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The role of biomarkers as a diagnostic tool in some neoplasms of pet animals

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ABSTRACT

Cancerous tumors are one of the main problems that cause deaths in pet animals. The objective of this paper was to describe the histopathological features of some neoplastic masses with confirmation using immunohistochemistry in pet animals. This study was done from May 2022 to August 2023 in Sharkia governorate, Egypt to correctly recognize 7 cases of pet animal neoplasms "5 cases of dogs and 2 queen case" based on the histopathological and immunohistochemical findings. The results revealed 2 cases of TVT in Penis with positive expressions for Iba2 and vimentin. 1 Case of sebaceous adenoma in Hock joint of male dog skin, 1 case of hepatoid carcinoma in perianal glands of male dog with positive immunolabelling for CK7. 1 case of chondroblastic osteosarcoma in 4 years old, male, Great Dane dog and 2 cases of queen with mammary gland adenocarcinoma with positive immunoreactivity for HER-2 and other mixed mammary neoplasm accompanied with sarcoma has positive staining for vimentin. Finally, we concluded that the histopathological evaluations by H&E stain is the backbone method for neoplasms diagnosis and using some biomarkers added a confirmatory tool for this diagnosis.

Introduction

Neoplasms consider life threaten of pet animals specially when had ability to metastases to critical organs (Yeruham *et al*., 1999). Many surgically excised animal neoplasms were not subjected to histopathological examination. However, with the increasing sophistication of veterinary practices and the laboratory services provided for them, this position is rapidly being rectified. as well as forming an important part of veterinary practice. The animal neoplasms provided an excellent model for the study of cancer in man. They approach the human situation very closely in that they arise spontaneously in an out bred population which in cases of the dog and cat, shares a similar environment to man (Gupta and Tiwari, 2009). Tumors affecting skin form the majority of neoplasms in veterinary field (Goldschmidt and Hendrick, 2002).

The most common types of malignant neoplasms in dogs were lymphoma, mast cell tumour, osteosarcoma, soft tissue sarcoma and mammary carcinoma (Bronden *et al*., 2010). Transmissible venereal tumor (TVT) in dogs represented < 0.6 % of skin neoplasms out of over 600 cutaneous neoplasms in some area of India (Chikweto *et al*., 2011). The sebaceous gland adenomas in some dog's neoplasms were 5% (Jakab, 2003). Perianal adenomas constitute > 80% of all perianal tumors and are the third most common neoplasm in male dogs due to their testosterone dependence (Withrow, 2001). Hepatoid gland adenocarcinomas (HGA) are uncommon and accounts for 3-7% of perianal neoplasm in dogs (Pisani *et al*., 2006). Osteosarcoma (OSA) is the most common primary tumor of the appendicular skeleton in dogs and cats representing about 85% of bone malignancies in dogs (Brodey and Riser, 1969). The incidence of OSA in dogs is twice more common in the forelimbs than in the hindlimbs with the highest incidence being in the distal radius and proximal humerus followed by distal and proximal femur and distal tibia (Morello *et al*., 2011). OSA is a locally aggressive neoplasm characterized by high metastatic rates; with approximately 90% of affected dogs dying early from hematogenous spread of the primary tumor mainly to the lungs (Ehrhart *et al*., 2012).

Mammary tumors represent 12% among cat's neoplasms. Moreover, mammary cancers in cats were ranked in the third most relevant neoplasms after cutaneous and lymphoid neoplasms (Misdorp and van der Heul, 2002).

Neoplastic biomarkers serve as differential diagnostic tools for certain undifferentiated cases (Teruya-Feldstein, 2010). Examples include cytokeratin 7 (CK7) in adenocarcinoma (Basturk *et al*., 2010). Vimentin (Marcos, 2006) and Iba1 (Pierezan *et al*, 2014) as a confirmatory tool for TVT in dogs. HER2 (De Maria *et al*., 2005) for poor prognosis of mammary carcinoma in cats and vimentin (Doyle and Hornick, 2014) for mesenchymal tissue associated with mixed mammary carcinoma. The goal of this paper was to describe histopathological features of some neoplasms affecting pet animals and using some biomarkers added confirmatory tools for diagnosis.

Materials and methods

Animals

Neoplastic growths of pet animals; 7 cases were recorded; 5 cases of different dogs breed and 2 queen cases, which were admitted to Zagazig Veterinary Teaching Hospital during the period of study from May 2022

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to August 2023. A full description data for each case about age, affected site, pet species, sex, and healthy condition were recorded. Furthermore, gross picture (shape, size, color, consistency, cut sections) of collected specimens were recorded.

Surgical intervention

The animal was prepared for aseptic surgery. The dogs and cats was pre-anaesthetized with (Atropine sulphate 1%): (1mg/kg B.wt) by I/M injection and the dogs were sedated using Xylazine Hcl (Xylaject ® 2%) (0.5 mg/kg B.wt) by I/M injection. General anesthesia for cats was maintained with Ketamine Hcl 5% (Ketalare ® 50mg/ml). 20 mg/kg B.wt mixed with Xylazine Hcl (Xylajct ® 2%)(0.5 mg/Kg B.wt) by I/M injection and for dogs by using Thiopental Na 2.5% at dose of 20-30 mg/kg B.wt by I/V injection. An elliptical incision was made on the tumor and the mass was excised by blunt dissection. All the bleeding vessels were cauterized, and the skin flap was closed after applying subcutaneous sutures. Animal was kept on Inj. Intacef: 25 mg/kg b.wt and Inj. Melonex: 0.2 mg/kg b.wt. for 5 days according to Vani *et al.* (2016).

