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RESEARCH ARTICLE

CHARACTERISATION AND SPECIFIC MARKERS EXPRESSION OF SOFT TISSUE SARCOMA: CASE REPORTS FOR THE DOG AT THE HO CHI MINH CITY VETERINARY CLINIC

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ABSTRACT

Treatment failure in removing canine soft tissue tumors, a locally invasive mesenchymal neoplasm, may occur in between 7 and 75 percent of patients, with recurrence consistently associated with lower overall survival for the dog. This study was to classify the tumor examine the occurrence of cancers in dogs reported at the Ho Chi Minh Veterinary Clinic by integrating blood physiological and histopathological of patient samples. In this study, the percentage of tissue sarcoma in the dog was 1.5%. Histopathological analysis characteristics of the ear lobe tumors displayed a strong proliferation of rhabdoid cells, presence of leukocytes infiltrating around blood vessels, and vaginal tumors showed normal tumor cell density with a strong collagen fibers formation in fibroblast cells and no strong proliferation and ferocity of cells. In particular, immuno-histopathological analysis of biopsies sample from breast tumor showed an overexpressing of vimentin marker (68.1%) in cells which indicated status of malignant metastasis. The metastatic tumor cells in breast tumor might cause the change in blood biochemical parameters together with sepsis, thus the dog was not eligible for surgery. In general, histological examination and expression levels of markers can help determine the malignant status of tumors in dogs and should be included medical examination to limit local recurrence following surgical resection.

KEYWORDS

Female dog, mammary tumor, oncology, veterinary

1. INTRODUCTION

The most prevalent cause of mortality in adult dogs is cancer. Soft-tissue sarcomas (STSs, soft-tissue sarcomas) are a diverse population of mesenchymal tumors with varying tissue development that account for 15% of all cutaneous and subcutaneous tumors in dogs (Liptak and Forrest, 2013). These tumors are more common in middle-aged and older canines, as well as large breed dogs. Up to 60% of STS in dogs is located in the limbs, with other anatomical regions showing less expression (35 percent trunk and 5% head or neck) (Liptak and Forrest, 2013; Bray et al., 2017). Soft tissue tumors (STS) can grow from nearly any anatomical location. They are the most frequent kind of cutaneous or subcutaneous tumor in dogs, accounting for 9 to 15% of all cutaneous or subcutaneous tumors (Dennis et al., 2011; Liptak and Forrest, 2013). Local recurrence following surgical resection is a common cause of failure in the therapy and management of STS, occurring in 7 to 75 percent of dogs, and continued recurrence is related with a reduction in the dog's overall survival. Treatment results for dogs with STS have improved over the last 30 years, according to recent publications, probably as a result of early screening (Bray et al., 2014). More crucially, unexpected resections are still quite prevalent, with no prior diagnostic testing to rule out probable malignancy (Bray et al., 2014).

Since the late 1980s, a three-level histological categorization for STS in dogs has been devised and recently evaluated (Dennis et al., 2011). The most prevalent type of STS is grade I (low grade), which is a slow-growing lesion with the lowest recurrence rate following surgical excision. Greater tumor grade was related with more physiologically invasive activity, resulting in a higher risk of local recurrence. According to a study, roughly 7% of low-grade cancers return following marginal excision, compared to 34% and 75% for intermediate and high-grade tumors, respectively (McSporran, 2009). Currently, accurate diagnostic techniques to establish the optimal surgical site size for a specific tumor are required, which might lead to a mismatch between therapy and illness. Analytical methods employed in this work include histopathological morphological features analyzed using a histochemical approach and the expression of several molecular markers analyzed using an immunohistochemical method frequently used in tumor diagnosis. Furthermore, the findings will be examined in order to offer appropriate prognostic and therapy orientations to help the improvement of STS management in dogs.

2. MATERIALS AND METHODS

2.1 Ethics Approval

All animal studies were carried out in accordance with the Department of Animal Health recommendations (reference number TCVN 8400:2019).

