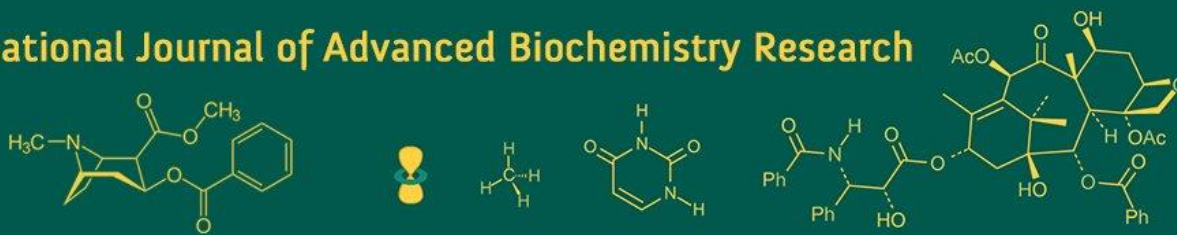


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; 8(1): 193-196
www.biochemjournal.com
 Received: 12-11-2023
 Accepted: 16-12-2023

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Cytopathological and histopathological study on canine fibrosarcoma

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i1c.345>

Abstract

Fibrosarcoma is a relatively uncommon yet alarming canine cancer that predominantly affects the dog's connective tissues. Its aggressive nature and the difficulties in treating it, fibrosarcoma diagnosis can be upsetting for dog owners. In contemporary Veterinary practice, Cytology especially Fine-needle aspiration cytology (FNAC) and impression smears are frequently employed methods for prompt diagnosis of neoplasms. This research aimed to determine the incidence, gross morphological, cytological and histopathological characteristics of Fibrosarcoma. So, in current investigation, tumours were diagnosed using both cytological and histopathological approaches. FNAC and impression smears were utilized for diagnosing palpable and non-palpable tumour masses from diverse dog breeds. Within this study, 58 cases of tumours were scrutinized and three instances of fibrosarcoma were analyzed. Grossly, varying sizes of the tumor masses, displayed an oval to irregular shape and a firm consistency. The cut surfaces exhibited a whitish-red appearance. The cytological smears unveiled the presence of pleomorphic fibrocytes, exhibiting spindle-shaped to plump forms, prominent nuclei with multiple nucleoli, coarse chromatin, and mitotic activity. The histological examination revealed characteristic features such as keratinization, a typical "whorling" pattern, round to oval nuclei with one or more prominent nucleoli, the presence of giant cells, anisokaryosis, and mitotic figures. These findings underscore the potential for cytology to become a more reliable and valuable diagnostic tool for assessing cutaneous and subcutaneous masses in small animal practice.

Keywords: Neoplasm, incidence, cytopathology, histopathology, fibrosarcoma, FNAC

Introduction

Cancer stands as a prominent cause of mortality among animals. In this context, an examination of the most frequent cutaneous neoplasms reveals the following statistics: hemangiosarcomas (19.1%), histiocytomas (8.6%), melanocytomas (8%), mast cell tumours (6.8%), lipomas (6.8%), hemangiopericytomas (6.2%), papillomas (5.6%), fibrosarcomas (5.6%), hemangiomas (4.9%), and squamous cell carcinomas (4.3%). Tumours of vascular origin and transmissible venereal tumours exhibit a higher prevalence among dogs. (Chikweto *et al.*, 2011) [4].

Presently, the global trend towards seeking faster and more cost-effective methods for diagnosing tumour. This is because the traditional histological method, which involves invasive sampling and lengthy processing of tissue samples, has its limitations. As an alternative, cytology has emerged as a valuable approach due to its speed, affordability, reduced discomfort for patients, and ease of repeatability. Cytology involves the microscopic examination of cells that either naturally exfoliated or has been artificially removed from the body or tissue mass. This methodology demands minimal specialized equipment and offers a relatively non-invasive procedure, eliminating the necessity for open biopsies. Additionally, the occurrence of cancer cell implantation following cytology is exceedingly rare. (Engzell *et al.*, 1971; Al-Abbadi, 2011) [8, 1].

Fibrosarcoma is a malignant soft tissue sarcoma that originates from mesenchymal cells and is occasionally observed in dogs. Fibrosarcoma is a relatively uncommon yet alarming canine cancer that predominantly affects the dog's connective tissues. These tumours typically emerge from fibrous structures like muscles and tendons and they can manifest in various regions of a dog's body, including the limbs, chest, abdomen, or head. The diagnosis of fibrosarcoma can be distressing for dog owners due to its propensity for aggressiveness

and the associated treatment challenges. (Withrow and Vail 2007) [18]. This article describes incidence, gross, cytological and histopathological features of canine liposarcomas.

