PATHOMORPHOLOGICAL INVESTIGATION OF FIBROBLASTIC OSTEOSARCOMA IN A DOG

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This report describes a case of spontaneous fibroblastic osteosarcoma in 6 years old male German shepherd dog, brought in Teaching and Veterinary Clinical complex with history of trauma and signs of lameness in right forelimb. Radiography revealed an osteolysis with sunburst appearance of distal radius and formation of triangle by periosteum in remaining portion of the radius. Impression cytology from the mass yielded a moderately cellular sample composed of loosely cohesive groups or single spindle cells with basophilic cytoplasmic tails, oval nuclei with central prominent multiple nucleoli, marked anysokariosis and mitotic figures. Histological examination revealed pleomorphic tumor cells embedded in intercellular strands of osteoid matrix with mitotic figures. Population of fibroblast-like cells associated with diffuse activation of osteoclastic giant cells was observed. Clinical and radiological findings including the growth rate of the lesion, and the histopathological appearance supported the diagnosis of fibroblastic osteosarcoma.

Key words: Fibroblastic radiography, Impression cytology, Sun burst appearance.

Osteosarcoma is a mesenchymal malignant tumor arising from osteoblasts. Osteosarcoma is relatively rare among domestic animals, but corresponds to 85% of all malignant bone tumors in dogs with aggressive behaviour (Farcas *et al.*, 2012). The large or giant dog breeds are almost exclusively predisposed to the disease and the mean age of affected animals is 8 years. The most common sites are femur (42%), tibia (19%), and humerus (10%). Other locations include the skull or jaw (8%) and pelvis (8%) (Jerome *et al.*, 2010). It is commonly seen in large and giant breeds with high predisposition towards increasing weight and height (Pool *et al.*, 1990). Though histopathology is routinely used for the diagnosis of tumors in veterinary pathology, little emphasis is placed on cytology, which is a very rapid and field diagnostic technique. In the present scenario, cytopathology plays a major role in diagnosing many tumours. Based upon the above facts, the present case was studied such that initial diagnosis was made by cytology and later it was compared with histopathology for confirmation of fibroblastic osteosarcoma.

The present case report describes a case of osteosarcoma in the a 16 years old male German shepherd dog, brought in Teaching and Veterinary Clinical complex, Veterinary College, LUVAS, Hisar with history of trauma and signs of swelling and lameness in right forelimb carpal joint and below, since 2 months. The animal was unable to bear weight in his right forelimb. Radiographic examination of the right limb was performed. The tumor was 26 x 12 x 10 cm, with an irregular smooth, brown surface and a generally firm rubbery in consistency with gritty to hard areas interspersed. The mass was poorly vascularized with peripheral necrotic areas. Blood was collected from the dog and sent to clinical pathology laboratory for haematological examination. The growth was removed surgically and tissue was processed for cytology and histopathology. Cell cytology was carried out with the help of impression smears stained with Field's stain. Impression smears were taken from cut surface of tissue in a clean glass slide and dried in air. Fixed smears were dipped into Field Stain B for 5-6 secs and it was washed with running tap water. Again that smear was dipped into Field Stain A for 20-30 seconds and washed with running tap water. Smears were air-dried and examined under oil immersion microscope.

Representative tissue was collected by the excision biopsy in 10% buffered formalin and decalcified in 5% nitric acid for histopathological studies. After proper fixation, tissues were cut into small sections

with thickness of 2-3 mm and washed overnight in running tap water. After removal of fixative, the tissues were dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin blocks. The paraffin embedded tissues were cut into 4-5 µ thick section and stained with Haematoxylin and Eosin.

In accordance to our study; Rodrigues *et al*., (2009) reported that male predisposition and neutering or spaying, regardless of gender, may increase the risk of developing appendicular osteosarcoma. Lameness and localized limb swelling is the most common owner complaint. Lameness is caused may be due to periosteal inflammation, microfractures, and occasionally pathologic fracture. Swelling usually results from extracompartmental extension of the bone tumor into adjacent soft tissue. Radiographic examination revealed an osteolysis with sunburst appearance of distal radius and formation of triangle by periosteum in remaining portion of the radius (Fig.-1). Haematological examination revealed a normal blood picture with all values lying with in a normal range. Externally the growth was very large and it has been extended into the entire right forelimb. Entire right forelimb was surgically amputed to give relief from ostesarcoma. The tumor was 26 x 12 x 10 cm, with an irregular smooth, brown surface and a generally firm rubbery consistency with gritty to hard areas interspersed. The mass was poorly vascularized with peripheral necrotic areas. Impression cytology with Field's stain from the mass yielded a moderately cellular sample composed of loosely cohesive groups or single spindle cells with basophilic cytoplasmic tails, oval nuclei with central prominent multiple nucleoli, abundant atypical mitotic figures and marked anysokariosis (Fig.-2). Tumor was diagnosed as osteosarcoma when osteoid was seen on impressions. Histological examination revealed malignant mesenchymal cells producing osteoid and woven bone with mitotic figures. There was extensive osteoblastic differentiation associated