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2.2 Animals and Tumor Sample Collection

Tumor-related clinical animals were examined and treated at TrustVet Veterinary Clinic, Go Vap, Ho Chi Minh City, from March to June 2021. Tumor samples from diseases are categorized in the following ways: (1) Dogs with visible tumor signs (> 3 cm); (2) diseased dogs that are lethargic, sluggish, or refuse to eat; (3) the tumor has spread to another part of the body; and (4) the histological diagnostic is considered to malignant or benign. Curative-intent surgical excision was planned as wide as feasible in respect to tumor location and features and defined using the Enneking method as marginal (surgical margin close to pseudocapsule), wide local (2–3 cm of lateral margins of healthy tissue and 1–2 deep fascial planes), or radical (excision of the whole compartment) (Kuntz et al., 1997; Enneking, 1980). After surgical excision, samples were placed in 10% formaldehyde before being sent to the ANAPAS Center for Histopathology and Immunohistochemistry in HCMC.

2.3 Subclinical Analysis

Before surgical removal, the animals were tested for hematology to rule out cases of hemophilia, with or without indications of infection. Tumors were postoperatively excised and fixed in a 10% neutral formalin solution. The tumor samples were then microscopically examined using a paraffin molding technique, sliced the slide, and stained with Hematoxylin - Eosin (HE). Cut with a Thermo Scientific HM 340 E automated slide cutter and examined with a Leica DM2500 optical microscope.

Immunohistochemical staining on tumor tissues was assessed by labeling with immunological markers. Vimnetin, Desmin, S-100, Smooth Muscle Actin (SMA), Actin Muscle, Cytokeratin (CK), and CD68 were derived from Dako EnVision Plus and Dako Autostainer (Dako Corporation, Carpinteria, CA) and used the respective tumor classifications as mesenchymal tumor, rhabdomycinoma, neuroendocrine tumor, vascular tumor, muscle tumor, epithelial tumor, and tumor of tumor derived macrophage. The technique of implementation was conducted in accordance with the ANAPAS Center for Histopathology and Immunohistochemistry, Ho Chi Minh.

3. Results and Discussion

3.1 Tumor Incidence in Dogs

In this study, there were 19 cases of dogs with tumors out of a total of 1260 dogs who came to the clinic for assessment and treatment. The recorded percentage of dogs with tumors was 1.5 percent. This result is consistent with previous study, which had showed a 1.44% of the total in Vietnam (Dang, 2008). However, this percentage is still low when compared to prior publications throughout the world since cancers in dogs might emerge with tumors inside the organ and an assessment study utilizing specific procedures such as CT, MRI. More specifically, more than 1 million new instances of cancer are believed to occur in dogs each year in the United States, and cancer was the most prevalent cause of death in retrospective studies reporting mortality in dogs. with an estimated frequency of 30% (Fleming et al., 2011; Alvarez, 2014).

Many canine malignancies have a cluster of spontaneous tumors, many of which are histologically comparable to human tumors. Non-Hodgkin lymphoma, malignant melanoma, osteosarcoma (OSA, osteosarcoma), bladder carcinoma, and other brain tumors are among the most common histologic lesions (Leblanc et al., 2016). The increasing number of cancer-affected dogs is regarded as a significant source of data for preclinical investigation of disease resistance, pharmacology, pharmacodynamic effects, and prospective efficacy of novel anticancer medicines. In this study, three dog cases with tumors on their bodies in different areas were chosen for further investigation, including (case 1), which had a tumor in the ear lobe that was around 8x5x3cm in size and had dense hair. There are scant, thin, tiny, short hairs on the tumor; (case 2) the tumor arises in the vagina and measures more than 1.5cm in diameter; (case 3) The tumor was first detected in the breast, but it later emerged in the armpit of the right front leg. Size at the breast is around 23x2.5x1cm, and the size at the armpit is approximately 4x3.5x1.5cm (Figure 1).

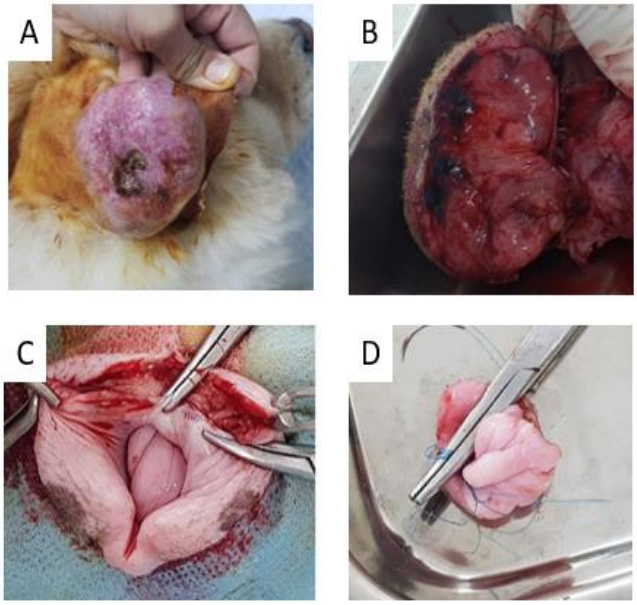

Figure 1: Various forms of dog tumors were analysed in this study. Fibrosarcoma tumor - malignant fibroma macroscopic images (A and B). Tumor in the vagina of a French Bulldog (C and D).

