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A CASE OF FIBROSARCOMA IN AN ADULT MALE GERMAN SHEPHERD DOG: DIAGNOSES AND MANAGEMENT

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ABSTRACT

This case documents the diagnosis and surgical management of fibrosarcoma in the medial surface of the right forelimb, distal to the humeral joint of an intact male German shepherd dog. The dog, weighing 32 kg and aged 2½ years, was presented at the Veterinary Teaching Hospital, Michael Okpara University of Agriculture, Umudike with a history of lameness and growth in the right forelimb. Lumpectomy was performed to obtain an excision biopsy. The surgical wound was thoroughly washed with sodium bicarbonate solution 8.4% w/v. Tension sutures were used to close the incision and to eliminate dead space in tissue. Histopathological examination of the biopsy confirmed the lump as fibrosarcoma. More than 24 months post-surgery, the growth did not re- occur. It was concluded that excision, coupled with surgical wound irrigation with sodium bicarbonate solution, may be an effective means of managing soft tissue fibrosarcoma in dogs. The clinical relevance of this case is that it provides support for the use of surgical excision in combination with sodium bicarbonate wound irrigation in the treatment of benign fibrosarcoma.

***Keywords*:** Fibrosarcoma, Male, Dog, Diagnosis, Management.

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INTRODUCTION

Fibrosarcoma is an aggressive mesenchymal, malignant or cancerous overgrowth of abnormal cells mostly arising from collagen-producing fibroblasts, often seen in fibrous connective tissues of the skin and the subcutaneous tissues. Fibrosarcomas are the most common soft tissue tumors in cats and dogs commonly noticed on the trunk and extremities, but are rare in other domestic animals [1]. Growths arising in the dermis may appear nodular, while those in the subcutaneous fat or adjacent tissues may be identified by palpation. Fibrosarcomas are firm and fleshy lesions affecting the dermis and subcutaneous fat often invading the musculature along facial planes [1,2,3].

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Tumors are idiopathic but are generally thought to result from many factors that cause genetic injury to cells. These factors include exposure to carcinogens (electromagnetic radiations and certain chemicals), infections, hormonal changes, and certain vaccinations more often seen in cats. The injured or mutated cells begin to divide and multiply uncontrollably at an accelerated rate forming a growth or lump. Fibrosarcomas are neither transmissible between pets nor zoonotic but may be primary or secondary. In dogs, primary fibrosarcoma is associated with defects or rearrangements in chromosomes 11 and 30 [1,4]. Primary fibrosarcomas are mostly found in older large breeds of dogs (e.g. Labrador, retriever, Rottweiler) arising from both bone (metaphyses of long bones, pelvis, and maxillary bone) and soft tissue in the oral cavity, pericardium, trachea, urinary bladder and skin (keloid fibrosarcoma). Fibrosarcomas are seen most often in middle-aged or older male dogs on the limbs and trunk. There have been incidences of an aggressive form of fibrosarcoma in dogs under 1 year of age, and in these cases, the prognosis is usually unfavourable [5,6,7, 8,9,10]. Secondary fibrosarcomas have been reported sequel to anti-rabies vaccinations (more often seen in cats), microchipping, and foreign bodies e.g. retained surgical swabs [11,12]. Fibrosarcomas often become ulcerated and, if they do, are prone to infection, so it is important to watch the tumor for any inflammation or bleeding, as dogs will often lick, scratch, and bite at the tumor or rub it against objects.

Treatment usually involves resective surgery with or without adjunct intralesional chemotherapy and or radiation [13]. Surgical treatment can range from lumpectomy to amputation of the affected limb, in extreme cases. Radiotherapy alone, or in addition to surgery (which is more often the case), can be of benefit, while chemotherapy is generally less effective [13]. The efficacy of lumpectomy in dogs depends on whether the abnormal cells are malignant and invasive and whether spread to other tissues has occurred or not. For non-invasive benign growths, resection usually resolves the condition, although lumps can recur occasionally. For invasive, malignant, tumors, outcome is more varied and surgical excision may require a combination with chemotherapy or radiation therapy to control abnormal cell growth. In these cases, prognosis is more guarded than favourable [13].