Histopathological examination

Different excisional biopsies were collected from all neoplastic masses. The tissue specimens were fixed in neutral buffered formalin 10% for 48 hours, dehydrated in grades of ethyl alcohol (70%-100%), cleared in xylene, embedded in paraffin. 5µm thickness of paraffin sections were obtained by using automated microtome then stained with routine Hematoxylin and Eosin (H & E). The bone neoplastic specimens were transferred to a formic acid solution for decalcification after fixation. After 6 days of decalcification the tissue samples were routinely processed (Suvarana *et al*., 2018).

Immunohistochemical examination

The immunohistochemical procedure was done according to Ali *et al*. (2015). The avidin-biotin-peroxidase complex (ABC) method (Hsu *et al*., 1981) was used with primary antibodies as the following: Cytokeratin 7 (CK 7) Monoclonal Antibody (Ks7.18) (Catalog # 61025 PROGEN), ThermoFisher scientific, US, 1:100 dilution for adenocarcinoma. Vimentin Monoclonal Antibody (V9) Catalog # MA5-11883, (ThermoFisher scientific, US. 1:50 dilution for TVT and Mixed mammary tumor. Anti-ErbB2 / HER2 antibody [H2Mab-139] (ab264541), 1/100 dilution (Abcam, Cambridge, UK) for mammary tumors. Anti-Iba1 antibody [EPR16588] (ab178846), 1/2000 dilution (Abcam, Cambridge, UK) for TVT. Sections (5 µ m in thickness) were placed on charged slides. Dewaxing, and rehydration were performed for antigen retrieval. After that the sections were incubated with the primary antibodies overnight at 4°C in a humidified chamber, slides were washed with phosphate-buffered saline (PBS) and incubated with the secondary antibody (Expose mouse and rabbit specific HRP/DAB detection kit, Abcam; Ready-to-use; Cat. #: ab80436) for 15 minutes at room temperature in a humidified chamber then rinsed with PBS. Staining was then performed using the DAB chromogenic agent for detection of immunoreactivity, counterstained with hematoxylin, dehydrated, and mounted in a synthetic medium. The staining index of immunohistochemical expression of all the markers was obtained as previously stated by multiplying the staining distribution and intensity scores (Heller *et al*., 2005).

Results

Seven cases of pet animal neoplasms were recorded during this study from May 2022 to August 2023 in Sharkia governorate, Egypt. Table 1 illustrates the results regarding animal species, age, sex, location of the tumor and their gross appearances.

The diagnosis of neoplasms was based on histopathological and immunohistochemical examination, which declared 2 cases of TVT on penis, 1 case of Sebaceous adenoma in hock joint of male dog skin, 1 case of hepatoid carcinoma in perianal glands of male dog. 1 case of chondroblastic osteosarcoma of male dog and 1 case of feline mammary gland adenocarcinoma with completely replacement of regional lymph nodes by metastatic foci and one case of mixed neoplasm in mammary gland in queen. The detailed pathological findings of these cases are described as follows:

Two male's dogs with 4.5 average years had cauliflower-like nodule at caudal part of glans penis (Fig. 1a). on gross examination, the neoplastic masses were red in color and soft, friable in its consistency. The initial appearance of the neoplasms was hyperemic papule that later progress to lobular multilobulated masses. In histopathology, there was subepithelial heavy infiltration of neoplastic lobules. Lobules consisted of dense impact of round, oval or variable shape neoplastic cells with pale basophilic cytoplasm that contained a single distinctive nucleus and scanty stroma (Fig.1b). The size of most nuclei was higher than the cytoplasm. Small, light, clear intracytoplasmic vacuoles were mostly visible (Fig.1c). Moreover, lymphocytes, plasma cells and macrophages are often observed. the arrangement of cells was in a diffuse pattern and supported by thin trabeculae of fibrovascular stroma. Mitotic figures averaged 2 per high magnification field.

TVT showed positive immunostaining reactions within cytoplasm of neoplastic cells by using Iba2 marker (Fig. 1d) and Vimentin marker (Fig.1e) as golden yellow granules.

Female dog (5 years old) with one nodular mass bulged on the skin of right leg at hock joint (Fig. 2a). the excised neoplasm dimension was 4.8x3.7x2.1 cm and weighed 38 grams. The mass was grayish in color, fleshy in consistency and homogenously grayish white by cut section.

Table 1. Description of pet animal neoplastic cases.

