Orginal article

Feline Injection Site Sarcoma (FISS): Clinicopathologic Findings in Cats

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Abstract

Feline injection site sarcoma (FISS) is a subcutaneous tumor were recognized among four cats aged between 5-7 years old. This study included four cases, which referred to the Veterinary Teaching Hospital, University of Tripoli from October 2011 to October 2013. All cats had a history of sudden growth of a subcutaneous mass located on the dorsal midline of the neck, just cranial to the shoulders.The diagnosis was confirmed based on characteristic histopathological finding. Grossly the tumor was infiltrative, irregular, lobulated, extensively ulcerated, necrotic with purulent and/or haemorrhagic exudation with different size among the cases. In all cases the microscopic examination revealed infiltratively growing pleomorphic tumor cells and mononuclear cell aggregations with prominent fibroblastic proliferation. The present investigation shows the first report on FISS in different local and imported cats in Libya

Keywords: Cats, Feline, Injection site fibrosarcoma, Histopathological findings

Introduction

Feline injection site sarcoma (FISS) is a common subcutaneous tumor that has been recognized for many years. In spite of a strong epidemiologic evidence that links the administration of inactivated feline vaccines and the subsequent development of sarcomas at the site of injection, sarcomas developing at sites of long-acting antibiotics, microchips, retained surgical sponge, corticosteroids and lufenuron administration, have also been reported occasionally (Gagnon 2000; Cohen *et al*. 200; Daly *et al*. 2008; Haddad *et al*. 2010).

Currently, it is believed that vaccines are not the only agents capable of causing sarcomas at the injection site; rather, virtually anything that produces local inflammation has the potential to cause the development of injection site sarcomas in susceptible cats (MacEwen *et al*. 2001).

Tumor types that have been reported at the sites of vaccination are fibrosarcoma, malignant fibrous histiocytoma, osteosarcoma, rhabdomyosarcoma, undifferentiated sarcoma, liposarcoma, and chondrosarcoma (Hendrick and Brooks 1994; De Man and Ducatelle 2007). The true incidence of sarcoma development after vaccination is unknown. Several studies reported that an incidence of sarcomas per vaccines administered (Macy and Hendrick 1996). Whereas others reported a prevalence of sarcoma per cat (Kass *et al*. 1993). The incidence was estimated to be between 1:1,000 and 1:10,000 of vaccinated pet cats (MacEwen *et al*. 2001; McEntee and Page 2001; Morrison *et al*. 2001; Ford 2004; Macy 2004).

However, the incidence is considered relatively low and vaccines frequently given to insufficient number of most cats population that's made a difficulty of any sort of correlation, with lack of attention of the vaccines as potential causative agents (MacEwen *et al*. 2001).

The precise pathogenesis of vaccine-associated sarcomas is unknown. The inflammation alone or in association with unidentified carcinogens or oncogenes leads to neoplastic transformation and tumor development (Morrison *et al*. 2001).

Feline injection site sarcomas are usually iatrogenic, difficult to treat, highly aggressive and invasive locally, making them more challenging to treat than non injection site sarcomas. However, they have a great tendency to be recurred after surgical excision than non injection site sarcomas (Hendrick *et al.* 1992).

To the best of our knowledge, there is no data available regarding to the incidence of FISS in cats in Libya. Thus, the goal of this study was to describe the microscopic and clinical feature of FISS in 4 cats and to determine criteria for etiology and prognosis.

Materials and Methods

Animals (patient selection and clinical examination).

Four cats aged between 5 – 7 years admitted to the Department of Surgery at the Faculty of Veterinary Medicine, University of Tripoli, Libya, between October 2011 and October 2013. The Cats were of both sex (3 females and one male), in the main time the case history, etiology, clinical signs, diagnosis, treatment, and prognosis of the disease were recorded. One case was referred and diagnosed by private veterinarian, while the other three cases were diagnosed at the department of surgery. For all cases final decision was

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to treat by radical excision with excluding of the chemotherapy.

Radiological examinations

Plain ventrodorsal and lateral thoracic radiographs were obtained for all presented cats .

