is usually associated with a high rate of mitotic activities, cell proliferation. Many dividing cells were present in all the samples but varying amounts. The percentage of dividing cells was calculated, and the highest number of dividing cells was seen in sample 6, while the lowest number of dividing cells was seen in sample 7.

Cytological evaluation of FNAC of CMT can be used as a pre-diagnostic tool based on the presence of dividing cells, nuclear size of the cells, collagen fibers, cell uniformity, chromatin pattern and other features that are different in cancer cells in comparison to normal healthy cells. Alteration in cellular morphology can be used as a diagnostic tool in accurate diagnosis of tumor cells before scheduling a treatment plan for the patient. Such evaluation provi-

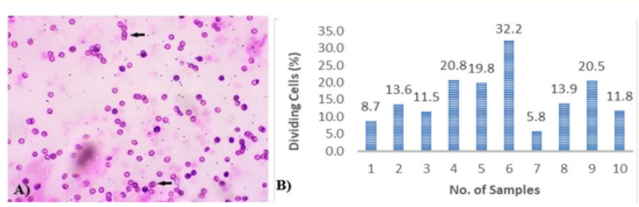

Figure 7: A) Presence of dividing cells (40X) image. B) Bar graph showing the percentage of dividing cells in FNAC of CMT.

des immediate results and is an easy, low-cost method, rapid and straightforward, even if the risk is minimal for the patients. There are certain limitations to the cytology, like an absence of tissue structure and variable cellularity resulting in misinterpretation of the results [20].

Conclusions

In conclusion, canine FNAC can potentially act as a first-hand diagnostic tool for the identification of mammary tumors. Setting up this technique in the veterinary hospital may be simple, easy, quick, non-invasive, and inexpensive way to decipher types of CMT without undergoing invasive biopsy, histology and expertise. Identification of simple vs. complex tumors or benign vs. malignant tumors possible using FNAC in majority of the CMT.

Bibliography

1. Gray M., *et al.* "Naturally-Occurring Canine Mammary Tumors as a Translational Model for Human Breast Cancer". *Frontiers in Oncology* 10 (2020): 617.

2. Schneider R., *et al.* "Factors Influencing Canine Mammary Cancer Development and Postsurgical Survival". *JNCI: Journal of the National Cancer Institute* 43 (1969): 1249-1261.

3. Davis BW and Ostrander EA. "Domestic Dogs and Cancer Research: A Breed-Based Genomics Approach". *ILAR Journal* 55 (2014): 59.

4. Egenvall A., *et al.* "Incidence of and survival after mammary tumors in a population of over 80,000 insured female dogs in Sweden from 1995 to 2002". *Preventive Veterinary Medicine* 69 (2005): 109-127.

5. Beaudu-Lange C., *et al.* "Prevalence of reproductive disorders including mammary tumors and associated mortality in female dogs". *Veterinary Sciences* 8 (2021).

6. Sontas BH., *et al.* "Fine-Needle Aspiration Biopsy of Canine Mammary Gland Tumours: A Comparison Between Cytology and Histopathology". *Reproduction in Domestic Animals* 47 (2012): 125-130.

7. Kuppusamy K., *et al.* "Cytological Grading of Breast Tumors-The Human and Canine Perspective". *Frontiers in Veterinary Science* 6 (2019): 283.

8. Fesseha H. "Mammary Tumours in Dogs and its Treatment Option- A Review". *Biomedical Journal of Scientific and Technical Research* 30 (2020).

9. Perez Alenza MD., *et al.* "Factors influencing the incidence and prognosis of canine mammary tumours". *Journal of Small Animal Practice* 41 (2000): 287-291.

10. Sorenmo K. "Canine mammary gland tumors". *Veterinary Clinics of North America: Small Animal Practice* 33 (2003): 573-596.

11. Sorenmo K., *et al.* "The estrogen effect; clinical and histopathological evidence of dichotomous influences in dogs with spontaneous mammary carcinomas". *PLoS One* 14 (2019): 1-24.

12. Kristiansen VM., *et al.* "Effect of Ovariohysterectomy at the Time of Tumor Removal in Dogs with Mammary Carcinomas: A Randomized Controlled Trial". *Journal of Veterinary Internal Medicine* 30 (2016): 230-241.

13. Casaubon JT., *et al.* "Fine needle aspiration of breast masses". *StatPearls* (2022).

14. Shafiee R., *et al.* "Diagnosis, classification and grading of canine mammary tumours as a model to study human breast cancer: an Clinico-Cytohistopathological study with environmental factors influencing public health and medicine". *Cancer Cell International* 13 (2013): 79.

15. Dolka I., *et al.* "Diagnostic efficacy of smear cytology and Robinson's cytological grading of canine mammary tumors with respect to histopathology, cytomorphometry, metastases and overall survival". *PLOS ONE* 13 (2018): e0191595.

16. Robinson IA., *et al.* "Prognostic value of cytological grading of fine-needle aspirates from breast carcinomas". *The Lancet* 343 (1994): 947-949.

17. Nerenberg PS., *et al.* "Collagen - A necessary accomplice in the metastatic process". *Cancer Genomics and Proteomics* 4 (2007).

18. Winkler J., *et al.* "Concepts of extracellular matrix remodelling in tumour progression and metastasis". *Nature Communications* 11 (2020): 1-19.

19. Muller PAJ., *et al.* "p53 and its mutants in tumor cell migration and invasion". *The Journal of Cell Biology* 192 (2011): 209.

20. Barger A. "Cytology of neoplasia: An essential component of diagnosis". *Today's Veterinary Practice* (2012).