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Histopathology and Immunohistochemistry Associated with Cutaneous Tumours in Canines

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ABSTRACT: The present study was designed to characterize and diagnose different spontaneously occurring canine cutaneous neoplasms. As there are different ways to diagnose and identify the tumours, it becomes important to determine the methodology for tumour detection. Conventional methods for diagnosis of tumours usually gives the idea whether the tumour is benign or malignant, however advanced methods such as immunohistochemistry and molecular tissue markers have also gained popularity due to the minute details they provide regarding the tumours. Twenty-nine tumour samples were collected from dogs irrespective of their sex, breed, age and were grouped as skin tumours based upon their anatomical location. Histopathological diagnosis of tumour samples evinced 12 benign skin tumours (perianal gland adenoma-5, trichoblastoma-4, trichofolliculoma-1, tricholemmoma-1 and haemangioma-1) and 17 malignant skin tumours (fibrosarcoma-6, mast cell tumour-3, apocrine adenocarcinoma-3, haemangiopericytoma-1, perianal gland adenocarcinoma-1, chondrosarcoma-1, melanoma-1, poorly differentiated carcinoma-1). A total of 10 cutaneous tumours were subjected to immunohistochemistry for various molecular markers, *viz*., F8, SMA, Desmin, S-100, p63 and ER. Various tumour subtypes were found positive for different IHC markers.

Keywords: Cutaneous neoplasms, Histopathological, Benign, Malignant, Immunohistochemistry.

INTRODUCTION

Neoplasm is defined as a new growth of cells, derived from normal tissues that undergoes certain changes and becomes unresponsive to normal growth controls of the body. Neoplasms can be benign or malignant. Benign neoplasms do not spread to the surrounding body tissues whereas malignant neoplasms grow rapidly and have tendency to metastasize to other parts of the body (Zachary and McGavin 2016). Many types of tumours are often detected on skin, subcutis and adnexa of dogs (Bronden *et al*., 2010) and are frequently submitted for histopathological diagnosis. In male and female dogs, cutaneous tumours are the most and second-most frequently reported tumors, respectively (Bronden *et al*., 2010; Dobson *et al*., 2002; Kok *et al*., 2019). In majority of cases, surgical removal of cutaneous tumour is the best option. However, surgical excision also depends on the type of neoplasm as well as its grade, stage, and location (Zachary and McGavin 2016). A profound and accurate knowledge of cancer provides information on possible causes and trends of conditions making it possible to establish timely and appropriate health care interventions (Camilla and Giuseppe 2019).

The diagnosis of neoplasms represents the major challenge faced by veterinary oncologists. Although an experienced clinician can sometimes make a guess as to the likely nature of a tumour according to its site, gross appearance and history, a definitive diagnosis can only be made by microscopic examination of representative tissue or cells from the tumour. Histopathology is often considered as the gold standard for diagnosis of tumours. However histopathology alone does not provide sufficient details of the cellular changes taking place in the tumour, so biological parameters such as tumour cell proliferation markers, different hormonal receptors and other biomarkers are important additives in its timely diagnosis as well as prognosis. Therefore, it is imperative to study carcinogenesis by using immunohistochemistry as well. Commonly used markers in immunohistochemistry involves Ki-67, estrogen receptor (ER), progesterone receptor (PR), c-erbB2, p53, Smooth muscle actin (SMA), Desmin and Vascular endothelial growth factor (VEGF) (Pawan *et al*., 2009). As these neoplasms are responsible for considerable animal morbidity and veterinary health services pursue due to their high frequency, exact diagnosis of cutaneous tumours remains important while planning the best line of treatment for the animal. Many recent developments have been also made in treatment of cancers however the efficacy of these treatments depends upon tumou type and its progression (Koji *et al.*, 2021).

MATERIALS AND METHODS

The present study was carried out at Department of Veterinary Pathology, Veterinary College Mhow and at

Department of Veterinary Pathology, SDAU, SK Nagar Gujarat. A total of 58 cases of canine tumours were collected during the study period from July 2018 to April 2019. Based on the anatomical location of tumours, 29 were grouped as cutaneous tumours. Blood and Serum samples from dogs having cutaneous tumours were also collected.