Materials and Methods

Source and Collection of Samples: The present research work was conducted at Department of Veterinary Pathology, Post Graduate Institute of Veterinary Education and Research, Jaipur from August 2020 to February 2021. Study material consisted of samples prepared from 58 spontaneous occurring tumour masses taken from various breed and aged dogs (both male and female). The tissue samples for proposed investigation were collected from Government Veterinary Polyclinic (Department of Animal Husbandry), Department of Veterinary Surgery and Radiology, Department of Veterinary Pathology, PGIVER, Jaipur and Veterinary Hospital (Help in Suffering Jaipur).

Gross Examination: Pre-operatively, gross examination of all the tumours were carried out. Tumour masses were examined for ulceration for its location on body part. After surgical excision, size (cm), shape (round, oval, irregular, multilobulated etc.), weight (g), consistency (soft, hard or firm, cystic etc.) and colour of cut surface of tumour were examined.

Cytological Examination: For cytological examination following techniques were used (Cowell and Valenciano, 2014) [5].

1. Fine needle aspiration cytology (FNAC): Pre-operatively, specimens for cytological study were obtained from each tumour by fine needle aspiration cytology (FNAC). Syringes of 5ml and 22 G needles were used to perform the FNAC.

2. Impression Smear / Touch Imprint Cytology

- Impression smears from tissues were collected at surgery or necropsy, the tissue to be imprinted was cut in half to have a fresh surface.
- Then fresh surface was blotted (e.g., paper towel, surgical gaze) to remove as much of the blood and

tissue fluid as possible.

- The fresh surface was touched to a clean glass slide, simply pressing down and lifting directly up the glass slide on tissue. This process repeated several times to collect multiple imprints of tissue.
- Then material was allowed to air-dry

Fixation and staining of cytological smears

Smears were immediately fixed using 95% ethanol (Allen *et al.*, 1986) [2]. Generally, smears were fixed for more than 10 minutes. Papanicolaou methods utilized wet-fixed smears, while dry-fixed smears were employed for Giemsa staining.

Histopathological Examination: Then dissected tissue samples preserved in the 10 percent buffered formalin for histopathology. The tissue samples were processed mechanically for paraffin embedding by Acetone and Benzene technique (Lillie, 1965; Culling, 1974) [11, 6]. Then, 4-6micron thick sections of tissue will be cut and stained by haematoxylin and eosin method (Luna, 1960; Culling, 1974; Suvarna *et al.*, 2008) [12, 6, 16] for histopathological evaluation. The tissue sections also stain with Masson's Trichrome stains for diagnosis of tumours (Luna, 1960) [12].

Results and Discussion

Three instances of fibrosarcoma were identified, revealing an incidence rate of 5.17%, a result that closely corresponds to the observations of Khan (2019) [9], who reported a 4.38% incidence. Regarding the gross characteristics, the tumour masses displayed an oval to irregular shape and a firm consistency. The cut surfaces exhibited a whitish-red appearance (Fig. 1). The size of the tumour masses varied from 4 x 3 x 3 to 13 x 10 x 10, with weights ranging from 40 g to 340 g (Table 1). These gross morphological findings partially aligned with the observations of Soujanya and Madhuri (2019) [14], who reported well-circumscribed, round, firm, whitish-grey-colored cut surfaces and nodular growth in the elbow region. Similar findings were noted by Leblanc (2017) [10], describing poorly circumscribed and nodular to irregular fibrosarcomas ranging from 1 to 15 cm in diameter.

Table 1: Gross morphology of Fibrosarcoma in dog

S. No.	Size (cm)	Breed	Sex	Age (year)	Weight (g)	Shape	Consistency	Colour of cut surface	Site of location
1.	4 x 3 x 3	Rottweiler	Male	4	40	Oval to irregular	Firm	Whitish red	Fore-leg
2.	6 x 5 x 4	Doberman	Female	5	70	Oval to irregular	Firm	Whitish red	Vagina
3.	13 x 10 x 10	Labrador retriever	Male	8	340	Oval to irregular	Firm	Whitish red	Mandibular region

Cytologically, the impression smear revealed pleomorphic, spindle-shaped to plump fibrocytes occurring individually and occasionally in sheet-like patterns. The cytoplasm, which extended from both ends, appeared eosinophilic, and a few binucleated cells were observed. Higher magnification disclosed plump nuclei with multiple nucleoli, along with anisocytosis, anisokaryosis, an increased nuclear-to-cytoplasm ratio, coarse chromatin, and mitotic figures (Fig. 2 and Fig. 3). Elongated spindle-shaped immature fibroblastic cells formed a distinctive "whorling" pattern (Fig. 4). These observations were consistent with the results reported by Cowel and Valenciano (2014) [5] and Subapriya *et al.* (2018) [15], while aligning more or less with the findings of Raskin and Meyer (2016) [13], who described fibrosarcoma as consisting of large, plump cells occurring

individually and in sheet-like patterns, often associated with pink, collagenous material and occasionally multinucleated giant cells.

