

Pathology and Immunohistochemistry of Mammary Tumours in Dogs

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Received: 8.05.2020 | Revised: 14.06.2020 | Accepted: 23.06.2020

ABSTRACT

Mammary tumours were most frequent tumours occurring in dogs. The study was carried to know the incidence and pathology of mammary tumours in dogs. A total of 25 tumour samples collected and analysed histopathologically, they were found to be mammary gland tumours which were further classified as simple adenoma -2, benign mixed mammary tumours-3, tubular adenocarcinoma -3, papillary adenocarcinoma 5, cystic papillary adenocarcinoma 4, solid carcinoma 2, anaplastic carcinoma 2, and malignant mixed mammary tumours 4. The highest incidence was noted in an age group of 7-12 years. Tumours were located mostly in the abdominal and inguinal mammary glands and in two cases thoracic mammary glands were involved. Most of tumours were malignant (20). Pure breed dogs were mostly affected. The highest Agyrophilic nucleolar organizer regions (AgNOR) count and (Proliferating cell nuclear antigen) PCNA index were observed in anaplastic carcinoma while lowest was observed in simple adenoma. A positive correlation was noticed between the PCNA expression and AgNOR counts and these proliferation markers were useful for predicting the outcome of the canine mammary tumours.

Keywords: AgNORs, Dogs, Mammary tumour, Immunohistochemistry, PCNA.

INTRODUCTION

Cancer is the major cause of morbidity and mortality in pet dogs and humans. Approximately 25% of humans and 30% of dogs develop cancer during their life time (Ettinger and Feldman, 1995). Mammary tumours account for approximately 50% of all tumours in female dogs and are the second most frequently encountered spontaneous neoplasms followed by those derived from

skin (Moulton et al., 1970). The incidence of mammary tumours is higher in dogs than in any other domesticated animal and is three times the incidence in humans (Hoskins, 2008).

Determining the prognosis of canine patient with a malignant mammary tumour is very important for the clinician, but it is often difficult because the biological behaviour of these tumours differ widely.

Cite this article: Thippani, C., Kumar, A., & Laxman, M. (2020). Pathology and Immunohistochemistry of Mammary Tumours in Dogs, *Ind. J. Pure App. Biosci.* 8(4), 46-53. doi: <http://dx.doi.org/10.18782/2582-2845.8085>

The major challenge is to find those prognostic variables that allow the prediction of disease behaviour in the individual case (Sarli et al., 2002).

The work is carried to know the incidence, gross & histopathology and to study the tumour prognostic factors such as proliferation markers like Agyrophilic nucleolar organizer regions (AgNORs) and Proliferating cell nuclear antigen (PCNA).

MATERIALS AND METHODS

About 25 tumour samples were collected from dogs that were coming to veterinary hospitals in and around Hyderabad after surgical incision. Samples were preserved in 10% neutral buffered formalin for the histopathology and immunohistochemistry. Formalin fixed paraffin embedded sections of 4 μ thickness were stained with haematoxylin and eosin, AgNOR staining was carried by following Krishnamurthi et al.,(1998) method and AgNOR dots in 100 consecutive nuclei were counted and mean number per nucleus was calculated. Same sections were taken on to poly-L-lysine coated slides. Sections were deparaffinised, rehydrated and antigen retrieval was done by boiling in 6M urea solution for 10 min. 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. After placing in PBS wash bath for 2 min, sections were incubated with 5% normal goat serum (Sigma, G9023) in PBS for 20 min. then rinse in PBS for 5 min and were incubated with PCNA monoclonal antibodies (1:3000 dilution in PBS with 1% BSA) at 4⁰C over night in a humidified chamber. After rinsing in the PBS, sections were incubated with biotinylated goat antimouse immunoglobulin IgG (1:20 dilution in PBS) for 30min and washed twice with PBS. Apply 1:20 dilution extra avidin peroxidase (Sigma Extra-2) to the section and incubate for 30min. 3-Amino-9-ethylcarbazol (AEC) staining kit (Sigma AEC-101) was used for the visualisation which gave brick red reaction product. Sections were counterstained for 3 min with Mayer's Haematoxylin (Sigma MHS-16), rinsed for 5min in running tap water and mounted with aqueous glycerol gelatine (Sigma GG-1).The immunoreactivity for PCNA was done by counting the positive cells in 10 randomly selected high power fields and the mean values were calculated (Karademir et al., 1998).