Surgical Techniques

The surgical excision of the masses was applied in addition to 2 cm of living tissue margins were removed intact. Then the whole excised masses were immediately fixed in 10% neutral buffered formalin, routinely processed, and paraffin embedded for pathological evaluation.

Histopathological evaluation

Biopsy specimens from cats were submitted to histopathological investigation which examined grossly. The characteristic lesion, specimens' size and dimensions were recorded. At the time of surgical excision of the lesions, specimens were placed on rigid paper to prevent wrinkling and the diameter of the tumor was recorded immediately.

Specimens were fixed in 10% neutral buffered formalin for 48 hours, dehydrated in increasing concentrations of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of 5-6 microns were stained with Hematoxyline and Eosin (H&E). Van Gieson stain was used to differentiate collagen and smooth muscle fibers in the tumors under study (Bancroft and Cook 1984).

Results

Signalment and case details

Feline injection site sarcoma observed in 3 females and one male. The occurrence of FIIS was predominant in aged admitted cases (5-8) years. The incidence of FISS was more frequent at the cervical portion was than at the shoulder region.

History and clinical presentation

All the four cats admitted to the clinic were obviously not physically affected. However in one of the cases the owner discover two subcutaneous nodules on the lateral right shoulder just above elbow joint after three weeks of previous surgical excision of a small subcutaneous masses at same region (Figure 1A), while, the other three cats showed a large hard (lobulated) swelling under the skin above the cranial thoracic vertebrate just cranial to the interscapular apace (Figure 1B).

Radiographic Diagnostic finding

Several plain radiographic X-Rays showed that no metastasis observed in the thoracic cavity, as well as no bone involvement was detected in all cases (Figure1C).

Treatment and outcome

Surgical treatment was successfully performed throughout the investigation and all admitted cats were recovered without any postoperative complications except in one case only, which was readmitted one month post surgical excision of the tumor with extensive recurrence. So we advised the owner to euthanize the affected cat. However, the surgical interventions were quickly and easily conducted. Whereas the long-term, follow-up (about 6 months) of the other cases revealed complete recovery and absence of complications at the surgical site.

Pathological Findings:

The largest mass was 8 × 5 × 4 cm size after surgical excision, it involved the skin and subcutaneous tissue just cranial to interscapular space, it was irregular, lobulated, extensively ulcerated, necrotic, purulent and/or haemorrhagic. (Figure 1D).

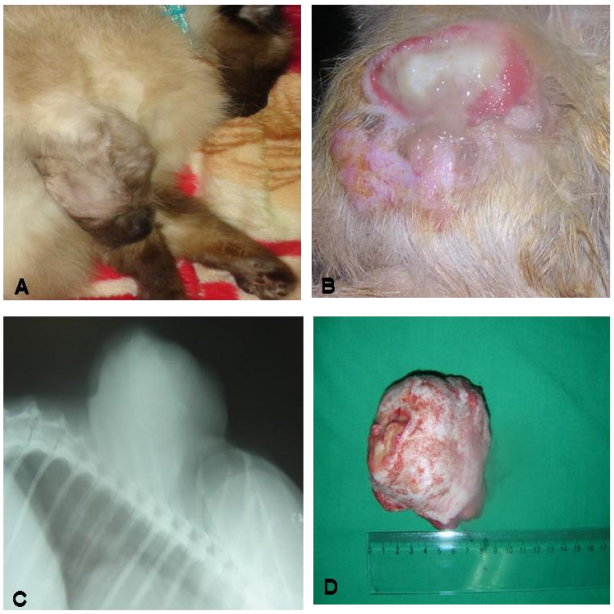

Figure1. (A)- Subcutaneous nodules on the lateral right shoulder just above elbow joint. **(B)**- Large freely movable subcutaneous fibrosarcoma located just cranial to the interscapular space of a cat with ulceration and hemorrhages at margins and purulent exudates in center. **(C)**- Plain radiographic X-Ray for the previous case. **(D)**- 8×5×4 cm subcutaneous mass involved the skin and subcutis (after surgical removed).