Histopathological examination revealed neoplastic fibrocytes with indistinct eosinophilic cytoplasm, arranged in sheets. Elongated spindle-shaped immature fibroblastic cells formed interlacing fascicles or interwoven bundles in a typical "whorling" pattern. The neoplastic cells exhibited a fusiform shape with poorly defined cytoplasmic borders. Hyperchromatic elongated, round to oval nuclei with one or more prominent nucleoli were observed. An increased nuclear-to-cytoplasmic ratio was noted (Fig. 5). Giant cells and mitotic figures were present. Masson's trichrome staining revealed red cytoplasm and collagen tissue between the anaplastic fibrocytes and fibroblasts, forming the tumour

parenchyma (Fig. 6). These histopathological findings concurred with the results reported by Bhadwal *et al.* (2005)^[3], Subapriya *et al.* (2018)^[15], Soujanya and Madhuri (2019)^[14], and Tanrisever *et al.* (2020)^[17].



Fig 1: Gross photograph showing whitish red cut surface of fibrosarcoma

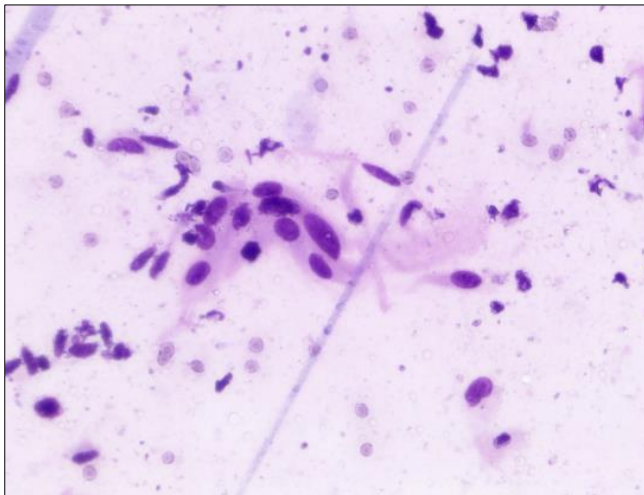


Fig 2: Photomicrograph of impression smear showing anisocytosis, anisokaryosis, plump prominent nuclei with multiple nucleoli, and mitotic figure. Giemsa stain, 400X

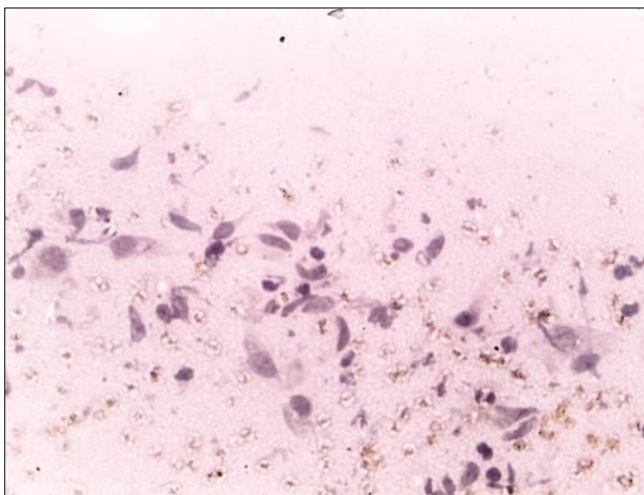


Fig 3: Photomicrograph of impression smear showing anisocytosis, anisokaryosis, plump prominent nuclei with multiple nucleoli, and mitotic figure. Papanicolaou stain, 400X

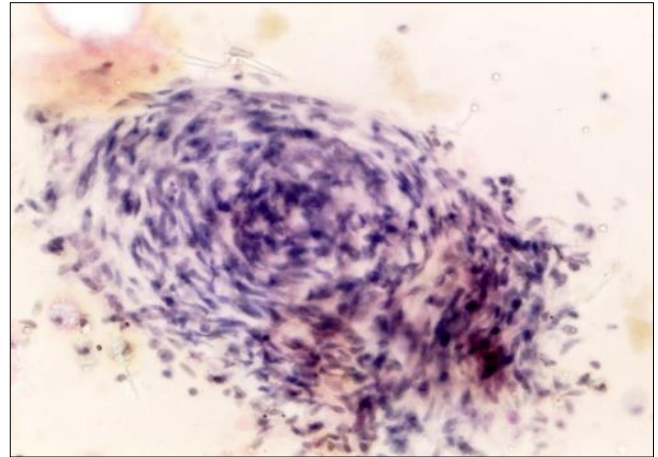


Fig 4: Photomicrograph of impression smear showing elongated spindle shape immature fibroblastic cells forming "whorling" pattern. Papanicolaou stain, 400X