Diagnostic markers	Main microscopic picture	Gross picture	average age	Animal species and gender	Organ	Neoplasm
Iba2, Vimentin	Variable shape neoplastic cells and contain colorless vacuoles in cytoplasm with scanty stroma	Cauliflower-shaped, Soft, friable masses, Creamy with hemorrhagic cyst	4.5 year	2 Male dogs	Penis	TVT
--	Proliferation of mature sebocytes or fully-lipidized cells to form large lobulated mass	One Nodular mass bulged on the skin, fleshy, homogenously grayish white	5 years	Female Dog	Skin at hock joint	Sebaceous adenoma
CK7	Neoplastic cells of large hepatoid cells with marked pleomorphism and distributed irregularly in the stroma	Irregular mass neighboring to anus, soft, Pale brown with hemorrhagic patches	8 years	Male Dog	Perianal gland	Hepatoid carcinoma
--	Osteoid and cartilaginous matrices between neoplastic cells, Mitotic figures, multinucleated giant cells, and hemorrhages	firm, grayish white in color with central reddish areas of friable consistency.	4- years	Male Dog	left forelimb	Chondroblastic osteosarcoma
HER2, Vimentin	Tubule formation, pleomorphism, mitotic activity, malignant cells of fibroblasts. cartilaginous tissue may be seen	Multinodular mass, soft, Yellowish Creamy	7 and 9 years	2 queens	Mammary gland	Adenocarcinoma with sarcoma

Microscopically, the swelling was formed from deep invasive neoplasm toward the dermis and reach muscles forming irregular lobules surrounded by interlobular collagen fibers in addition to subepithelial inflammatory cells infiltrations mainly lymphocytes (Fig. 2b). The mass revealed round cell with vacuolated cytoplasm. Proliferation of mature sebocytes or fully-lipidized cells lining sebaceous gland (Fig. 2c). Moreover, Cystic dilatation of some glands was seen.

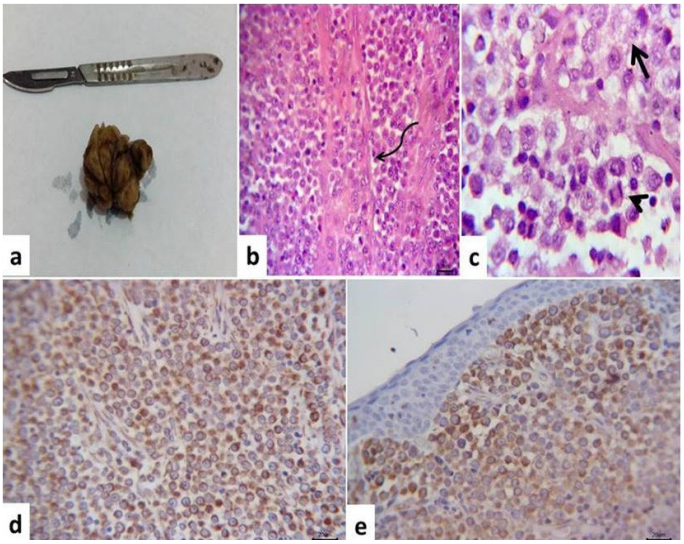

Fig. 1. (a) Formalin fixed cauliflower-like nodule excised from caudal part of glans penis. (b) Lobules of dense amount of round, oval or in variable shape neoplastic cells and contain a pale basophilic or colorless cytoplasm with scanty stroma (curved arrow) H &E bar 20 µm. (c) Microscopic picture showing numerous mitotic index (arrowhead) with small, clear intracytoplasmic vacuoles (arrow). H&E x1000. (d) Photomicrograph of TVT showing positive immunostaining reactions within cytoplasm of neoplastic cells by using Iba2 marker as golden yellow granules. IHC counterstaining with Mayer's haematoxylin. Bar 20µm. (e) Photomicrograph of TVT showing positive immunostaining reactions within neoplastic cells by using Vimentin marker as golden yellow granules. IHC counterstaining with Mayer's haematoxylin. Bar 20µm.

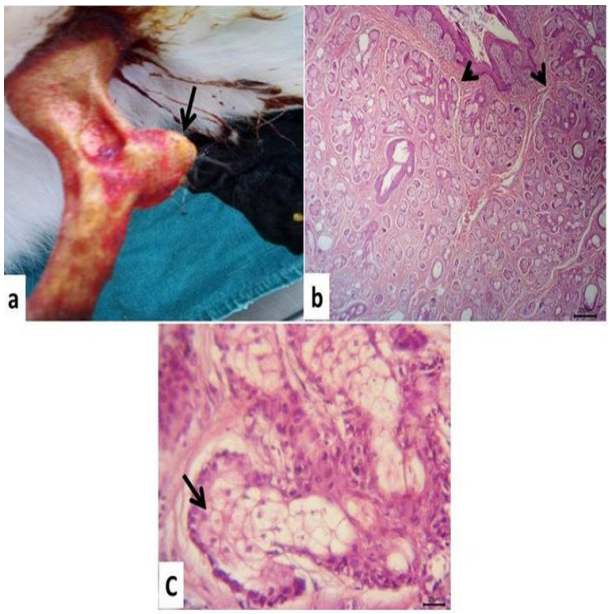

Fig. 2. (a) Nodular mass bulged on the skin of right leg at hock joint (arrow), (b) Microscopic picture showing deep invasive glandular neoplasm (arrowhead) toward the dermis. H &E bar 20 µm. (c) Photomicrograph showing proliferation of mature sebocytes (arrow). H &E bar 200 µm.