Materials:

Equipments. The following equipments were utilized in the execution of this research

- Spencer Microtome (American optical company)
- Automatic knife sharpner (Reichert Jung, USA)
- Hot air oven (Thelco)
- Hot water bath (Scientech)
- Trinocular Microscope (Leica)
- Incubator
- Centrifuge machine
- Automatic tissue stainer
- Humidity Chamber

Chemicals. Following chemicals were used for performing histopathology

- 10% neutral buffered formalin
- 0.9% sterile normal saline
- Acetone
- Benzene
- Alcohol
- Xylene
- Harris Hematoxylin and 2% aqueous eosin
- Saturated salt solution
- Giemsa stain
- Toluidine blue stain

Polyclonal antibodies and conjugates. The polyclonal antibodies used for immunohistochemistry were of Santa-Cruz Biotechnology, USA and from Sigma Aldrich, USA. Anti-rabbit HRPO, Anti-mouse HRPO and anti guinea pig-HRPO conjugates (Santa Cruz Biotechnology, Inc.) were used for immunohistochemistry**.**

Methodology:

Histopathology. Representative tissue pieces (approximately 0.5 cm each) were collected from multiple (at least 3) sites from excised cutaneous tumour masses after surgery and immediately fixed in 10% neutral buffered formalin (NBF) for 48-72 hours with 2-3 changes of formalin. After fixation in 10% NBF, tissue samples were trimmed to 1.5mm thickness and given overnight washing under running tap water. The tissue samples were then dehydrated by passing through ascending grades of ethyl alcohol, cleared in xylene and embedded with paraffin wax (melting point 58°C) for block making. The sections were cut at 4-5µm thickness and stained by Haematoxylin & Eosin (H&E) stain as per standard procedure (Luna, 1968). Duplicate sections were kept for special staining and immunohistochemical staining.

Special histological stainings

For presence of mast cells: Toluidine blue staining. The hydrated tissue sections were stained by toluidine blue working solution for 2-3 minutes, washed in three changes of distilled water, differentiated quickly through 95% ethanol (1 dip each since stain fades quickly in alcohol), cleared in 2 changes of xylene (3 minutes each) and mounted with resinous mounting medium.

For presence of collagen: Van Gieson's staining

The deparaffinized and hydrated tissue sections were first put in Haematoxylin solution for 5 minutes. After that the sections were washed briefly under running tap water and given two changes of distilled water. The tissue sections were then exposed to Van Gieson's solution for 3 minutes. The sections were then dehydrated using ascending grades of alcohol followed by their clearing in xylene. The sections were further mounted by using DPX and were analysed for the presence of collagenous material.

Evaluation of expression of molecular biomarkers in tissues by immunohistochemistry

Immunohistochemical staining of tissues for molecular biomarkers. Expression of tumour markers and their expression pattern were determined by IHC using formalin-fixed paraffin- embedded tissue sections following standard methods (Zhao *et al*., 2009).

RESULTS AND DISCUSSION

Canine Cutaneous Tumours. A total of 29 skin tumours were recorded on different locations like face, neck, anus, limbs, toe and tail. The animals with localized skin tumours were apparently healthy without any derangement of physiological activities. Tumours varied in size from multiple small nodular masses to single large round pedunculus mass. In some cases tumours were covered by intact hairy skin and in other cases, tumours were ulcerated and bleeding. The case-wise details of tumours are given in Table 1.

Histopathology. In the present study, out of the 29 cases of canine cutaneous tumours, 12 were benign and 17 were malignant tumours.

Benign skin tumours (n=12)

Perianal gland adenoma (n=5)

Five cases showed enlarged tumour mass generally devoid of hair in the perianal region. The tumour masses were excised surgically. The sizes of the masses ranged from 4cm×3cm to 8.5cm×4.5cm and weighed from 30gm to 150gm. Grossly the tumour masses were soft in consistency and cut surface was smooth and pale in colour. In one case the tumour mass was superficially ulcerated and haemorrhagic.

Histopathologically, the tumour was mainly composed of the large cells resembling hepatocytes (hepatoid cells) arranged as cords of variable sizes infiltrating into the loose connective tissue stroma. The cells were polyhedral in shape having centrally located, large, ovoid, vesicular nuclei with a central small nucleolus, abundant eosinophilic cytoplasm and distinct cell borders. At the periphery of the lobules a rim of small basaloid reserve cells, generally of one cell layer thick, having small hyperchromatic nuclei and little cytoplasm was present. Mitotic activity index was 14 to 16 per 10 hpf (Fig. 1).

Perianal glands are non secretory abortive sebaceous glands found near anus of dog and also in prepuce, tail, hind limbs and trunk. The tumour is common in aged male dogs and affects the normal bowel movement and defecation leading to health problems in dogs. Similar type of tumours and their histopathology has been

reported by other researchers (Meuten, 2002; Rao, 2004; Devi *et al*., 2012).

Trichoblastoma (n=4). There were four cases of trichoblastoma ranging from 2cm×2cm to 4cm×2cm in size and 15gm to 30gm in weight. All four tumour masses were excised from the facial area of different dogs. Grossly the tumour masses were small and single round masses covered by hairy skin. In one of the case, the tumour mass was nodular and ulcerated.