CASE HISTORY

An intact adult male German Shepherd Dog aged 2½ years and weighed 32 kg was presented at the Veterinary Teaching Hospital, Michael Okpara University of Agriculture, Umudike with a superficial, circular growth in the medial surface of the right forelimb, just distal to the humeral (humerotibioulnar) joint presenting the pet with lameness. The client had noticed the lump when it was small-sized but ignored it thinking it would resolve with time only to observe some months later that the swelling had enlarged. The animal had good appetite and was active.

On general examination of the patient, the vital parameters were as follows: rectal temperature was 40ºC (relatively higher than normal range of 37º-39ºC), heart rate was 80 b/min (70-120b/min), pulse rate 88b/min (70-120b/min), respiratory rate 12c/min (18-34c/min), mucous membrane colour pink (normal), and capillary refill time <2 seconds (normal). The tumor on the medio-lateral aspect of the right forelimb of the dog was irregularly shaped, firm and tender to touch, pink in colour, pendulous, and had ulcerated surfaces (Fig. 1). The patient was alert with good hair-coat and bright eyes. There were no ectoparasites on the animal. Exudate from the growth was collected with sterile swab sticks and sent for microbiological examination. The entire lump was surgically excised and submitted for histopathology.

SURGICAL INTERVENTION

The patient was starved of water and food for 12 and 24 hours respectively prior to surgery. The surgical site was clipped, scrubbed with soapy water, mopped dry, and finally cleaned with 50% ethanol solution. The patient was pre-medicated with intramuscular injection of atropine sulphate (Pauco Atropine®, Jiangsu Huayang Pharmaceutical, Jiangsu China) at 0.02 mg/kg and xylazine hydrochloride (XYL –M2®, VMD, Belgium) at 2.0 mg/kg. Induction of general anaesthesia was achieved using intrasmucular ketamine Hydrochloride (Ketanir®, Aculife Healthcare, India) injection at 10 mg/kg. Thereafter, the

patient was placed on lateral recumbence with the affected limb up and draped properly. Lignocaine hydrochloride (2% solution) with adrenaline (Lignolab®, Laborate Pharmaceutical, India) was injected around the base of the lesion for analgesic and haemostatic purposes.

The outgrowth was carefully undermined with a circumferential incision at the tumor base. Bleeding was controlled by clamping, ligation, digital pressure and the use of plain adrenaline hydrochloride solution. The lump was completely excised together with some healthy surrounding tissues in order to forestall spread of abnormal cells (Fig. 2). The excised lump and other tissues were preserved in formalin and forwarded for histological analysis. The surgical wound was mopped of blood with sterile dry gauze, irrigated copiously with adrenaline, mopped again with gauze, and then generously washed with sodium bicarbonate solution (8.4% w/v, POM, Martindale). The muscles were apposed with horizontal mattress (retention) sutures using catgut, leaving no dead space in tissue, while the skin was approximated with nylon also in a horizontal suture pattern (Fig. 3).

Post-operatively, the patient was treated with a single dose (at 10 mg/kg) of long-acting oxytetracycline (Oxytetra 200 LA®, Pantex, Holland); dichlofenac sodium (Dicloecnu Injection®, Ecnu Pharmaceutical, Shandong China) (at 1 mg/kg) for two days and 1 ml of B-complex vitamins (Yikang Pharmaceutical, China) for three days. All the treatments were given intramuscularly. Oxytetracycline spray was applied topically and the surgical wound was then protected with a bandage. Elizabethan collar was placed on the patient to prevent auto-mutilation of the wound. The bandage was removed 2 days post-operation. Three days post-operation, the animal was placed on intramuscular penicillin injection (Antipen®, Biochemie, Austria) at 20,000 iu/kg for five days. Following wound healing, the skin sutures were removed on day 14 post surgery (Fig. 4).

