HISTOLOGY AND IMMUNOHISTOCHEMISTRY OF CANINE MESENCHYMAL TUMOURS

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ABSTRACT

Cancer is the major cause of mortality in pet dogs and humans. Assessment of the cell kinetics is a field of interest in modern oncology and it indicates the prognosis. Out of 68 cases screened, histopathologically 19 were found to be mesenchymal tumours. Among 19 cases, fibroma 3 (15.78%), fibrosarcoma 2 (10.52%), fibromatous epulis 2 (10.52%), lipoma 2 (10.52%), osteosarcoma 2 (10.52%), hemangiopericytoma 2 (10.52%), chondrosarcoma 1 (5.26%), myxoma 1 (5.26%), leiomyoma 1 (5.26%), rhabdomyosarcoma 1 (5.26%), lymphangiosarcoma 1 (5.26%) and hemangioma 1 (5.26%). The mean number of AgNOR dots and PCNA counts in benign tumours was significantly ($P<0.05$) lower than malignant tumours and both indices together give good prognostic value rather than following a single.

KEY WORDS: Mesenchymal tumours, Fibroma, Chondroma, Osteosarcoma, Canine tumours

INTRODUCTION

Cancer is one of the major cause of mortality in pet dogs and humans. With increasing health awareness by pet owners there has been tremendous increase in the interest on cancer malignancies. The study of the parameters that reflect the cell cycle, phase of the neoplastic cell has been shown useful for the evaluation of biological behaviour of the tumours. This study is carried out to know the gross pathology, histopathology and to evaluate the proliferation markers like AgNOR count and PCNA indices as prognostic indicators.

MATERIALS AND METHODS

To study the pathology of canine neoplasms, 68 tumour samples were collected from the various surgical wards in and around Hyderabad. The collected samples were preserved in 10% neutral buffered formalin for the histopathology and immunohistochemistry. Routine tissue processing has been carried out. Sections of 5μ thickness were taken and stained with haematoxylin and eosin. Special staining techniques viz. argyrophilic nucleolar organizer regions (AgNOR) and proliferating cell nuclear antigen (PCNA) were performed to evaluate the prognosis of tumours.

The tissue sections were stained by modified silver colloid staining followed by Krishnamurthi *et al.* (1998). The AgNOR dots in 100 non overlapping nuclei were counted under oil immersion objective (1000x magnification) and mean number of AgNOR dots per nucleus (AgNOR index) was calculated for each specimen.

Immunohistochemistry of proliferating cell nuclear antigen was done as per procedure by Pawaiya *et al.,* (2006). Formalin fixed paraffin embedded sections of 5 μ thickness were taken on to poly-l-lysine coated slides. Endogenous peroxidase was blocked by placing the sections in 3% hydrogen peroxide for 10 min and rinsed with 0.01M phosphate buffer saline (PBS) at pH 7.4 and incubated with 5% normal goat serum (Sigma, G9023), then with PCNA monoclonal antibodies. The immunoreactivity for PCNA was done by counting the positive cells (brown colour nucleus) in 10 randomly selected high power fields (x200) and the mean values were calculated (Karademir *et al.,* 1998).

RESULTS AND DISCUSSION

Out of 68 cases screened, histopathologically 19 were found to be mesenchymal tumours. These included benign tumours like fibroma 3 (15.78%), fibromatous epulis 2 (10.52%), lipoma 2 (10.52%), hemangiopericytoma 2 (10.52%), myxoma 1 (5.26%), leiomyoma1 (5.26%) hemangioma 1 (5.26%) and malignant tumours like fibrosarcoma 2 (10.52%), osteosarcoma 2 (10.52%), chondrosarcoma 1 (5.26%), rhabdomyosarcoma 1 (5.26%) and lymphangiosarcoma1 (5.26%).