Histopathological examination revealed trichoblastoma ribbon type pattern in three cases and trichoblastoma medusa type pattern in one of the case. In ribbon type, there was presence of long chords of two cells thick cords with nuclei arranged in a palisaded fashion. Nuclei were hyperchromatic and individual neoplastic cells had scanty cytoplasm with indistinct cell borders. Connective tissue separating the neoplastic cells was hyalinized. Mitotic index was variable and ranged from 15 to 20 per 10 hpf (Fig. 2).

In trichoblastoma medusa type, there were cords of cells radiating from a central island of densly packed cells. The cords of epithelial cells had abundant eosinophilic cytoplasm and connective tissue matrix was hyalinized. Mitotic index was 14 per 10 hpf.

Trichofolliculoma (n=1). One tumourous mass hanging from the muzzle region of a dog measuring 8cm×5cm and weighing 100gm was surgically excised. The gross surface of the mass was blackish in colour and covered with hair. There was also presence of some nodular growth on the apex of the mass.

Upon histopathological examination of tissue, cysts lined by squamous epithelium resembling infundibular epithelium and containing keratin and hair fragments were seen. Hair follicles were found attached to the wall of cyst and presence of infiltrated fibrous connective tissue was also noticed. Mitotic activity index was very less (3 per 10 hpf) (Fig. 3).

Tricholemmoma (n=1). One tumour mass measuring 2cm×2cm and weighing 15gm was surgically excised from the lumbar region of a dog. Grossly, the tumour mass was hard in consistency and bleeding.

Histopathology revealed presence of islands of neoplastic cells involving the hair follicle isthmus. There was a central lumen filled with keratin and lined by large squamous cells with pale eosinophilic cytoplasm. Some cysts were also present in between the keratinized islands of epithelial cells. A granulomatous inflammatory response was seen in surrounding tissue due to release of keratin. Mitotic activity index was 12 per 10 hpf.

Primarily an involvement of hair matrix cells, infundibulum along with isthmus is found in trichoblastoma, tricholemmoma and trichofolliculoma respectively. All are uncommon benign dermal tumours with breed, hair coat and infectious etiologies.

Cutaneous haemangioma (n=1). One case of cutaneous haemangioma excised from anal area was found in the study. The tumour mass was 8.5cm×3cm in size and weighed around 80gm. Grossly the tumour mass was nodular in consistency and cut surface was haemorrhagic. There was also presence of dark reddish coloured fluid inside the tumour mass.

Histopathology revealed large blood filled spaces in deep dermis. The spaces were lined by single layer of flattened endothelial cells. Large number of capillary type blood vessels was also present in the upper dermis. Occasionally blood vessels with thrombus were also seen. Mitotic activity index was very low (1-3 per 10 hpf).

Cutaneous haemangiomas are common in dogs and variants of these tumours have been called cavernous or capillary based on the size of vascular channels. Mitotic figures are rare as described by other researchers (Meuten, 2002; Zahoor, 2016).

Malignant skin tumours (n=17)

Fibrosarcoma (n=6). Six cases of fibrosarcoma excised from different locations were found during the study. The sizes of the tumour masses varied from 5cm×3cm to 17cm×15cm and weighed 50gm to 650gm. Grossly, the tumour masses were hard and nodular in consistency. Cut surface was smooth and whitish, however in one case haemorrhagic areas were also observed.

The tumours showed proliferation of spindle shaped cells arranged haphazardly and frequently forming whorl-like pattern. The nuclei of proliferating cells were hyperchromatic and elongated to oval in shape. The cytoplasm was scanty and nucleoli were not visible. Mitotic figures (30 to 33/10hpf) were also present. The duplicate sections stained with Van Gieson's stain revealed spindle shaped fibroblasts with pink cytoplasm and black nucleus surrounded by yellow collagenous substance (Fig. 4).

Fibrosarcomas are usually of low malignant type and metastasis is usually uncommon. However recurrence occurs with speed after excision. Similar histopathological appearance has been described by other research workers (Meuten, 2002; Kumar *et al.,* 2004).

Mast cell tumour (n=3). Of the three cases of mast cell tumours, one was noticed in the lateral aspect of abdomen and the other two were present on the forehead. Grossly the tumours were large round masses of sizes ranging from 3cm×2cm to 6cm×3cm and weighed from 20gm to 100gm. One of the tumours was ulcerated while the other two were covered by intact skin. Cut surfaces of the tumours were smooth and pale in colour.