MICROBIOLOGICAL ANALYSIS

Samples of the exudates from the tumor ulcer were collected using a swab stick and subjected to microbiological evaluation. The swab sample was inoculated into nutrient (enrichment) agar using the streak method, and incubated at 37ºC for 24 hours. Thereafter, a loopful of the colony was picked and inoculated into Mannitol salt and MacConkey media using the modified streaking method [14]. Moderate growth of discrete colonies of each sample was subjected to modified gram reaction [15]. Subsequent microscopy at x1000 magnification revealed bunches (from MacConkey colonies) and clusters (from Mannitol colonies) of Gram positive cocci suggestive of *Staphylococcus aureus* as the stains were picked (Figs 5 and 6).

Catalase and coagulase biochemical tests were also carried out using the slide method [16,17]. In the catalase test, a drop of normal saline was placed on a clean glass slide and mixed thoroughly with a loopful of the colony using an inoculating loop. A drop of hydrogen peroxide was carefully added on the smear. Presence of bubbles was observed, thus, confirming the organism to be *Staphylococcus aureus* [16]. In the coagulase test method, a drop of distill water was placed on a slide. Loops of the colonies were emulsified on blood agar plate into a smooth suspension. To the suspension was added a drop of citrated plasma and mixed with a needle. There was clumping of coccal organisms, which further showed that the organism was *Staphylococcus aureus* [17].

Antimicrobial susceptibility test using the unicellular disc (Oxoid, Bakingsloke, UK) showed that the *Staphyloccocus* organism was highly sensitive to penicillin and gentamicin as indicated by inhibition zones of 26 cm and 24 cm respectively, but moderately susceptible to ampicillin, and resistant to tarivid, ciprotab, ciproflox, septrin, and perfloxacin.

HISTOPATHOLOGY

The excised lump was fixed in 10% neutral buffered formal saline. The histological technique was done as described by Gridley [18]. The fixed tissues were dehydrated in graded alcohol (70%, 80%, 90%,

absolute 1, and absolute 2), cleared in xylene, infiltrated and embedded in paraffin wax. The embedded tissues were sectioned at 5µm thickness using sliding microtome (KD 202, Kedi, China). The sections were floated on a warm water bath (45ºC) to remove the wrinkles on the cut surfaces and were collected with slides. The slides were stained using haematoxylin and eosin (H&E) technique.

Figure 1: The outgrowth (arrow).

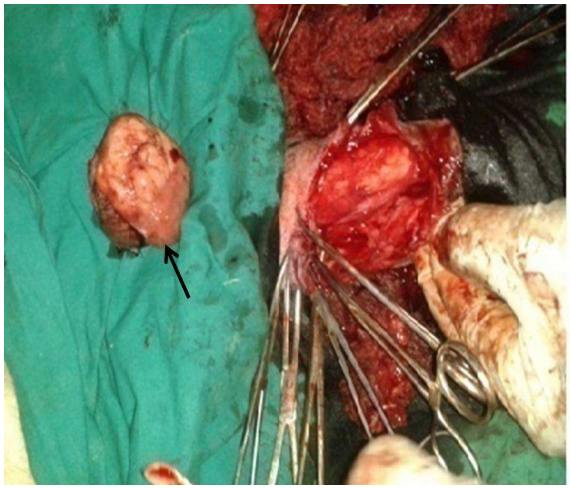

Figure 2: The excised tumor (arrow).