Eight years old male dog with irregular mass neighboring to anus on ventral aspect of the tail (Fig. 3a) with defecation problems related to the mass. Grossly, the mass was grayish in color with ulcerated hemorrhagic areas. soft in consistency, dimensioned 4x2.5x2 cm, weighed 52 grams, Pale brown with hemorrhagic patches on cut sections.

By histologic evaluation, the cells of tumor mass were morphologically similar to hepatocytes which referred to hepatoid glands. These neoplasms are less well organized into distinct lobules and trabeculae with areas of hemorrhage and edema (Fig. 3c). The neoplastic cells were often polyhedral cells having pale and eosinophilic cytoplasm with vacuoles. The cells were presented with marked pleomorphism and distributed irregularly in the stroma and were arranged in an acinar pattern (Fig. 3c). In many areas, the tumour cells were separated by delicate strands of fibro-vascular stroma. Small basophilic cells (basaloid cells) found at the periphery of the tumour mass had greater nuclear pleomorphism with prominent nucleoli and hyperchromatic. Mixture of both basaloid and hepatoid cells were noticed in majority of tumour mass. Furthermore, there were foci of squamous metaplasia and sebaceous differentiation. Inflammatory cell infiltrations containing many neutrophils were observed beneath the epidermis in association with serous exudate. The most important feature noted on histology is mitosis of differentiated hepatoid cells and/or invasion of neoplastic cells into the connective tissue around the tumor and into lymphatics. This is an indicator of malignancy. The neoplastic cells were mildly immunolabelling with CK7.

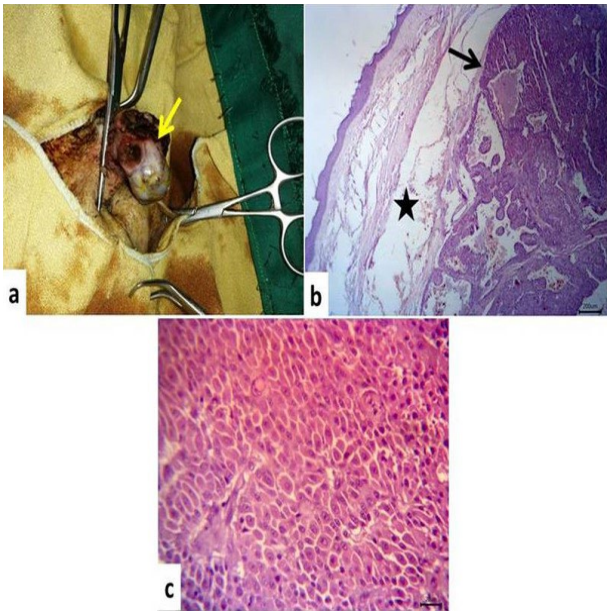

Fig. 3. (a) Dog with Irregular mass neighboring to anus on ventral aspect of the tail (arrow), (b) Photomicrograph showing neoplasms with distinct lobules (arrow) and trabeculae with areas of hemorrhage and edema (star). H &E bar 200 µm. (c) Photomicrograph showing neoplastic cells of large hepatoid cells with marked pleomorphism and distributed irregularly in the stroma. H &E bar 20 µm.

Table. 2. Staining distribution and intensity for used biomarkers.

Criteria	Staining distribution	Staining intensity
Anti- Vimentin [1]	4	++
Iba1[1]	3	+++
Anti- Vimentin[2]	2	++
Iba1[2]	3	++
CK7	1	+
HER2[3]	4	+++
Vimentin[3]	3	+++
HER2[4]	3	++
Vimentin[4]	2	+

1: 1 case of TVT, 2: 2 cases of TVT, 3: Mammary gland adenocarcinoma, 4: Mixed mammary gland adenocarcinoma.
The staining distribution was scored from 0 to 4, with 0 = 0%, 1 = < 10%, 2 = 10–30%, 3 = 31–60%, and 4 = > 61% of cells staining positive. The staining intensity was defined as the strength of the signals for the positive-staining tumours, with – = no signal, + = weak signal, ++ = moderate signal, and +++ = strong signal.

A 4-year old male, Great Dane dog with a history of lethargy, in-appetence and left forelimb lameness. On clinical examination, the patient showed severe left forelimb lameness with swelling in the area of left dis-

tal radius and ulna (Fig. 4a). Cranio-caudal x-ray imaging of distal radius showed a sunburst appearance suggestive of mixed bone lysis and new bone formation (Fig. 4b). Per the owner, the dog died 10 days later, but unfortunately consent for postmortem necropsy couldn't be obtained. Macroscopically, the excised specimen was moderately firm, grayish white in color with central reddish areas of friable consistency. Histopathological evaluation of the mass revealed, densely cellular, infiltrative mesenchymal neoplasm, composed of round, oval and elongated cells arranged in bundles and whorls on a fine fibrovascular matrix (Fig. 4c). Neoplastic cells had indistinct borders and small to moderate amount of eosinophilic cytoplasm. Among the neoplastic cells, osteoid and cartilaginous matrices were identified (Fig. 4d, e). Mitotic figures, multinucleated giant cells, and hemorrhages were detectable (Fig. 4f).