3.2 Case 1: An Earlobe Tumor

Bo Chi, a castrated male dog of the Chow Chow breed, is nearly two years old. Weighing 25kg, he had a history of treating dermatitis at the clinic for two months and was entirely healed. Furthermore, the owner has thoroughly vaccinated the animals since childhood. At the time of the inspection, the dog had a tumor that had been there for more than a year. While the tumor was on the ear lobe, the ill animal ate well and functioned regularly. Sick animals are housed on the grounds, with limited interaction with dogs outside. The findings of the hematological analysis revealed a modest drop in lymphocytes and a rise in MID, suggesting that the patient had minor inflammation. Antibiotics were given during and after surgery to keep patients from becoming infected. A slight increase in RBC levels suggests that the animal is anemic, and the body displays indications of moderate dehydration.

The tumor's microscopic analysis showed an extensive proliferation of rhabdoid cells that varied in size and shape. The cells are organized in parallel bands that cross each other in certain places. The tissue structure was necrotic, the cells proliferated and grouped, and there were infiltrative leukocytes surrounding the blood vessel, including necrotic disintegrating nuclei (Figure C). In this instance, surgery is still a viable therapy and management method for tumors. Standard guidelines for tumor excision with a margin of at least 3 cm (Bray, 2014). The blood indications are stable after examining the hematological components, allowing surgery to remove the tumor, including the ear lobe harboring the tumor, to proceed. The case of the ChowChow dog showed strong signals of healing following surgical removal and no recurrence after 3 months.

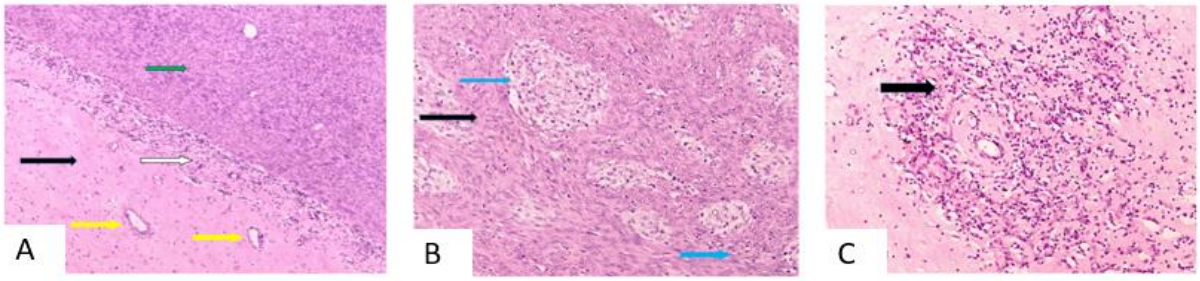

Figure 2: The tumor on the canine auricle is depicted as a slice picture (100X HE). (A) Necrotic dissolving nuclei divide the necrotic tissue from the cellular portions (white arrows). The necrotic tissue loses its cell structure and transforms into a pink-colored homogeneous mass. A large number of blood arteries enter the tumor to feed it. (B) Fibroblasts (black arrows) surround tumors, which include rhombus cells of varying sizes (blue arrows). There are several cell foci of various sizes. (C) Leukocytes infiltrate surrounding blood arteries, including necrotic disintegrating nuclei.

3.3 Case 2: Identifying and Distinguishing Vaginal Tumors

Poli, a diseased French BullDog breed, is more than a year old, weighs 8.6kg, and is reproducing for the second time. Historically, the dog has no history of infectious infections or surgery. There were no peripheral lymph nodes seen on the body. Vaginal discharge is seen in animals during the mating season. It is unclear when the tumor first developed. The results of blood tests reveal that the indicators are at an average level, slightly dehydrated, allowing surgery to remove the tumor to proceed.