RESULTS AND DISCUSSION

A total of 25 mammary tumours were collected. The tumours were histologically classified under light microscopy according to WHO classification (Goldschmidt et al., 2011) shown in table 1.

Table 1: Incidence of tumours and their histological classification

S.no	Histological classification	Number found	% percentage
1	Benign neoplasms	5	20%
	Simple adenoma	2	8%
	Benign mixed tumours	3	12%
2	Malignant epithelial neoplasms-carcinoma	16	64%
	Tubular adenocarcinoma	3	12%
	Tubulo Papillary adenocarcinoma	5	20%
	cystic Papillary adenocarcinoma	4	16%
	Solid carcinoma	2	8%
	Anaplastic carcinoma	2	8%
3	Carcinoma -Malignant mixed mammary tumours	4	16%

Mammary tumours were more common in females, Most of tumours were malignant. Similar observations have been reported by previous workers also (Reddy et al., 2009; Chavan et al., 2016 Raval et al. 2018).

The age wise incidence of tumours was as follows: 4-6 years were 8, 7-9 years was 8 and above 9 years was 9. Age is the prominent factor which determines the susceptibility to cancer as it provides the required time for accumulation of cancer events allowing for recognition of transformation. Highest incidence of mammary tumours found in the age group of 7-11 years, which is recorded by others also (Maria, 2003 and Nair et al., 2007).

The affected breeds were German Shepherd (5), Pomeranian (4), Labrador (3), Dachshund (3) and non descriptive dogs (10). Growths were located mostly in the abdominal and inguinal mammary glands and in two cases thoracic mammary glands were involved. In previous studies the caudal mammary glands were mostly affected compared to cranial glands, pure breed dogs were mostly affected and small breeds were mostly affected than large breeds (Krithiga et al., 2005; Itah et al., 2005; Stratmann et al., 2006 and Airas et al., 2007)

Pathology

Simple Adenoma:

Out of 25 mammary tumours two were simple adenoma, one was located at abdominal mammary gland of 7 year old German shepherd (Fig.1). Microscopically, tumour revealed cuboidal to columnar shaped well differentiated luminal epithelial cells.

Benign mixed tumour:

Grossly the tumours were well circumscribed, firm and cream to white coloured. The tumours consisted of benign cells resembling luminal epithelium and myoepithelium mixed with mesenchymal cells; they had produced fibrous tissue in combination with bone, cartilage or fat.

Carcinoma:

Grossly tumours were usually lobulated. The tumour consisted of both epithelial and myoepithelial components. The amount of

stroma varied considerably. In most of tumours necrotic areas were observed.

Tubular adenocarcinoma:

Histopathologically, tumour revealed predominant tubular arrangement of neoplastic cells (Fig.2). The tubules were lined by single to multilayered cuboidal to short columnar cells with eosinophilic cytoplasm. AgNOR stained mitotic figures can be observed (Fig.3) Anaplasia was noticed in most of cells.

Tubulo Papillary adenocarcinoma:

The tumours were characterized by formation of tubules with papillary projections (Fig.4). The stromal component also showed proliferation. Tubules were lined with cuboidal to columnar epithelium and some polyhedral neoplastic cells. The cytoplasm was eosinophilic, with hyperchromatic prominent nucleus.

Cystic Papillary adenocarcinoma:

Tumour revealed cystic spaces of varying sizes, which were lined by flattened to cuboidal epithelium. Papillae were projected into the cystic spaces (Fig.5). Cells with eosinophilic cytoplasm with hyperchromatic nuclei were observed.

Solid carcinoma:

In this, tumour cells were arranged in solid nests (Fig.6). The small amount of stroma was observed in the tumour.