The microscopic examination of biopsies or the mass in all cases revealed that the neoplastic cells displayed different growth arrangements with cellular and nuclear pleomorphism which increase toward tumor edge with hyperchromasia (Figure 2a), the lesions also revealed the existence of moderately differentiated intense fibroblastic proliferation which had oval to fusiform nuclei with indistinct cytoplasmic borders and moderate amounts of fibrillar, lightly eosinophilic cytoplasm and numerous mitotic figures (Figure 2b). Foci of necrosis were seen, hemorrhage and lymphocytic aggregation were also found, mostly perivascular. However, the neoplastic cells were often surrounded by an eosinophilic fibrillar matrix that stained red with Van Gieson stain and was interpreted to be collagen fibers (Figure 2c&2d).

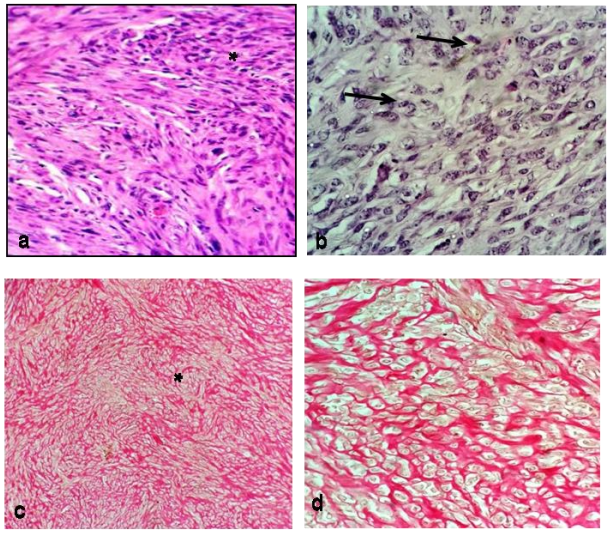

Figure 2. (a) Neoplastic fibroblasts showing pleomorphism and hyperchromacia and are arranged in bundles of different directions (asterisk). H&E stain; × 200. **(b)** Histopathology of the same tumor note bundles of spindle- shape cells (fibroblast) with ovoid nuclei and mitotic figures (arrows), H&E stain; × 400.**(c)** Neoplastic fibroblasts separated by diffuse red-colored extracellular collagen fiber (asterisk).Van Gieson stain; × 100 **(d)** Spindle shape cells surrounded by collagen Fiber. Van Gieson stain; × 200.

Discussion

Feline injection-site sarcomas (FISS) or vaccination-associated sarcomas are a disease entity in cats that has alarmed the veterinary world because of its possible connection to vaccines and vaccination procedures. FISS are subcutaneous tumors that develop aggressively at the site of injection and were first described in cats by Hendrick *et al.* (1992). In this study, four cats with FISS were documented over a period of 2 years based on histopathological findings.

Injection site sarcomas are a challenge to the veterinary profession on a number of levels: they are iatrogenic, difficult to treat, and potentially preventable (Lappin *et al*. 2002). In all cases of FISS aggressive therapy is indicated. At this moment, wide surgical excision of the tumor with or without adjunctive therapy is the most effective treatment (Davidson *et al.* 1997). Surgical excision as the sole modality of treatment has been shown to be inadequate in a number of studies, although the importance of surgery in a multimodality approach is unquestionable. A retrospective analysis of 61 cats treated with surgical excision reported a median overall time to recurrence of 94 days, with only 11% of cats remaining tumor-free for at least 1 year (Hershey *et al*. 2000). Cats with appendicular tumors (most commonly treated with amputation) had a longer time to recurrence than cats with tumors at other sites (Martano *et al*. 2011), similarly in our investigation a good prognosis was recorded after the limb amputation in a case with aggressive recurrence.

There have been a few smaller studies evaluating more radical excision of injection site sarcomas with promising results. Lidbetter *et al* (2002) described 6 cats that underwent a lateral body wall resection for this tumor. Three cats had preoperative radiation therapy and at a mean follow-up of 17.2 months, no cats had evidence of recurrence. Kuntz (2000) reported on a modified wide local excision technique, in which 5-cm margins in all directions and two muscle planes deep were taken at surgery. Histopathologically, 23 of 24 cats had complete excisions, and follow-up of these patients is continuing to determine the effectiveness of this approach. At the present study 3 to 5cm margins around injection site sarcomas were excised, removing as much of the surrounding tissue as possible while still leaving the cats able to function relatively normally.