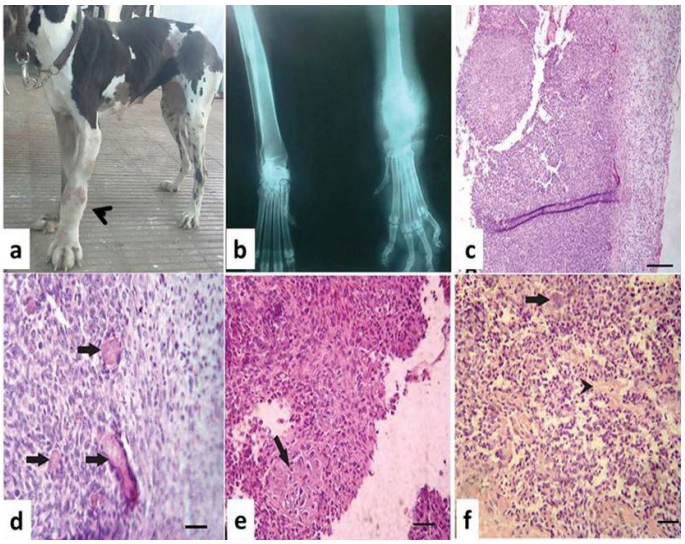

Fig. 4. (a,b) Clinical and radiographical findings: a) The left forelimb of the dog shows a swelling in the area of the distal radius (arrowhead). b) Cranio-caudal x-ray image of the forelimbs showing a sunburst appearance of the bone at the left distal radius metaphysis. c) A photomicrograph of the mass showing a highly cellular mesenchymal neoplasm, where the neoplastic cells are arranged in bundles and whorls. H &E bar 100 µm., d) A higher magnification of the previous photomicrograph depicting the presence of the osteoid matrix (arrows) among the neoplastic cells.H &E bar 20 µm. e) In addition to the osteoid matrix the neoplastic cells also produced chondroid matrix (arrow). H &E bar 20 µm. f) Multinucleated giant cells (arrow) and hemorrhages (arrowhead) were observed among the neoplastic cells. H &E bar 20 µm.

Two cases of multiparous queen (7 and 9 years old) with solitary mass on its left axillary crainoventral mammary gland (Fig. 4a). the other case appeared with multinodular masses on 4 quarter of mammary tissues. The majority masses were soft in consistency and yellowish creamy in color but 2 masses from the 2nd case were hard consistency and gritty sound. Thoracic x-rays were performed to verify possible pulmonary metastasis. Complete mastectomy with excised regional lymph nodes was performed. According to TNM system, the 2 neoplastic cases were III degree of malignancy depending upon their diameter more than 3 cm (large size tumor), 1 node involvement, without lung and other organ metastasis. Microscopically, the first neoplastic case had infiltrative growth with tubular or gland-like pattern which was composed of pleomorphic cells with vesicular nuclei and prominent nucleoli. Most neoplastic Cells showed elongated or oval nuclei containing inconspicuous nucleoli resembling mesenchymal cell with scant cytoplasm. An average mitotic index was three mitoses/40x field and bizarre mitoses were observed. The intertubular stroma consists of myxoide matrix with minor vessels. In some areas, an infiltrate by plasma cells, lymphocytes, and macrophages were seen. The majority of associated lymph nodes were replaced by metastatic adenocarcinoma with presence remnant of lymphoid cells (Fig. 5b, c). The adenocarcinoma was accompanied with some sarcomatous lesions which investigated through pleomorphic malignant cells of fibroblasts and positivity with vimentin marker (Fig. 5d). The 2nd neoplastic case showed cartilaginous tissue beside obviously characters of adenocarcinoma (Fig. 5e.).

The heterogeneous cell population showed strongly and diffusely positive immunolabeling for HER2 antibodies (Fig. 5f) for both cases.