The vaginal tumor had a typical density of tumor cells, including mature fibroblasts that generated many collagen fibers, according to microscopic inspection (Figure 3, black arrow). There was no evidence of a robust and aggressive multiplication of cells. The cell nucleus is very consistent in size, and the elongated nucleus has an oval, oval shape. Histological examination has verified the clinical diagnosis of vaginal fibroids. Fibroids may be found in all domestic animals, however horses are more likely to have them than dogs or cats (Rahal and Sampaio 1997). Vulvar and vaginal tumors constitute for 2.4 percent to 3 percent of all tumors in dogs (Vali and Withrow, 2007). They are the second most prevalent type of tumor after breast tumors. The vast majority of these tumors are not cancerous.

Subclinical analysis of a vaginal tumor after removal revealed that it is a benign tumor with very few mitotic areas. Vaginal fibroids are benign tumors with the presence of fibrous connective tissue on imaging based on histological parameters of the tumor such as grade, histological type, and mitotic count. In particular, for benign soft tissue tumors, excision remains the most successful technique in the therapy of benign fibrous tumors in general and soft tissue tumors

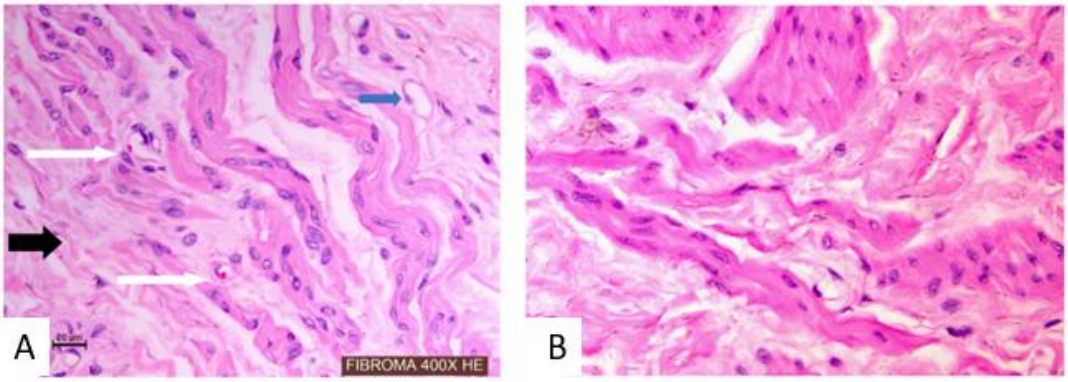

Figure 3: Histological picture of a vaginal tumor in a French Bulldog (400X HE). (A) Mature fibroblasts, numerous blood vessels arrive to nourish the tumor, and white blood cells surround the vessel wall. The boundaries between cells are difficult to determine. (B) The collagen fibers are pale pink, whereas the cytoplasm is dark pink.

3.4 Case 3: Histiocytic Positive Expression of Vimentin in Breast Tumors

The unhealthy Lak (20kg) is an 8-year-old female Husky it has been neutered. Body temperature was increase to 39.4°C and express weak responsibility. Clinical examination resulted in blood parasite infection via a rapid test for the *E. canis*. Previously, dog had been treated for tumors at another veterinary laboratory. The tumor, on the other hand, grew more and larger with each passing day. When the tumor first forms in the breast and spreads to the armpit, this dog feeds regularly and walks properly. However, the owner reported a powerful and rapidly developing breast tumor in the breast with considerable swelling, expanding in the right breast row. Dog was sluggish to move, generally tough, and rarely play.