FIBROMA:

Three (15.78%), out of 19 cases were Fibromas. The affected dogs were 10 year old male Great Dane, 7 year old female non descriptive and 6 year old male German Shepherd. Fibromas were noticed at knee, abdomen and vagina. Grossly tumours were 0.2-1.5 cm in diameter, circumscribed, round to oval and white to cream coloured. Histopathologically, sections revealed the collagenous fibres which were repetitive and arranged in interwoven fascicles or whorls (Fig.1). Fibrocytes were uniform, fusiform shape with oval normochromic nuclei and indistinct cytoplasm that blends into the extracellular collagen stroma. Results were inaccordance with Gold Schmidt *et al.* (2002) and Krithiga *et al.* (2005).

Fig.1: Fibroma showing

FIBROSARCOMA:

Fibrosarcoma was observed in 2 (10.52%) case
year old male Doberman dog and right knee of
well circumscribed, 2 cm in diameter with white cut surface and firm in consistency. Microscopically, tumour consisted of spindle shaped cells arranged in interwoven pattern. Cytoplasm was scanty and nuclei were elongated to oval with inconspicuous nucleoli. Mitotic figures were seen. Also note AgNOR dots in nucleus (Fig.2) of tumour cells. Histological findings were in line with findings of Thangathurai *et al.* (2008).

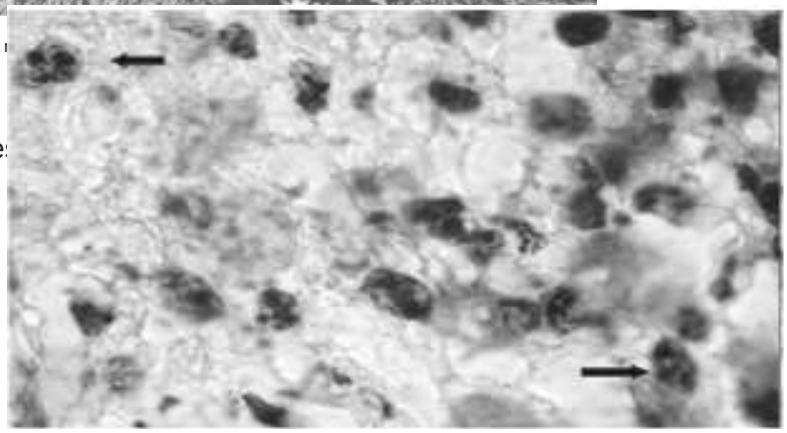

Fig.2 Fibrosarcoma showing diffusively scattered dark stained AgNOR dots. AgNOR x 1000

FIBROMATOUS EPULIS:

Two (10.52%) out of 19 cases were fibromatous epulis. Dogs affected were 4 year old male German shepherd and 7 year old female Pomeranian. Tumour masses were 1-2 cm in diameter, round to ovoid, pink and rubbery in consistency. Histopathology of tumour consisted of proliferation of stellate and spindle shaped cells with interlacing bundles of collagen. Cytoplasm was eosinophilic with elongated to oval, vesicular nucleus and single nucleolus.

Also note the PCNA positive immunolabelling (Fig.3). This was in accordance with Krithiga *et al.* (2005).

LIPOMA:

Two cases (10.52%) of lipoma were observed in 8 year old female Doberman and 10 year old male Pomeranian on ventral abdomen and flank region respectively. The tumours were soft, circumscribed, yellowish coloured with oily cut surface. Microscopically, tumour revealed lipoma cells which were identical to normal adipose tissue. Large clear vacuoles replace the cytoplasm with peripheralisation and compression of nuclei. The tumour at the flank region shows the infiltration of lymphocytes

along with above cell characters (Fig.4). These features were in accordance with the findings of Goldschmidt *et al.* (2002) and Krithiga *et al.* (2005).

CHONDROSARCOMA:

Chondrosarcoma was observed in one (5.26%), 9 year old, male Labrador dog at the distal end of femur. The tumour was 1 cm in diameter, nodular with glistening cut surface. Histologically, the chondrocytes had less anaplastic features. Hyalinization of much of the cartilage matrix gave the appearance of bone tissue giving the false impression of mixed tumour of bone and cartilage (Fig. 5). These features were in accordance with the findings of Goldschmidt *et al.* (2002) and Krithiga *et al.* (2005).