Anaplastic carcinoma:

Histologically, anaplastic carcinoma showed diffuse infiltration of large, pleiomorphic cells with bizarre nuclei that were rich in chromatin. Neoplastic cells were arranged in cords and clusters (Fig.7) with moderate amount of stroma. Cells had eosinophilic cytoplasm. Areas of necrosis and haemorrhage were also observed. Also note the PCNA positive nuclei in tumour cells (Fig.8).

Malignant mixed tumours:

Grossly tumour was well circumscribed, hard and greyish white in cut surface. Microscopically, tumour revealed malignant epithelial cells and cells of connective tissue component.

Both gross and histomorphology of present lesions were compared well with those who reported by earlier workers (Misdrop, 2002 ; Krithiga et al., 2005; Reddy et al., 2009; Chavan et al., 2016 Raval et al. 2018 and patel et al.,2019).

Proliferation markers: AgNOR count and PCNA index

The AgNOR counts and PCNA index in different tumours were given in table 2. The mean AgNOR counts in individual tumours varied from 2.81 ± 1.2 to 10.58 ± 2.67 . The highest AgNOR count was observed in anaplastic carcinoma while lowest was observed in simple adenoma. The AgNOR dots were large round and less in numbers in benign tumours compared to malignant ones. Invasive tumours had high AgNOR count than non invasive tumours. Benign mixed tumours had smaller AgNOR area than malignant mixed tumours. The AgNOR dots were large round and less in numbers in benign tumours compared to malignant ones.

Jelesijevic et al., 2003 reported the mean number of AgNOR in benign tumours was significantly lower than the malignant tumours. Giraldo et al., 2003 noticed AgNOR dots were large, round, less in number in benign tumours compared to malignant tumours. Invasive tumours had high AgNOR count than non invasive tumours (Bostock et al., 1992). Benign mixed tumours had smaller AgNOR area than malignant mixed tumours (Destexhe et al., 1995). In canine tumours higher number of mitosis is known to be the histological criteria which could be evaluated with higher number of AgNOR's. The nucleolar organizer regions being the DNA loops were transcribed to ribosomal RNA, vital need for synthesis of proteins, thus their smaller size and high numbers are correlated with the aggressive proliferative activity of the cell, while large size, low number indicate minimum proliferative activity of cell (Egan and Crocker 1992).

The PCNA index in individual tumours varied from 14.23 ± 1.22 to 336.95 ± 6.98 . The highest PCNA index was observed in anaplastic carcinoma while lowest was observed in simple adenoma. PCNA positive cells were high in malignant mammary tumours compared to benign tumours. Anaplastic and solid mammary adenocarcinomas showed highest PCNA counts than cystic papillary and tubular adenocarcinomas suggesting that neoplastic cells with highest proliferating activity tend to form the solid pattern with high cellular densities (Funakoshi et al., 2000).

Proliferating cell nuclear antigen (PCNA, 36 KDa), also known as cyclin, is an auxiliary protein of DNA polymerase 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 the G1 phase of the cell cycle, is most abundant during S phase and declines during the G2/M phase (Braco et al., 1987 and Morris and Mathews, 1989). The predominant distribution of PCNA appears to change with the stage of the cell cycle. 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 up regulate the production of this protein (Preziosi et al., 1995). Therefore immunohistochemical labelling of PCNA may be a reliable marker for judging the malignancy of canine tumours (Preziosi et al., 1995).

In canine tumours highest number of mitosis is known to be the histological criteria which could be evaluated with higher no of AgNOR counts and PCNA index and there exist a significant positive correlation between the two indices in our study.

Table 2: AgNOR counts and PCNA index in different tumours

S.No.	Type of tumour	AgNOR counts 1000x (Mean±S.E)	PCNA index 200x (Mean±S.E)
1	Adenoma	2.81±1.2	14.23±1.22
2	Papillary adenocarcinoma	5.34±0.39	82.34±1.87
3	Papillary cystic adenocarcinoma	6.21±0.41	93.15±7.89
4	Tubular adenocarcinoma	6.47±0.83	77.42±5.74
5	Solid carcinoma	7.68±0.04	221.38±4.78
6	Anaplastic carcinoma	10.58±2.67	336.95±6.98
7	Benign mixed mammary tumours	3.01±0.60	32.40±1.54
8	Malignant mixed mammary tumours	7.65±0.21	94.56±4.98

Note: AgNOR counts and PCNA indices were positively correlated (P<0.05).