Histopathology revealed round to polygonal cells with round darkly stained nuclei and moderate, pale pink cytoplasm containing blue granules. The neoplastic cells were found in deep dermis and in subcutaneous tissues arranged in diffuse and solid pattern. Eosinophils were also found distributed within the tumour as the predominant infiltrating cell type. Mitotic figures were less and averaged 15 per 10 hpf. Toluidine blue staining showed large number of violetcoloured neoplastic mast cells with metachromatic granules in cytoplasm (Fig. 5).

Mast cells are present around blood vessels in the connective tissue. Tumours are normally nodular and pedunculated with frequent ulceration. The size and shape of the cells vary according to the stage of development. The present description of mastocytoma

resembles with the documentation of other researchers (Meuten, 2002; Reddy *et al*., 2009).

Apocrine adenocarcinoma (n=3). Three tumourous masses of size ranging between 5cm×5cm to 7cm×5cm and weight 100gm to 120gm were found in study. The masses were located at tail, interdigital space and at left hind limb. Grossly the tumour masses were having smooth surface, semi-solid consistency and on the cut surface, irregular areas of necrosis and hemorrhages were seen.

Histopathological examination revealed multiple tubules surrounded by fibrovascular stroma. The tubules were lined by multilayered epithelium with numerous mitotic figures (39-43/10hpf). Pink coloured hyaline secretion was also seen in the neoplastic tissue. Dermal lymphatic invasion by neoplastic cells was also found along with occasional melanocytes (Fig. 6).

Apocrine glands have a secretory tubule surrounded by myoepithelial cells and a excretory duct, both voiding into the follicular infundibulum. Some modified apocrine glands empty directly on to skin surface. Apocrine carcinomas appear histologically as solid, tubular or cystic tumours with infiltration of dermal lymphatics with variable mitotic rate.Similar description has been documented by eminent researchers (Meuten, 2002).

Cutaneous haemangiopericytoma (n=1). One case of haemangiopericytoma measuring 7cm×5cm and weighing 180gm was surgically excised from interdigital space. Grossly the tumorous mass was having smooth surface with fat deposition on its cut surface.

The proliferating tumour cells (pericytes) were arranged in interlacing bundles varying from few to many concentric layers surrounding the capillaries and separated by variable amounts of collagenous stroma. Cellular pleomorphism and mitotic activity was high (Fig. 7).

The hallmark of this neoplasm is the presence of perivascular whorls of fusiform cells. Cellular pleomorphism, mitotic activity and metastatic potential is low in primary tumour but increases with each recurrence. The present histopathological findings of cutaneous haemangiopericytoma are in concordance with the previous documentations (Meuten, 2002; Namazi *et al*., 2013).

Perianal gland carcinoma (n=1). One case of perianal gland carcinoma was recorded during the study. The tumour mass was present on the left side of anus and measured 7cm×4cm in size and weighed around 130gm. The tumour mass was nodular and appeared pink, fleshy with ulcerated growth on its surface.

Histopathology revealed lobules with disorderly arranged proliferating hepatoid cells. Reserve basaloid cells with hyperchromatic nuclei were not restricted to the periphery and were present throughout the lobules intermixed with hepatoid cells. The lobules varied in shape and size. In some lobules squamous metaplasia of hepatoid cells was also seen. Mitotic activity index was 32 per 10hpf.

Perianal glands are found in the wall of two anal sacs between the external and internal sphincter muscles and empty via a short duct into anal sacs. The frequency of perianal gland carcinomas are relatively less as compared to adenomas and are characterized by a high mitotic activity of proliferating hepatoid cells. Similar histopathological findings have been documented by others (Meuten, 2002).

Chondrosarcoma (n=1). One large tumour mass measuring 15cm×14cm and weighing 400gm was excised from the hock joint of the dog. The tumour mass was hard in consistency and had many nodular growths over its surface. Some areas of necrosis were present over the cut surface of the tumour mass.

On histopathological examination, immature spindle shaped cells were seen along with highly pleomorphic cells at the periphery. The centre showed poorly differentiated cartilage cells. Multiple areas of hemorrhages and necrosis were also seen. Mitotic activity was variable and quite high in all the type of cell population. Large area of tumour was necrosed and calcified (Fig. 8).

Chondrosarcoma is one of the rare tumours in animals, with dog being the most commonly affected animal. The tumour masses are large in size, multinodular and some may show areas of degeneration. Immature spindle shaped cells and cartilage cells with frequent mitosis are commonly seen. The present microscopic appearance of chondrosarcoma is similar to the description documented by other research workers (Meuten, 2002).

Melanoma (n=1). One case of melanoma excised from the lumbo sacral area of a dog. The tumour mass 9cm×6cm in size and weighed around 250gm. Grossly the tumour mass was hard with bleeding surface.