OSTEOSARCOMA:

Osteosarcoma was observed in 2 (10.52%) cases, one was 6 year old male German Shepherd, other was 9 year old female Pomeranian

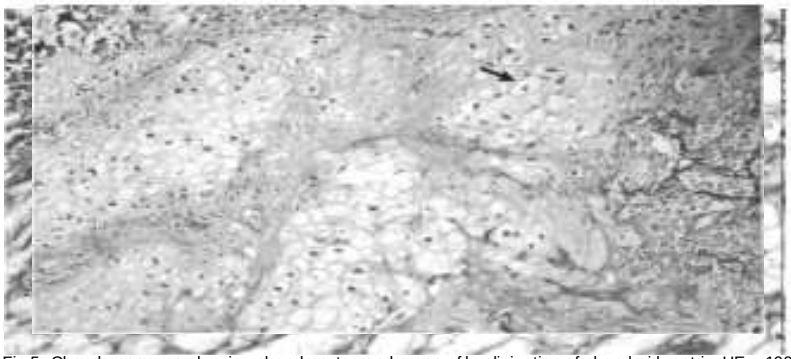

Fig.5: Chondrosarcoma showing chondrocytes and areas of hyalinization of chondroid matrix. HE x 100

Fig.4: Lipoma showing cells with vacuolated cytoplasm and eccentric flat nuclei and areas of infiltration. HE x 100

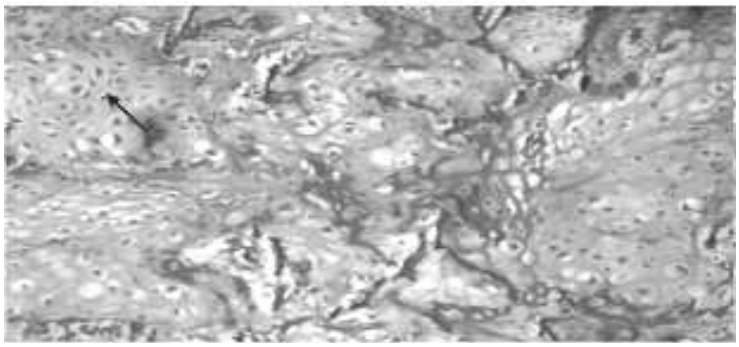

Fig.6: Osteosarcoma showing neoplastic round and spindle shaped cells with small osteoid areas. HE x 100

at the distal metaphysis of radius. Tumours were hard, 0.5 cm in diameter, round to spherical in shape. Histologically, osteoblasts appeared as pleiomorphic, spindle shaped cells resembling fibroblasts with basophilic cytoplasm and eccentric hyperchromatic nuclei. Areas of osteoid formation were also seen (Fig. 6). These findings were similar to Malie *et al.* (2007).

MYXOMA:

One (5.26%) case of myxoma was observed at the radio carpal joint of the 8 year old male Doberman dog. The tumour was soft, spherical, white coloured with lobulations. Microscopically, tumour revealed the stellate to spindle shaped cells loosely arranged in a myxoid matrix. Matrix was blue coloured, with low cellularity and nuclei were small hyperchromatic with basophilic cytoplasm. Also note the PCNA positive cells (fig. 7). It was correlated with the records of Gold Schmidt *et al.* (2002) and Machida *et al.* (2003).

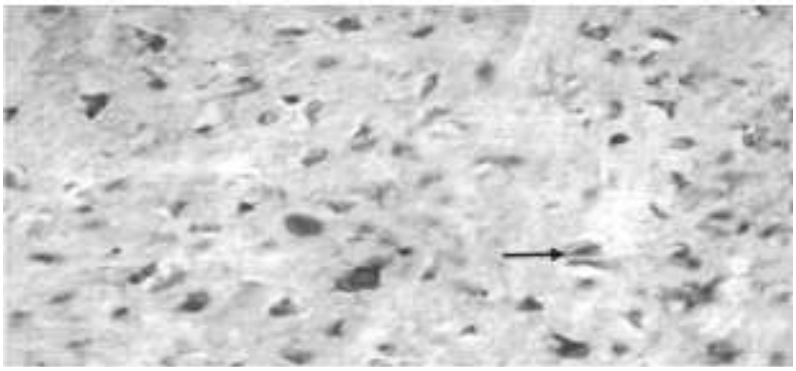

Fig 7.Myxoma showing stellate cells with nuclear PCNA immunolabelling. IP- AEC- MH x 400