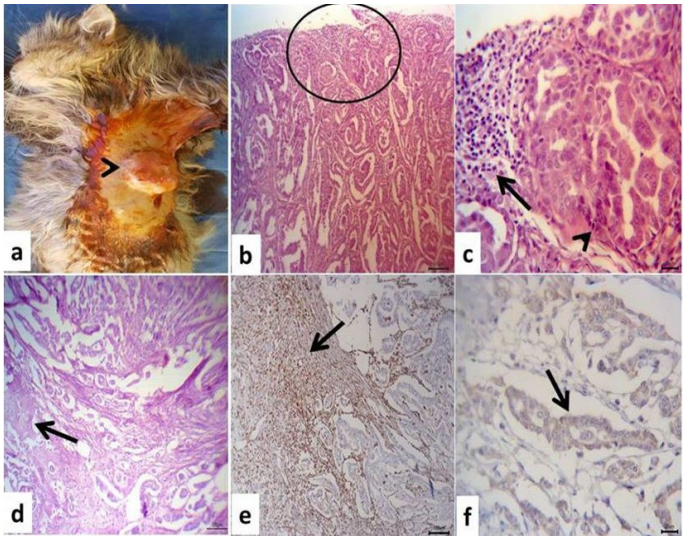

Fig. 5. (a) multiparous queen (7 years old) with solitary mass on its left axillary crainoventral mammary gland (arrowhead). (b) The associated lymph nodes showing complete replacement by metastatic adenocarcinoma. (c) High magnification of the previous figure showing Tubule formation, pleomorphism, and mitotic activity (arrowhead) with presence few lymphoid cells (arrow). H &E bar 100 µm. (d). Photomicrograph of mixed mammary adenocarcinoma showing cartilaginous stroma (arrow) beside obviously characters of adenocarcinoma (thick arrow). H &E bar 100 µm. (e) Photomicrograph of mammary gland adenocarcinoma with sarcoma showing positive immunostaining reactions within cytoplasm of pleomorphic malignant cells of fibroblasts by using vimentin marker aa golden yellow granules. IHC counterstaining with Mayer's haematoxylin. Bar 20µm. (f) Photomicrograph of mammary gland adenocarcinoma with sarcoma showing positive immunolabeling for HER2 antibodies as golden yellow granules. IHC counterstaining with Mayer's haematoxylin. Bar 20µm.

Discussion

TVT appeared as small hyperemic papule at initial stage which later progresses to nodular, papillary, multilobulated, and cauliflower-like pedunculated mass. The previous findings agreed with Eze *et al.* (2007). There is no metastasis recorded in our study so the prognosis of dog with TVT is excellent after surgical excision as mentioned by Slatter (2002). tumor cells of TVT tumor cells secrete cytotoxic proteins which cause apoptosis of B-lymphocyte during the neoplastic progression phase (Liao *et al.*, 2003). TVT was classified as being in a progressing growth phase due to high mitotic activity and low number of lymphocytes as Hill (1984) recorded.

Sebaceous adenoma is usually less lobulated and greater than 1 cm in diameter, while sebaceous hyperplasia is commonly papillated, and 2-5 mm in diameter (Pullet and Stannard, 1990). In the case described here, the tumor was 4.8x3.7x2.1cm in dimensions and this macroscopic feature can be used as a diagnostic tool for distinguishing sebaceous adenoma from sebaceous hyperplasia. Our findings were in accordance with Lorsirigool *et al.* (2020) who reported mass at left front toe 7-year-old dog with focally expansion of the dermis by a highly cellular well delineated, expansile, exophytic proliferation of neoplastic epithelial cells showing sebocyte differentiation. The sebaceous gland plays a role in secreting sebum to coat the fur and skin of animals. Tumors exhibit many external features such as a dome-shape, pedunculated papule, or nodule (Yoon and Park, 2016).

The odour of perianal gland secretion helps in recognition and communication between dogs (Goldschmidt and Hendrick, 2002). Perianal adenomas comprise more than 80% of all perianal tumors and are the third most common tumor in male dogs because of their testosterone dependence. The perianal adenocarcinomas look like adenoma, but they tend to grow faster, ulcerate frequently, are firmer and reoccur frequently (Withrow, 2001) as we reported grossly. Histopathology confirmed the presence of anal gland tumor which are correlated with Yumuşak *et al.* (2016) which found cells of hepatoid gland carcinoma were presented with marked pleomorphism and distributed irregularly in the stroma. Manipulation of tumor was minimal during surgery to minimize embolization of tumor cells.

Chondroblastic OSA in in a 4 years old Great Dane is the main primary malignant and non-hematopoietic bone tumor in dogs representing about 85% of all bone tumors. Certain dog breeds are at significantly higher risk for developing OSA, and Great Danes are one of these highly

susceptible breeds (Davis and Ostrander, 2014). The common large size that these breeds share and the higher prevalence in the long bones suggested an association between bone growth and the development of osteosarcomas, which was further supported by an investigation done in 2004 demonstrating the expression of insulin growth factor-1 (IGF-1) receptor and its ligand in canine OSA cells (MacEwen *et al*., 2004). The x-ray imaging of the swollen limb showed the characteristic sunburst appearance commonly seen in OSA (Gorlick and Khanna, 2010) and suggesting the concomitant production and lysis of osseous matrix which was further supported by the observation of friable hemorrhagic centers of the neoplastic mass macroscopically and the presence of hemorrhage and necrosis microscopically. Our definitive diagnosis for the case was based on the production of both osteoid and chondroid matrices by the neoplastic cells, where the presence of the osteoid matrix ruled out chondrosarcoma as another possible diagnosis. In accordance with several previous descriptions of canine OSA (Kirpensteijn *et al*., 2002), histologically the neoplastic cells were pleomorphic, arranged in whorls and bundles and had scant to moderate cytoplasm, with stippled hyperchromatic nuclei and a single prominent nucleolus. The presence of tumor giant cells and mitotic figures are also classical findings in OSAs.