Histopathology revealed proliferation of round cells in the dermis. The nuclei of the proliferating cells were dark and hyperchromatic and cytoplasm of some of the cells contained blackish granular pigment (melanin). The proliferating cells penetrated into the deep dermis and replaced the connective tissue. Mitotic activity was variable.

The melonocytic tumour arises from the specialized cells that produce melanin and occur in all domesticated animals. Grossly these tumours vary in size and may be black or brown in colour. Histological section of melanomas consists of pigment laden melanoblasts amidst fibrous connective tissue. Similar descriptions of melanoma have been reported by other researchers (Kumar *et al*., 2004).

Poorly differentiated carcinoma (n=1). A tumour mass measuring 4cm×2cm in size and weighing around 50gm was excised from the anal area of a dog. Grossly the tumour mass was nodular in consistency and cut surface was oily and smooth.

Histopathologically, tumour cells were cubodial to polygonal in shape with eosinophilic cytoplasm and poorly differentiated nucleoli. Small foci of keratinization were also present in the tissue. Intraluminal necrotic cells were also seen. Mitotic activity was highly variable in all the cells.

Poorly differentiated carcinomas have undifferentiated histogenesis and show high mitotic activity index indicative of malignancy (Pawan *et al*., 2010).

Immunohistochemistry. A total of 10 skin tumour samples were subjected to Immunohistochemistry for

confirmatory diagnosis of tumour type. Different markers such as Factor 8(F8), Smooth muscle actin (SMA), Desmin, S-100, p63, Pan Cytokeratin (PCK) and Cytokeratin (CK5/6) were used (Fig. 9, 10). Details of the markers along with the cases are given in Table 2. Molecular mechanisms which underlie altered cell cycle progression and proliferation of cells serve as better prognostic markers. Genes and proteins regulating the transition of the GI to S phase of the cell cycle have been implicated in the development of human and animal cancers. In present study, a panel of different Immunohistochemistry markers were used as an additional diagnostic criteria to confirm and differentiate tumours sharing certain common histological features.

A total of 10 skin/cutaneous tumours were subjected to different IHC markers namely SMA, Desmin, PCK, CK5/6 and S-100. All cases of fibrosarcomas were found positive for SMA and Desmin which aid in labeling of tumours of smooth and striated muscle origin. Perianal gland adenomas and apocrine adenocarcinoma were found positive for PCK and CK5/6 markers. A single case of cutaneous haemangiopericytoma was confirmed by SMA and S-100 markers. S-100 is a mutigene family of low molecular weight calcium binding proteins of 19 members. S-100A6 is expressed by fibroblasts, smooth muscle and heart muscle cells. These findings are in concordance with the reports of other research workers (Kumar *et al.*, 2004; Al- Daraji *et al.*, 2009; Shivani *et al.*, 2017).

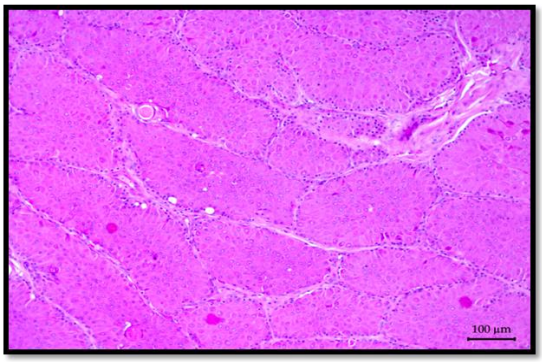

Fig. 1. Perianal gland adenoma: Multiple closely packed lobules of hepatoid cells with a peripheral single layer of small dark reserve cells (H&E × 100).

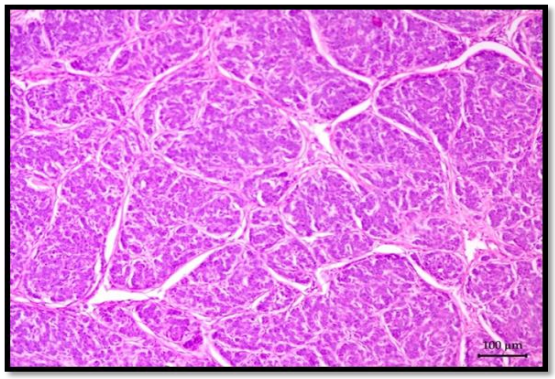

Fig. 2. Trichoblastoma: Double layers of long epithelial chords arranged in a palisaded ribbon like fashion with hyalinized connective tissue (H&E × 100).