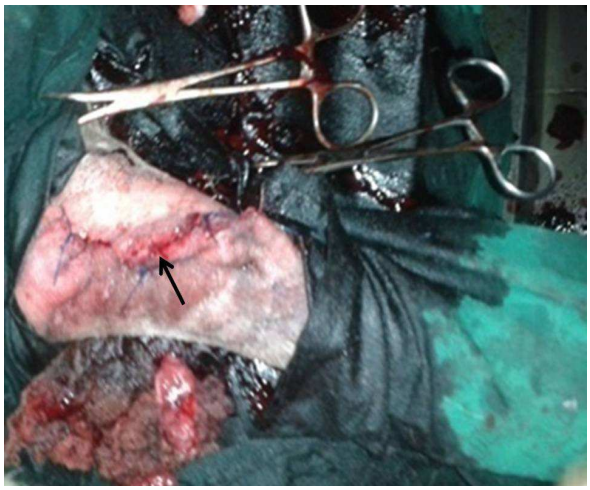

Figure 3: The sutured wound (arrow).

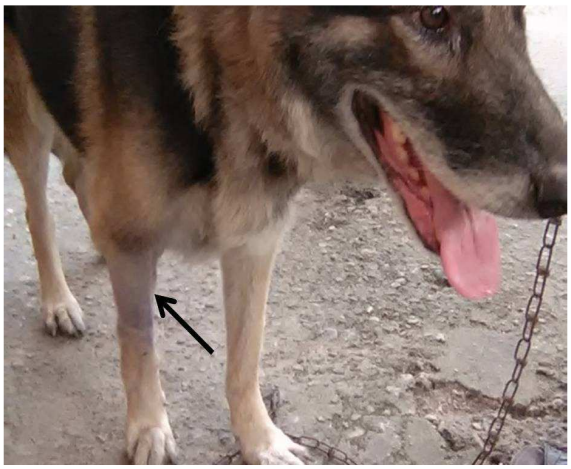

Figure 4: The wound healed (arrow).

The examination revealed spindle-shaped and plumb malignant cells (Fig. 7) and whorls of fibroblasts which are immature and arranged haphazardly (Fig. 8); these features are consistent with the diagnosis of fibrosarcoma. At the surface of the lesion, there were increased vascularization, haemorrhages, and marked cellular infiltration especially with neutrophils (Figs. 9 and 10). The increased vascularization observed is a common feature of neoplasm due to angiogenesis. The haemorrhages are likely the result of the new blood vessels formed which are porous because they lack endothelial cell junctions. Presence of increased neutrophils at the surface of the lesion is indicative of a response to secondary bacterial infection sequel to the rupture of the tumor. Furthermore, the haemorrhages at the surface of the lesion may have contributed significantly to necrosis leading to increased inflammatory cell infiltration into the ruptured and necrosed area of the tumor.

Based on the findings (Figs 7 - 10), the tumor was a well-differentiated fibrosarcoma with orderly herringbone pattern. The growth was low grade (grade 1) according to the tumor grading system of the National Cancer Institute [19]. The prognosis of low grade tumors is usually favourable since they have limited or zero metastatic ability [20].

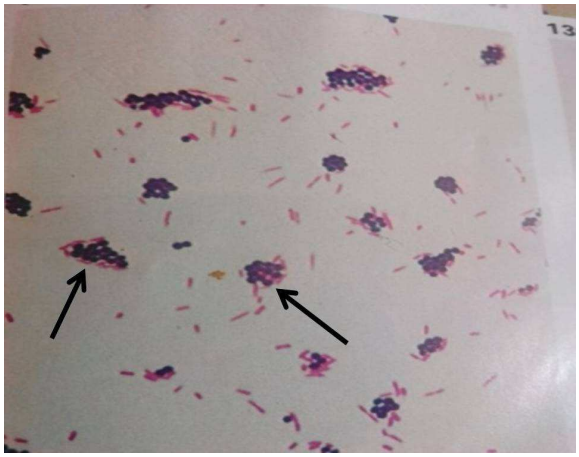

Figure 5. Clusters of *S aureus* from Mannitol x1000 (arrows).