Mammary adenocarcinomas (MACs) are common tumors in cats. The postexcisional survival period of affected cats is inversely proportional to tumor size, but the median survival periods for different tumor size categories is variable. Cats with MACs and mixed mammary neoplasm with greater diameter than 3 cm had a 12-month median survival period, whereas those with MACs less than 3 cm in diameter had a 21-month survival period. Some terminologies were used such as Survival period " the time from first neoplasm detection to time of death ". Postexcisional survival period " the time from neoplasm excision to time of death ". Tumor free interval " the time from neoplasm excision to time of neoplasm first recurred" (Viste *et al*., 2002). In our study, MACs and mixed mammary neoplasm appeared with greater diameter than 3 cm, the 1st case had a 9 months survival period and 8 months post excisional period.

Human epidermal growth factor receptor 2 gene (HER2/neu) is overexpressed in 10% to 40% of breast carcinoma in women (Millanta *et al*., 2005). Because feline mammary tumors generally behave aggressively, identifying HER2 overexpression in these neoplasms indicated for poor prognosis. Recent investigations have demonstrated HER2 expression to be low (25%) in benign mammary tumors and absent in nontumor samples. In contrast, 41% to 90% of feline mammary carcinomas express HER2 (De Maria *et al*., 2005). The previous reports were in agreement with our results due to the overexpression of MAC malignant cells with HER2 reported in this study. Using vimentin as (Doyle and Hornick, 2014) for mesenchymal tissue associated with mixed mammary carcinoma.

We confirmed the 2 cases of TVT by using Vimentin which were given 100% positivity immunostaining for TVT as (Marcos, 2006) seen. Iba1 considers a good discriminatory marker for TVT among non-histiocytic leukocytic tumors (Pierezan *et al*., 2014). Using cytokeratin 7 (CK7) biomarker as a confirmatory tool in hepatoid adenocarcinoma as recorded by Basturk *et al*. (2010).

Conclusion

Histopathological evaluations by H&E stain is the backbone method for neoplasms diagnosis and using some biomarkers added a confirmatory tools for these diagnosis. Staining intensity and distribution of used biomarkers were also evaluated.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ali, A.G., El mowalid, M., Abdel-Glil,T., Sharafeldin, F., Abdallah, S., Mansour, A., Nagy, Ahmed, B., Abdelmoneium, M., 2015. Etiology and pathology of epidemic outbreaks of avian influenza H5N1 infection in Egyptian chicken farms.Pol.J.Vet.Sci.18,779-786.

Basturk, O., Farris, A.B., Adsay, N.V., 2010. Immunohistology of the pancreas, biliary tract, and liver. In Diagnostic Immunohistochemistry. Theranostic and Genomic Applications, 3rd edn (ed. D.J. Dabbs). Saunders, St. Louis, MO, 508–592.

Brodey, R.S., Riser, W.H. 1969. Canine osteosarcoma. A clinicopathologic study of 194 cases. Clin. Orthop. Relat. Res. 62, 54-64.

Bronden, L.B., Nielsen, S.S., Toft, N., Kristensen, A.T., 2010. Data from the Danish Veterinary Cancer Registry on the occurrence and distribution of neoplasms in dogs in Denmark. Vet. Record. 19, 586-590.

Chikweto, A., McNeil, P., Bhaiyat, M.I., Stone, D., Sharma, R.N., 2011. Neoplastic and non neoplastic cutaneous tumors of dogs in Grenada, west Indies. ISRN Vet Sci.12, 85-89

Davis, B.W., Ostrander, E.A., 2014. Domestic Dogs and Cancer Research: A Breed-Based Genomics Approach. ILAR J. 55, 59-68.

Doyle, L.A., Hornick, J.L., 2014. Immunohistology of neoplasms of soft tissues and bone. In Diagnostic Immunohistochemistry. Theranostic and Genomic Applications, 4th ed. (ed. D.J. Dabbs). Saunders, St. Louis, MO, 73–129.

De Maria, R., Olivero M., Iussich S., Nakaichi, M., Murata, T., Biolatti, B., Di Renzo, M.F., 2005. Spontaneous feline mammary carcinoma is a model of HER2 overexpressing poor prognosis human breast cancer. Cancer. Res. 65, 907-912.

Ehrhart, N.P., Ryan, S.D., Fan, T.M., 2012. Tumors of the Skeletal System. In: Withrow and MacEwen's Small Animal Clinical Oncology: 5th ed., 463-503.

Eze, C.A., Anyanwu, H.C., Kene, R.O., 2007. Review of Canine Transmissible Venereal Tumour in dogs. Nig. Vet. J. 28, 54-70.

Gorlick, R., Khanna, C., 2010. Osteosarcoma. J Bone Miner Res. 25, 683-691.

Goldschmidt, M.H., Hendrick, M.J. 2002. Tumours of the skin and soft tissues. In: Textbook of Tumours in Domestic Animals (DJ Meuton, editor). 4th ed. Iowa, Iowa State Press. 45-118.

Gupta, N., Tiwari, S.K., 2009. Studies on Incidence, Histopathological features and Surgical management of Neoplasms in Canine. Vet. World. 10, 392-395

Heller, D.A., Clifford, C.A., Goldschmidt, M.H., Holt, D.E., Shofer, F.S, Smith, A., Sorenmo, K.U., 2005. Cyclooxygenase-2 expression is associated with histologic tumour type in canine mammary carcinoma. Vet. Pathol. 42, 776–780.