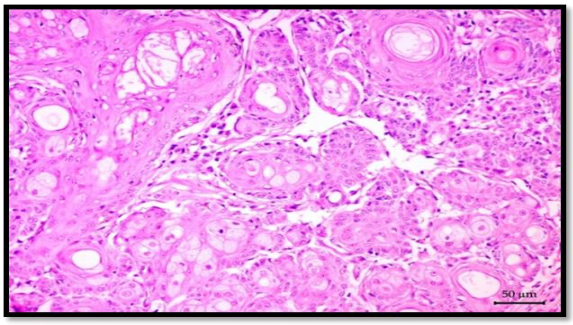

Fig. 3. Trichofolliculoma: Hair follicle cysts lined by squamous epithelium with keratin, hair fragments and presence of fibrous connective tissue infiltrated by mononuclear cells (H&E × 100)

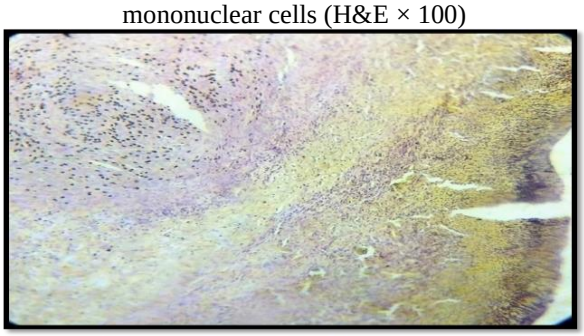

Fig. 4. Immature fibrous tissue stained yellow by Van Gieson (VG 100x)

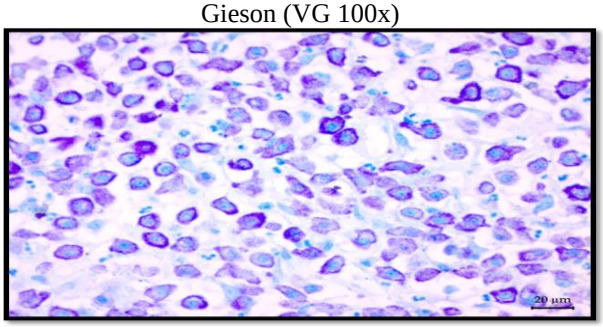

Fig. 5. Mast cell tumour showing round blue nuclei of cells and intracytoplasmic purple granules (Toluidine Blue × 400)

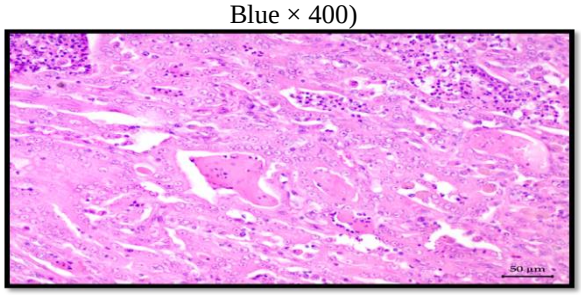

Fig. 6. Apocrine adenocarcinoma: Presence of multilayered tubules with pink coloured hyaline secretion and lymphatic invasion (H&E × 200).

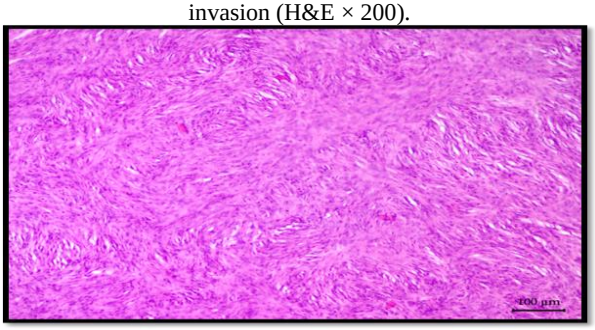

Fig. 7. Haemangiopericytoma: Interlacing, concentric bundles of proliferating pericytes surrounding capillaries (H&E × 100).

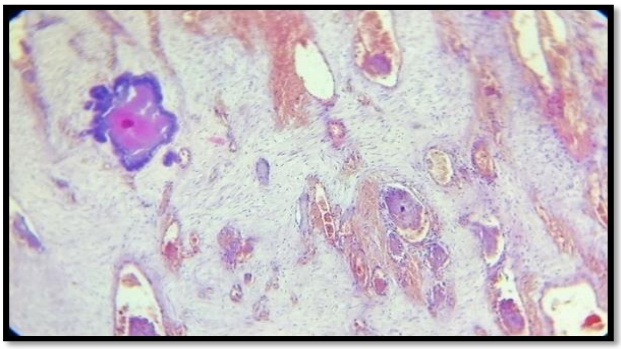

Fig. 8. Chondrosarcoma: Poorly differentiated chondroblasts with areas of haemorrhage and necrosis (H&E × 100).