RHABDOMYOSARCOMA:

Rhabdomyosarcoma was 1 (5.26%) out of 19 cases in a 11 year old male Rottweiler located at the axillary region. The tumour was oval to spherical shaped with firm consistency. Histologically, tumour revealed large, deeply eosinophilic round to polygonal cells intermixed with smaller cells with less cytoplasm, conspicuous and hyperchromatic nuclei. Results were in accordance with Ji-Young Yhee *et al*. (2008).

LEIOMYOMA:

Leiomyoma was 1 (5.26%) out of 19 cases in 12 year old female Collie located at the vagina. The tumour was 2 cm in diameter, irregular in shape, firm in consistency and cream coloured. Histologically, tumour revealed interlacing bundles of respectable strap like smooth muscle fibers which intersected at right angles. Stroma was minimal. Cells had cigar shaped nucleus with blunt ends (Fig.8). The findings were similar to Cooper *et al*. (2002).

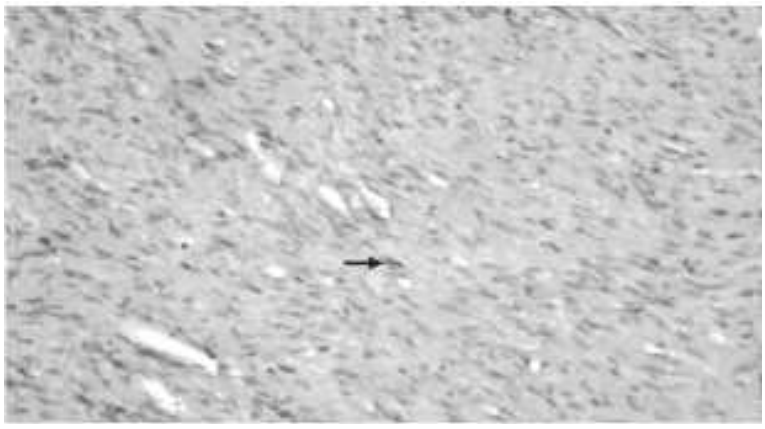

Fig.8: Leiomyoma showing interlacing bundles of smooth muscle fibres having elongated nuclei with blunt ends. HE x 100

HEMANGIOMA:

Hemangioma was observed in one (5.26%) case of dog, on the left fore leg at shoulder region of 6 year old male Doberman dog. The tumour was red coloured with clear demarcation and encapsulated. The cut surface revealed lobulations. Microscopically, tumour composed of vascular spaces of various sizes filled with erythrocytes and were lined by single layer of uniform endothelial cells. These findings are similar to the Singh *et al.* (2004).

LYMPHANGIOSARCOMA:

One case (5.26%) of lymphangiosarcoma was observed in 6 year old male Pomeranian at the base of ear, about 3 cm in diameter with soft consistency and had clear serous fluid. Histologically, neoplastic cells resembled normal epithelial cells, however cell grow directly on bundles of dermal collagen dissecting them and forming numerous clefts and channels. The majority of clefts were devoid of cells but occasionally erythrocytes were seen. The cells lining the clefts and channels had more rounded nuclei with hyperchromatism. The recordings were in agreement with Goldschmidt *et al*. (2002) and William *et al*. (2005).