Hill, D.L., Yang, T.J., Watchel, A., 1984. Canine transmissible venereal sarcoma: tumor cell and infiltrating leukocyte ultrastructure at different growth stages. Vet Pathol. 21,39–45.

Hsu, S.M., Raine, L., Fanger, H., 1981. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 4, 577–580.

Jakab, C., 2003. Histopathological analysis of tumours of the sebaceous gland in spaniels. Kisallat. Praxis. 4, 36-38.

Kirpensteijn, J., Kik, M., Ruttman, G.R., Teske, E., 2002. Prognostic Significance of a New Histologic Grading System for neoplasm. Vet. Pathol. 39, 240-246

Liao, K., Hung, S., Hsiao, Y., Bennett, M., Chu, R., 2003.Canine transmissible venereal tumor cell depletion of B lymphocytes: molecule(s) specifically toxic for B cells. Vet. Immunol. Immunopathol. 92, 149-162.

Lorsirigool, A., Chantrarasmee, C., Roongsitthichai, A., 2020. Sebaceous adenoma on the toe of a dog at an animal hospital in thonburi area, bangkok Province, Thailand. Inter. J. of Vet. Science 10, 144-147.

MacEwen, E.G., Pastor, J., Kutzke, J., Tsan, R., Kurzman, I.D., Thamm, D.H., 2004. IGF-1 receptor contributes to the malignant phenotype in human and canine osteosarcoma. J. Cell. Biochem. 92, 77-91.

Marco, R., Santos, M., Marrinhas, C., Rocha, E., 2006. Cutaneous transmissible
venereal tumor without genital involvement in a prepubertal female dog. Vet. Clin. Pathol. 35,106–109

Millanta, F., Calandrella, M., Citi, S., 2005. Overexpression of HER-2 in feline invasive mammary carcinomas: an immunohistochemical survey and evaluation of its prognostic potential. Vet. Pathol. 42, 30-34.

Misdrop, W., van der Heul, R.O., 2002. Carcinosarcomas of the dogs. Vet. Pathol.17, 53-57.

Morello, E., Martano, M., Buracco, P., 2011. Biology, diagnosis and treatment of canine appendicular osteosarcoma: similarities and differences with human osteosarcoma. Vet. J. 189, 268-277.

Pierezan, F., Mansell, J., Ambrus, A., Hoffmann, A.R., 2014. Immunohistochemical expression of ionized calcium binding adapter molecule 1 in cutaneous histiocytic proliferative, neoplastic and inflammatory disorders of dogs and cats. J. Comp. Pathol. 151, 347–351.

Pisani, G., Millanta, F., Lorenzi, D., 2006. Androgen receptor expression in normal, hyperplastic and neoplastic perianal glands in the dog. Res. Vet. Sci. 81, 231-236.

Pullet, L.T., Stannard, A.A., 1990. Tumor of the Skin of Soft Tissues. In:
Moulton J.E., Ed. Tumors in Domestic Animals. 3rd ed., University of California Press, California, 23-87.

Slatter, D., 2002. Textbook of small animal surgery, 3rd ed. Saunders, USA, 2439

30.Suvarana, S.K., Layton, C., Bancroft, J.D. 2018. Bancroft's theory and practice of Histological techniques.8thed., Churchill Livingstone. Elsevier, England.

Teruya-Feldstein, J., 2010. The immunohistochemistry laboratory. Looking at Molecules and preparing for tomorrow. Arch. Pathol. Lab. Med. 134, 1659–1665.

Vani, G., Saibaba, M., Devaratnam, J., Reddy, K.S., 2016. Surgical Excision of Sebaceous Adenoma in a dog. Inter. J. of Science, Engineering and Technology Research (IJSETR). 5, 3285-3287.

Viste, J.R., Myers, S.L., Singh, B., Simko, E., 2002. Feline mammary adenocarcinoma: tumor size as a prognostic indicator. Can. Vet. J. 43, 33–37.

Withrow, S.J., 2001. Small Animal Clinical Oncology. 3rd ed. WB Saunders, Philadelphia, 346-353.

Withrow, S.J., MacEwan, E.G., 2001. Small animal clinical oncology. 3rd ed. Modified World Health Organization TNM Classification. Philadelphia, Pa: WB Saunders Co., pp. 346-470,

Yeruham, I., Perl, S., Orgad, U., Yakobson, B., 1999. Tumours of the vulva and vagina in cattle - a 10-year survey. The Vet. J. 158, 237-239.

Yoon, J., Park, J., 2016. Immunohistochemical characterization of sebaceous epithelioma in two dogs. Iran. J. Vet. Res. 17, 134.

Yumuşak, N., Çalişkan, M., Kutsal, O., 2016. Fine needle aspiration cytology (FNAC) in the diagnosis of canine hepatoid gland tumors- A comparative study with histopathology. Ankara. Üniv. Vet. Fak. Derg. 63, 259-266.