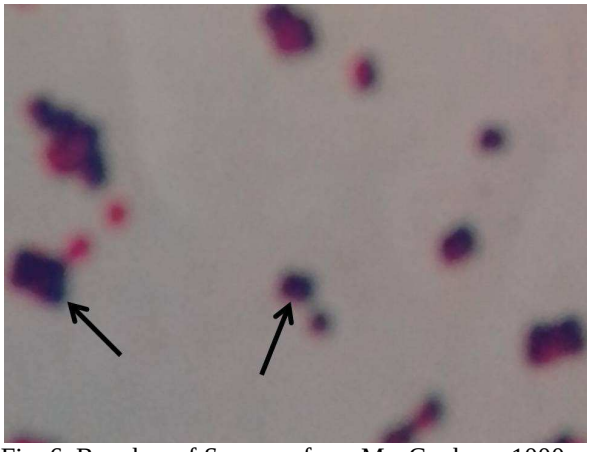

Fig. 6. Bunches of *S aureus* from MacConkey x1000 (arrows)

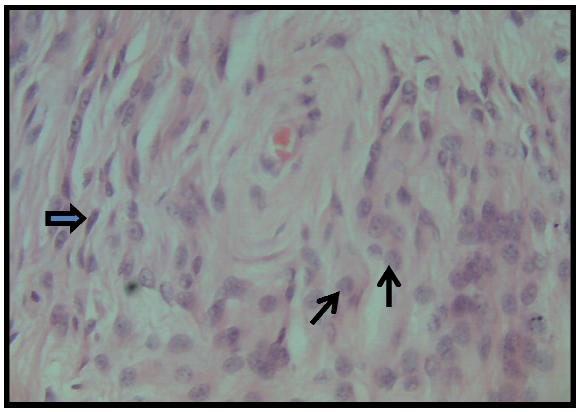

Figure 7. Plump and spindle shaped skin cells stained in H & E (Arrows). Magnification ×40

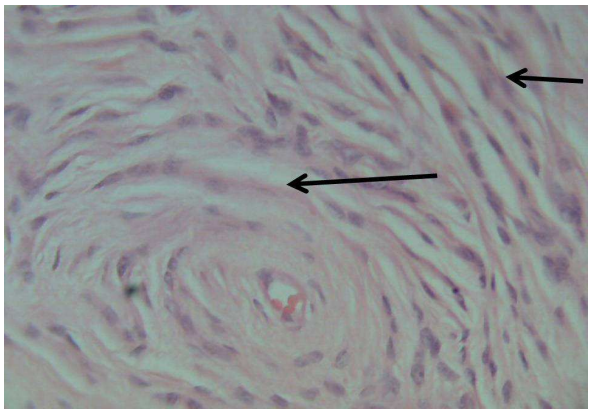

Figure 8. Whorls of fibroblasts of skin tissues stained in H & E (Arrows). Magnification ×40.

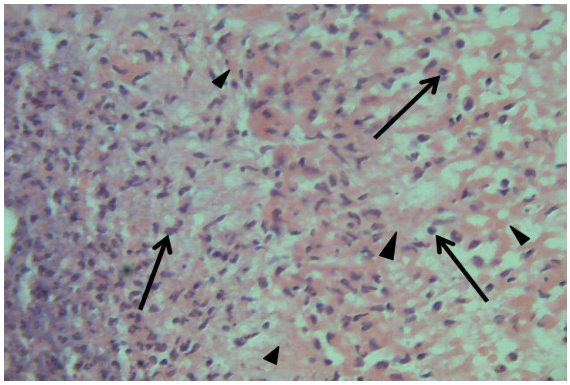

Figure 9. Haemorrhages (arrow-heads) and cellular infiltration (arrows) at the tumor surface ×40mag (Arrows). Stain used: H & E.

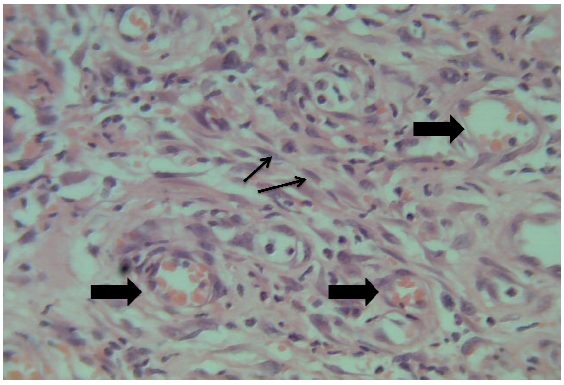

Figure 10. Surface of tumor cells with fibroblasts (thin arrows) and more vascularization (fat arrows) stained in H & E.