HEMANGIOPERICYTOMA :

Out of 19, two (10.52%) were hemangiopericytoma, located at the knee joint of forelimbs in 7 year old male nondescriptive dog and 5 year old female German Shepherd. Tumour was round to oval with shiny mucoid material. Histologically, tumour consisted of loosely arranged interlacing streams and storiform bundles of spindle cells that often formed distinct whorls around the capillaries. Also note the PCNA positive cells (Fig.10). Results were in accordan

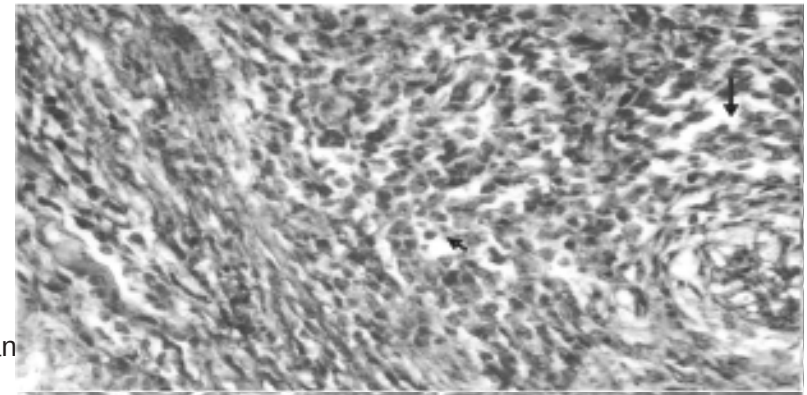

Fig.10. Hemangiopericytoma showing positively stained PCNA nuclei. IP- AEC- MH x 200

Fig.9: Hemangioma showing capsule of vascular spaces containing red blood cells lined by inconspicuous endothelial cells. HE x 100

AgNOR and PCNA counts

The number of AgNORs had been associated wit
from 3.29±0.43 to 7.25±1.30 in individual tumours. The highest AgNOR count was observed in rhabdomyosarcoma while lowest was observed in hemangiopericytoma. The AgNOR dots were large round and less in numbers in benign tumours compared to malignant ones (Giraldo *et al.,* 2003). The mean number of AgNOR in benign tumours was significantly ($P<0.05$) lower than the malignant tumours (Vajdovich *et al., 2004*). In canine tumours higher number of mitosis is known to be the histological criteria which could be evaluated with higher number of AgNOR's and it can be used as proliferation marker and further to know the cell kinetics and prognosis of tumours.

Proliferating cell nuclear antigen (PCNA 36 KDa), also known as cyclin, is an auxiliary protein of DNA polymearase δ that is essential for DNA replication during S- phase. The protein is present in nucleoplasm of continually cycling cells throughout the cell cycle. PCNA begins to accumulate during S phase and declines during the G2/M phase. The predominant distribution of PCNA appears to change with the stage of cell cycle. In early S phase, PCNA has a variety granular distribution

and is absent from the nucleoli, while at the late S phase, prominent presence in the nucleoli is evident. In normal tissues, PCNA positive cells are limited to proliferative compartment. In tumours, the proportion of PCNA- positive cells exceeds that expected for the proportion of proliferating cells. It has been postulated that increased expression of PCNA in tumours is due to growth factors that regulate the production of this protein. The mean PCNA positive nuclei in tumour tissues varied from 8.23±1.25 to 192.64 ±2.66. The highest PCNA index was observed in fibrosarcoma while lowest was observed in myxoma. In the present study malignant tumours shows significantly (P<0.05) higher PCNA index than benign tumours. These findings were consistent with the results of previous studies of canine tumours (Manjunath Reddy *et al.,* 2007).

Table.1 AgNOR counts and PCNA index in different tumours:

S.No.	TYPE OF TUMOUR	No of tumours	AgNOR at 10 (Mean
1	Fibroma	3	3.87 ±
2	Fibrosarcoma	2	7.02±
3	Fibromatous epulis	2	4.65 ±
4	Chondrosarcoma	1	3.31±
5	Osteosarcoma	2	4.98 ±
6	Myxoma	1	3.71 ±
7	Rhabdomyosarcoma	1	7.25±
8	Leiomyoma	1	3.53±
9	Hemangiopericytoma	2	3.29 ±
10	Lipoma	2	3.33±
11	Hemangioma	1	
12	Lymphangiosarcoma	1	6.24±

The mean number of AgNOR dots and PCNA counts in benign tumours was significantly (P<0.05) lower than malignant tumours. The mean AgNOR dots per nucleus and PCNA counts are given in the table.1. The AgNOR and PCNA counts may be the reliable marker for judging the malignacy of canine tumours.

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