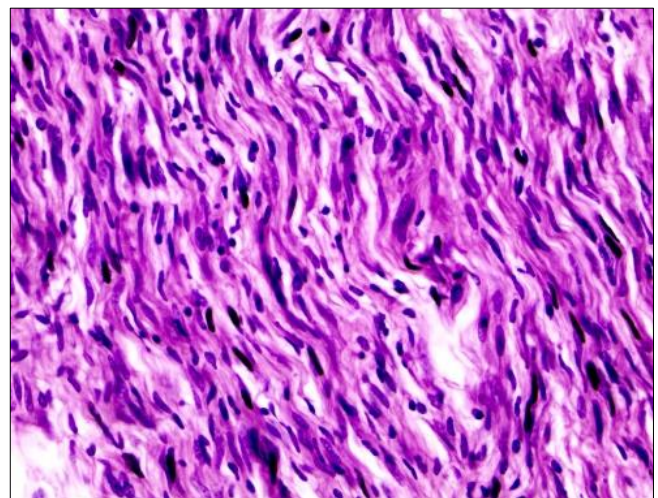


Fig 5: Photomicrograph showing fusiform shape neoplastic cells, hyperchromatic elongated, round to oval nuclei with prominent nucleoli. H & E stain, 400X

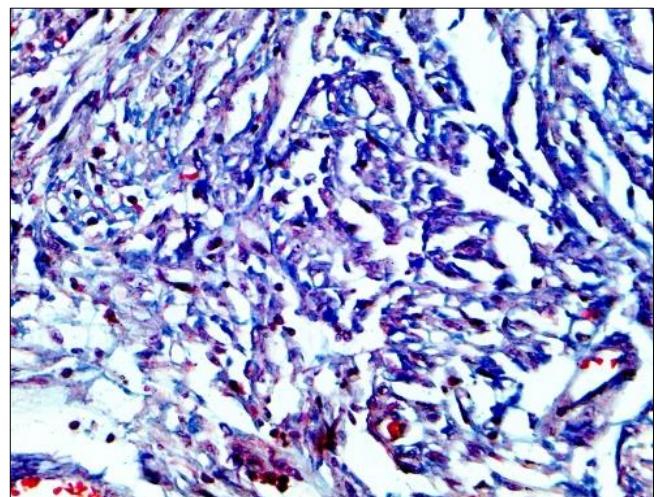


Fig 6: Photomicrograph showing giant cells and mitotic figures, collagen tissue between anaplastic fibrocytes and fibroblasts forming the tumour parenchyma. Masson's trichome stain, 400X

Fig. 1 Gross photograph showing whitish red cut surface of fibrosarcoma. Fig. 2 Photomicrograph of impression smear showing anisocytosis, anisokaryosis, plump prominent nuclei with multiple nucleoli, and mitotic figure. Giemsa

stain, 400X. Fig. 3 Photomicrograph of impression smear showing anisocytosis, anisokaryosis, plumpy prominent nuclei with multiple nucleoli, and mitotic figure. Papanicolaou stain, 400X. Fig. 4 Photomicrograph of impression smear showing elongated spindle shape immature fibroblastic cells forming "whorling" pattern. Papanicolaou stain, 400X. Fig. 5 Photomicrograph showing fusiform shape neoplastic cells, hyperchromatic elongated, round to oval nuclei with prominent nucleoli. H & E stain, 400X. Fig. 6 Photomicrograph showing giant cells and mitotic figures, collagen tissue between anaplastic fibrocytes and fibroblasts forming the tumour paranchyme. Masson's trichome stain, 400X

Conclusion

In our current investigation, among the 58 cases under scrutiny, we identified 13 instances of Mesenchymal cell tumours, which accounts for approximately 22.41% of the cases. Among these Mesenchymal cell tumours, 3 cases (representing about 5.17% of the total cases) were diagnosed as fibrosarcoma. The cytological assessment revealed the presence of pleomorphic cells, showing spindle to plump shapes, and characterized by prominent nuclei with multiple nucleoli. Additionally, anisokaryosis, anisocytosis, coarse chromatin, and mitotic figures were observed. In histopathological examination emphasized a distinct "whorling" pattern in the sections, which served as a prominent feature of fibrosarcoma. Cytology plays a pivotal role as a primary diagnostic tool in establishing the presence of disease processes and neoplastic growth in various bodily tissues. This technique is both straightforward and swift, rendering it a cost-effective and dependable method that can be performed on outpatient basis without the need for anesthesia or sophisticated equipments.

Acknowledgment

The authors are thankful to the Vice Chancellor, RAJUVAS, the Dean and Head, Department of Veterinary Pathology, PGIVER, Jaipur (Rajasthan University of Veterinary and Animal Science), Bikaner, Rajasthan for providing facilities to carry out the research work.

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