Similar to previous reports (Hendrick*et al*. 1992; Hendrick and Brooks 1994; Kass *et al*. 2003; and Kirpensteijn 2006), FISS in the current study involved older cats. Similarly, most cases of FISS were reported in the dorsal thoracic region in between the shoulder blades, a site corresponding with the most common location for either vaccination or other injection.

The diagnosis of FISS is usually confirmed by fine needle aspiration cytology, ultrasonographic examination and histological examination of biopsy specimen. Radiographs of the lungs should be performed to check for distant metastases (Briscoe *et al*. 1998). In the present study all thoracic radiographs showed no evidence of distant metastases in the thoracic cavity of all cases. The additional use of computed tomography and magnetic resonance imaging will significantly improve margin determination prior to surgical and radiation therapy (Morrison and Starr 2001); unfortunately, the financial costs associated with such techniques are high which preclude them from being used in the present study.

Recent studies suggest that, FISS and chronic inflammatory lesions have been described at sites of previous vaccination in cats (Epslin and Campbell 1995; Hendrick and Brooks 1994 and Douglas *et al*. 2007) a similar reaction pathren was observed in the present study. Moreover, Douglas *et al*. (2007) suggests that many virus as herpesvirus / human herpesvirus-8 (KSHV/HHV8) may reprogram the target cell, thus masking the cell's true origin and possible that the original target cell is an uncommitted progenitor. In addition Kass (1993) suspected that the chronic inflammation caused by vaccines, particularly feline leukemia virus and rabies vaccines cause a derangement in tissue repair mechanisms resulting in neoplasia, The sites of vaccination which commonly used by veterinarians in the present study and histopathologic findings in the biopsy specimen are consistent with those previously described in cases of vaccination-site sarcoma (VS) in cats (Epslin and Campbell 1995). In 1992 the first sarcomas were reported and found in sites of vaccination and include dorsal neck/inter-scapular area, dorsolateral thorax, hind limb, and dorsal lumbar region Hendrick *et al.* (1992).

The use of Van Gieson stain in this study showed aggregations of the ECM which appeared as pink to dark red staining of the extracellular structures making the differentiation between collagen and smooth

muscle in tumors clear (Bancroft and Cook 1984). Additionally, lymphocytic inflammatory infiltration in tumor specimens was mainly located at the tumor periphery such result was reported by Vascellari *et al.* (2003) which detected a similar distribution pattern from presumed injection sites of fibrosarcomas of both dogs and cats. Moreover, the prominent peri-tumoral lymphoid aggregates contained numerous T lymphocytes (Couto *et al*. 2002) a similar reaction was observed in the present study.

The presence of necrosis, mitotic activity and pleomorphism in tumor specimens, are characteristic features of aggressive tumors (Doddy *et al*. 1996). In these cases, the hemorrhage was evident in tumor specimens; this finding has been previously described in cat fibrosarcoma (Mahir *et al*. 2012). Moreover previous studies showed that the peripheral vascularity was significantly higher than the central vascular density but no difference was found in tumor cell proliferation rates between the two areas. Furthermore, centrally located, fluid-filled micro- or macrocavitations were frequently observed in the large vaccine sarcomas and could probably be formed due to rapid tumor growth with consequent central necrosis which is similarly reported by Couto *et al.* (2002).

Our data suggest that local inflammation could be caused by aluminium or other potentially irritant inoculated substances, may predispose tissues to tumor development. Doddy *et al*.(1996) mention that the inflammatory response is one of the distinctive features of the feline post-vaccinal fibrosarcomas. Furthermore, feline fibrosarcomas found in vaccine sites are histologically identical to those observed in previously traumatized areas (Smith, 1995).

In conclusion, further in *vivo* and in *vitro* studies are needed to elucidate the complex process of vaccines products and sites of vaccination as well as the role of chronic inflammation as predispose factor lead to derangement of their fibrous connective tissue repair response that eventually results in neoplasia formation in cats. Additionally we needed to determine the incidence of metastasis which lack in this study, and more characterize the spectrum of biologic behavior of vaccination-site sarcomas .

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