DISCUSSION

Fibrosarcomas are slow-growing, malignant (cancerous) tumors most often found in the connective tissue of the skin and beneath the skin, in which fibroblasts are the most predominant cell type. The overgrowth of cells results in tumors [1]. These tumors can be successfully removed by surgery but they may frequently recur post-surgery. Fibrosarcomas rarely metastasized to other parts of the body. Radiographic evaluation may be necessary, especially in boney fibrosarcomas, but a definitive diagnosis is generally achieved by immunohistochemistry (Tumors often label positively for certain cellular proteins including vimentin, calponin and alpha-smooth muscle actin) and/or histopathology (microscopic examination of the tumor cells) [21].

There are different methods of sample collection that can be used to acquire these cells, namely: (a) Fine-needle aspiration (FNA): Using a syringe and needle to withdraw deep cells from the tumor. Generally no sedation is required for this procedure. Fine needle aspiration is not typically used to diagnose fibrosarcomas because it is difficult to aspirate the cells needed for identification. (b) Punch biopsy: Using a scalpel or an actual punch (a circular-shaped knife) to obtain a small biopsy that will include skin and underlying tissue for examination. This procedure sometimes requires light-to-moderate sedation. (c) Excisional biopsy: An excisional biopsy involves complete surgical removal of the tumor, as well as a wide area of skin surrounding the tumor. Anesthesia is required for this procedure. In the present case, the tumor mass was harvested by excisional biopsy under general anaesthesia while diagnosis of the lesion was confirmed histopathologically.

Fibrosarcomas are generally graded as high or low, indicating a visibly high or low number of dividing cancerous cells. Grading of malignancy can be determined by the use of minichromosome maintenance proteins, produced in about 70% of fibrosarcomas in the dog [11,12, 21, 22]. The pathologist's report should also provide the prognosis of the disease and opinion on whether or not the margins of the tumor at removal were adequate, and whether or not the lump was completely excised.

Tumor management is usually attempted by the combined use of surgery, radiation, and chemotherapy (often called the cut-burn-poison approach). In the present case, surgery alone with alkaline wash was the treatment option employed. The entire lump (about 3.2 cm in diameter) was excised (excisional biopsy) enveloped completely in normal tissues to prevent metastasis of abnormal cells. Since tumor cells create and proliferate in acidic environment but do not survive in high alkaline extracellular pH [23, 24], the wound was flooded and washed repeatedly with sodium bicarbonate solution to kill off any remnant of abnormal cells even though the lump was entirely extirpated. Haemorrhage was controlled by the use of haemostats, ligatures, digital pressures, and adrenaline. The surgical wound was closed routinely beginning with muscular and subcutaneous closure with catgut and then the approximation of the skin using nylon materials.

The infiltration of neutrophils identified histopathologically was a cell-mediated response to the *Staphylococcus aureus* infection, confirmed microbiologically. Previous studies [25,26] equally isolated *Staphylococcus aureus* from clinical cases of abscesses, external wounds and ulcers. The antimicrobial susceptibility test showed that the *Staphyloccocus* organism was highly sensitive to penicillin and gentamicin. Following the sensitivity test result, the patient was treated with penicillin injection for five days. The skin sutures were removed in two weeks following complete healing of the wound. The lesion did not recur after more than twenty-four months post-surgery.

The owner was advised to disallow habitual exposure of the pet to harsh sun (ultraviolet) rays, by providing shade or using sunscreens formulated for dogs when necessary. The damage to skin from sun exposure can thus be minimized. In addition, the client was told to report any form of anomaly to a veterinarian as soon as possible.

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