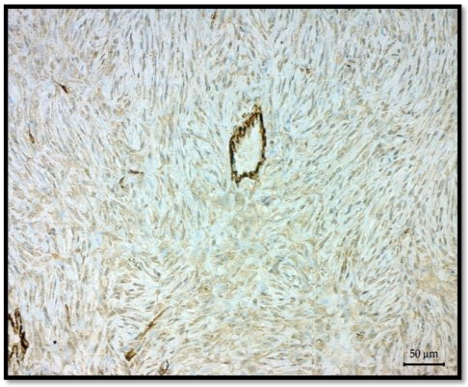

Fig. 9. Cutaneous haemangiopericytoma: Increased nuclear expression of SMA in vessel walls (DAB × 100).

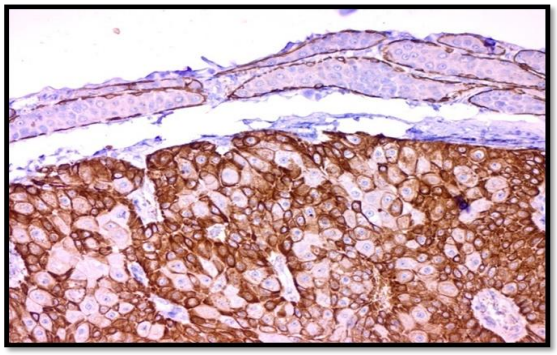

Fig. 10. Perianal gland adenoma: High expression of PCK in neoplastic cells (DAB ×100).

Table 1: Case wise details of Canine Skin Tumours (STs).

Sr. No.	Location	Morphometry
1.	Below anus	8.5cm×3cm,130gm
2.	Thoracic region	6cm×4cm,80gm
3.	Thigh	8.5cm×4cm,150gm
4.	Thigh	10cm×5cm,200gm
5.	Lumbar region	4cm×3cm,30gm
6.	Forehead	4.5cm×2.5cm,20gm
7.	Anus	7cm×4cm,130gm
8.	Interdigital space	7cm×5cm,100gm
9.	Hock joint	15cm×14cm,400gm
10.	Interdigital space	7cm×5cm,180gm
11.	Anus	8.5cm×4.5cm,150gm
12.	Lateral side of abdomen	6cm×3cm,100gm
13.	On the base of tail	7cm×4cm,120gm
14.	Anus	6cm×4cm,80gm
15.	Anus	4cm×2cm,50gm
16.	Neck	3cm×2cm,20gm
17.	Neck	2.5cm×2cm,15gm
18.	Elbow joint	17cm×15cm,650gm
19.	Lumbo-sacral area	9cm×6cm,250gm
20.	Leg	5cm×5cm,100gm
21.	Forehead	3cm×2cm,20gm
22.	Cheek	5cm×3cm,50gm
23.	Muzzle	8cm×5cm,100gm
24.	Forehead	4cm×2cm,30gm
25.	Near shoulder region	7cm×5cm,80gm
26.	Behind ear	2cm×2cm,15gm
27.	Base of tail	7cm×4cm,120gm
28.	Anus	6cm×3cm,80gm
29.	Lumbar area	2cm×2cm,15gm

Table 2: Immunohistochemistry markers used in different cutaneous tumours.

Sr. No.	Markers Used	Markers found positive	Confirmatory Diagnosis
1.	F8,SMA	F8	Cutaneous haemangioma
2.	SMA,F8,Desmin,S-100	SMA, Desmin	Fibrosarcoma
3.	SMA,F8,Desmin,S-100	SMA, Desmin	Fibrosarcoma
4.	SMA,F8,Desmin,S-100	SMA, Desmin	Fibrosarcoma
5.	PCK,p63,CK5/6	PCK	Perianal gland adenoma
6.	S-100,SMA,Desmin	S-100,SMA	Cutaneous haemangiopericytoma
7.	PCK,CK5/6	CK5/6	Perianal gland adenoma
8.	PCK,ER, HER2	PCK	Apocrine adenocarcinoma
9.	SMA,S-100,Desmin	SMA, Desmin	Fibrosarcoma
10.	SMA, Desmin	SMA, Desmin	Fibrosarcoma

CONCLUSIONS

It was deduced from the present investigations that advanced technique of immunohistochemistry alone, cannot aid in the proper grading, staging and characterization of tumours of the animal body. Although, it is comparatively easier to diagnose different benign tumours by cytology and histopathology but a combination of cytology, histopathology and immunohistochemistry are required for malignant tumours diagnosis. However molecular techniques are relatively very costly as compared to the conventional techniques of histopathology and cytology, hence its extensive use for veterinary purpose is still a challenge.