Fig.- 1 Radiographic examination showing osteolysis and sunburst appearance of distal radius

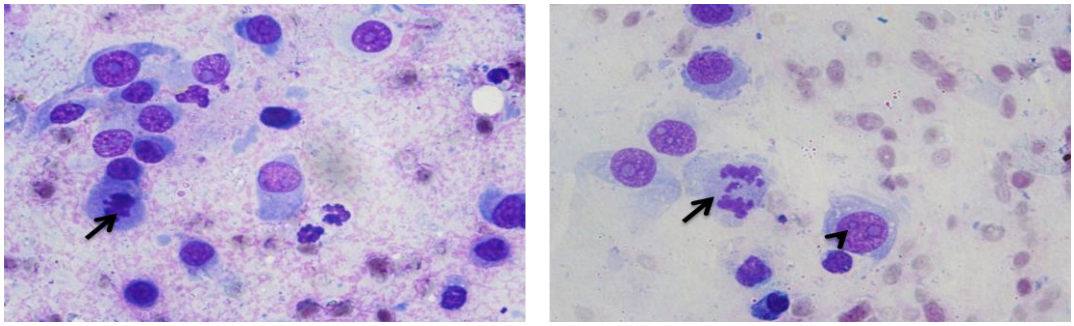

Fig.- 2 Impression cytology smears showing highly anaplastic cells containing multiple nucleoli (arrow head), atypical mitotic figures (arrow) and intercellular strands of osteoid Field stain 1000 X

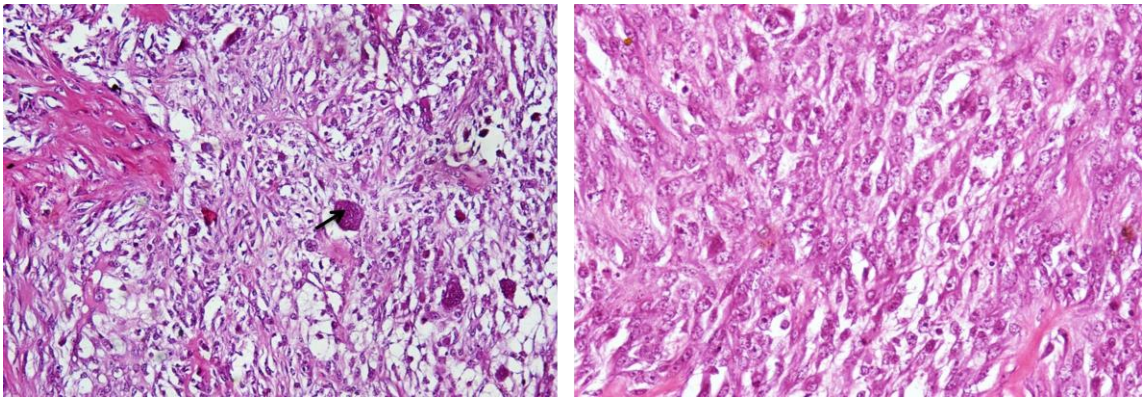

Fig.-3 Fibroblastic osteosarcoma showing irregular small islands of osteoid matrix without mineralization surrounded by malignant osteoblasts forming a fusiform pattern and giant cells (arrow) H & E stain 400 X

with the production of collagen, as well as a population of fibroblastic-like cells associated with diffuse activation of osteoclastic giant cells (Fig.-3). Neoplastic cells were large, pleomorphic and had hyperchromatic nuclei and prominent nucleoli as similarly reported by (Leonardi *et al*., 2014).

Conclusions

Diagnosis of the specific subtype of osteosarcoma i.e. fibroblastic osteosarcoma can only be reached by a multidisciplinary approach including clinical examination, radiology, impression cytology and histopathology.

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