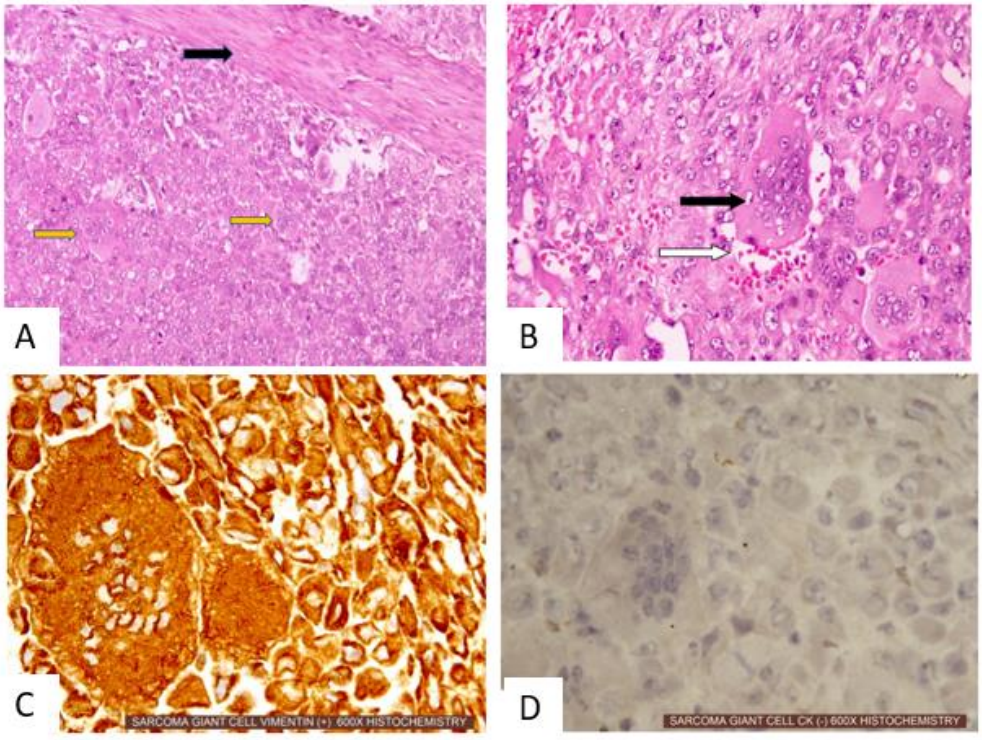

Figure 4: A histological examination of a breast tumor in a Husky dog. (A) A breast tumor section surrounded by fibroblasts (black arrows). Yellow arrow indicated for multinucleated giant cells (200X HE). (B) Tumor giant cells (black arrows) and red blood cells pick up red near blood arteries (white arrows) (400X HE). (C) Vimentin positive expression indicated by cytoplasmic dark brown staining (600X). (D) CK-negative expression in tumor indiacted by nuclei stained with Hematoxylin (600X).

A histological microscopic examination of the tumor showed a significant density of cells which were rapidly proliferating. In addition, the tumor was surrounded by a layer of fibroblasts and large size in round, big vacuoles (Figure 4). In particular, in immunohistochemical analysis

indicated that cells strongly expressed for vimentin but not for other exanimated markers (Figures 4C and D). The overexpression of vimentin (68.1 % by Image J software analysis, Figure 4C) in section of breast tumor is consistent with previous study that showed vimentin expression in breast cancer cells (MCF7) and HeLa cells, as well as in other epithelial cells (Messica et al., 2017; Saunus et al., 2018; Liu et al., 2015).

The expression of cytokeratins and vimentin in breast cancer is linked to malignant tumor (Heatley et al., 1993). Although markers were employed and referred to prior investigations, this work has not yet completed on additional markers that can be used for confirmation (de Faria Lainetti et al., 2020). For example, vasculogenic mimicry (VM) proteins include VE-cadherin, tumor groeth factor 1 (TGF-1), and EpCam. Thus, it might benefit from evaluating these indicators in future study breast tumor in dog by using cross-reactivity of commercially available anti-human monoclonal antibodies with canine proteins.

4. Conclusion

In this study, dogs with tumors accounted for 1.5 percent of the 1260 animals assessed at TrustVet veterinary clinic (Ho Chi Minh City, Vietnam). Despite the fact that cancer therapy for dogs in Vietnam is still poor. These findings will help to pave the road for future studies into cancer in dogs. In particular, precisely categorizing cancer cells will aid in the selection of the most appropriate intervention approach. Histopathological and immunohistochemical approaches in tumor diagnostics play an essential role in (1) categorizing and assessing tumors, distinguishing malignant tumors and benign tumors based on the degree of polymorphism of the tumor cells, mitotic activity, and degree of tumor necrosis; and (2) marker identification (eg, vimentin) will effectively categorize the origin of poorly differentiated tumors and the degree of malignancy.

Routine staining is difficult to detect in malignant tumors that are mostly poorly differentiated. According to the above research, the combination of two methods of histology and immunohistochemistry in tumor identification and classification aids in accurate tumor therapy and prognosis and has the following suitable treatment guidelines. Tumor recurrence is reduced with surgery.

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