FUTURE SCOPE

There is a high potential in the present study as these techniques could be extended to other animal species also for cancer diagnosis.

Conflict of Interest. None.

REFERENCES

Al-Daraji, W., Husain, E., Zelger, B. G. and Zelger, B. A. (2009). Practical and Comprehensive Immunohistochemical Approach to the Diagnosis of Superficial Soft Tissue Tumors. *International Journal of Clinical and Experimental Pathology*, *2*, 119-131.

Bronden, L. B., Eriksen, T., Kristensen, A. T. (2010). Mast cell tumours and other skin neoplasia in Danish dogs-data from the Danish Veterinary Cancer Registry. *Acta Veterinary Scandinavica* (2010).

Bronden, L. B., Nielsen, S. S., Toft, N. and Kristensen, A. T. (2010). Data from the Danish veterinary cancer registry on the occurrence and distribution of neoplasms in dogs in Denmark. *Veterinary Record*, *166*, 586-90.

Camilla, M. and Giuseppe, L. (2019). Current Cancer Epidemiology. *National Centre for Biotechnology Information*, *9*(4), 217-222.

Devi, N. A., Hadi, M.A., Venkateshwarulu, Priya. V. and Babu, V. L. (2012). Design and Characterization of Floating Controlled Release Tablets of Imatinib Mesylate for site specific Drug delivery. *International Research Journal of Pharmacy*, *3*(9), 2230-2245.

Dobson, J. M., Samuel, S., Milstein, H., Rogers, K. and Wood, J. L. N. (2002). Canine neoplasia in U.K: Estimates of incidence rates from a population of insured dogs. *Journal of Small Animal Practice*, *43*, 240-2246.

Koji, A., Qingjiang, H., Yuta, K., Eiji, O. and Masaki, M. (2021). Recent developments in cancer research: expectations for a new remedy. *Annals of Gastroenterological Surgery*, *5*(4), 419-426.

Kok, M. K., Chambers, J. K., Tsuboi, M., Nishimura, R., Tsujimoto, H., Uchida, K. and Nakayama, H. (2019). Retrospective study of canine cutaneous tumours in Japan. 2008-2017. *Journal of Veterinary Medical Science*, *81*(8), 1133-1143.

Kumar, Abbas and Fausto (2004). Pathologic Basis of Disease. 7thEdn. Robbins and Cotran. Reed Elsevier India Private Limited, India.

Luna, L. G. (1968). Manual of Histologic Staining: Methods of the Armed Forces Institute of Pathology, 3rdEdn., Blakiston Division, McGraw-Hill, U.S, 1968; 258p.

Meuten, D. J. (2002). Tumors in domestic animals. John Wiley & Sons.

Namazi, F., Hasiri, A., Oryan, A. and Moshiri, A. (2013). Hemangiopericytoma in a young dog: Evaluation of histopathological and immunohistochemical features. *Veterinary Research Forum*, *5*, 157-160.

Pawan, K., Kumar, R. and Pawaiya, R. S. (2010). Puttaswamy MB. Diagnostic significance of mitotic index and AgNOR count in canine mammary tumours. *Brazilian Journal of Veterinary Pathology*, *3*(1), 41-45.

Pawan, K., Reddy, S., Kumar, R., Singh, N. D. (2009). Canine skin tumours: Occurrence and histopathology. *Indian Journal of Veterinary Pathologyf*, *33*(2), 200-203.

Reddy, G. B. M., Kumar, R., Kumar, P., Sharma, A. K. and Singh, N. D. (2009). Canine skin tumours: Occurrence and histopathology. *Indian Journal of Veterinary Pathology*, *33*(2), 200-203.

Shivani, G., Arvind, K. and Goswami, K. C. (2017). Histomorphological Spectrum of Hepatic Lesions in Liver Biopsies – A Prospective Hospital Based Study. *Journal of Medical Science and Clinical Research*, *6*(7), 322-325.

Zachary, J. F and McGavin, M. D. (2016). Pathologic Basis of Veterinary Disease. *Elsevier Science Health Science*. 6th edition, pp. 322.

Zahoor, A. W. (2016). Pathology of Spontaneous Canine Cutaneous/ Visceral Tumours and Evaluation of Some Cell Cycle Regulators. Thesis M.V.Sc. (Veterinary Pathology) Submitted to the Deemed University, Indian Veterinary Research Institute Izatnagar (U.P.), India; 2016.

Zhao, C., Vinh, T. N., McManus, K., Dabbs, D., Barner, R. and Vang, R. (2009). Identification of the most sensitive and robust immunohistochemical markers in different categories of ovarian sex cord-stromal tumors. *American Journal of Surgical Pathology*, *33*(3), 354-366.

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