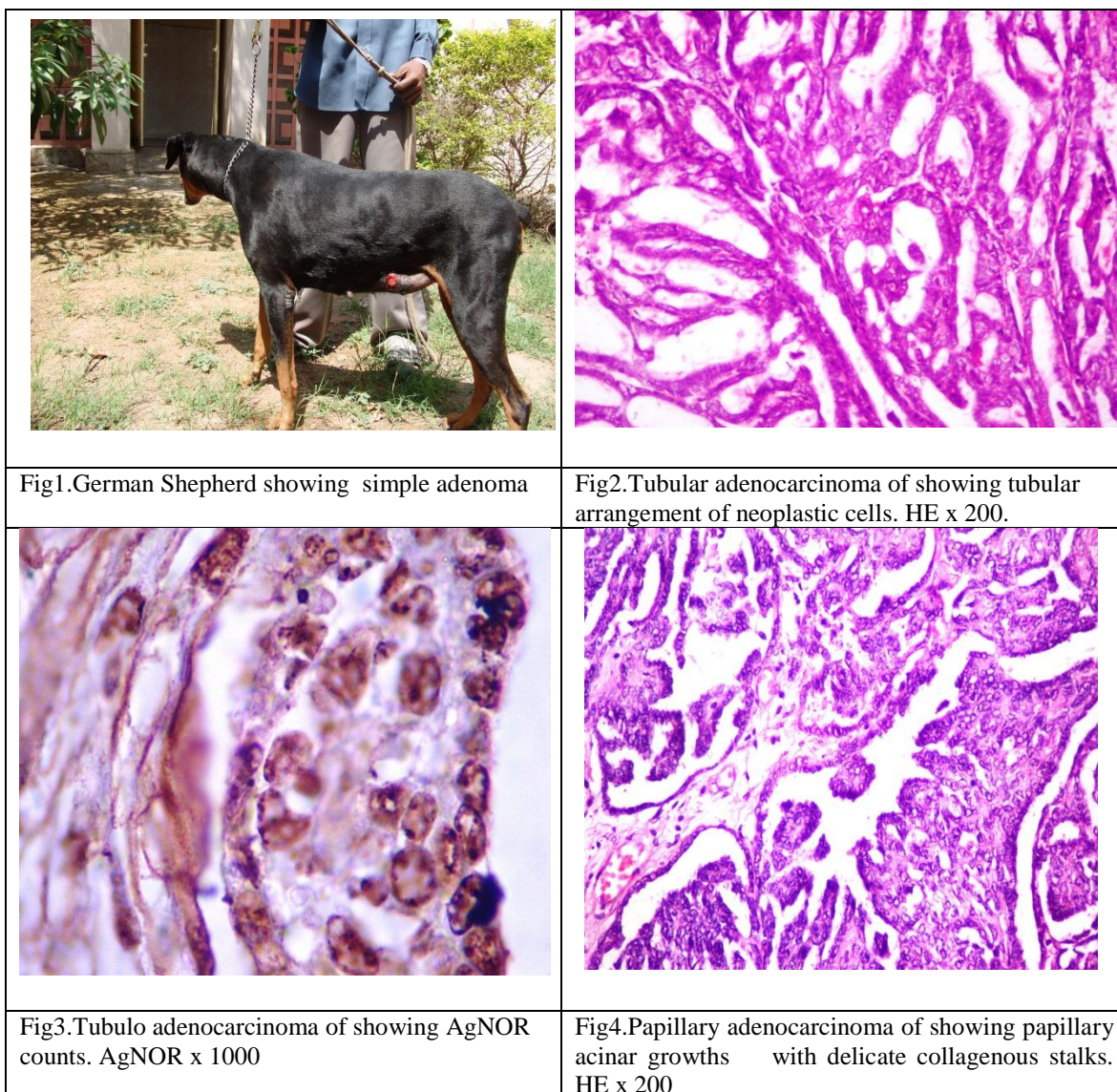


Fig1.German Shepherd showing simple adenoma

Fig2.Tubular adenocarcinoma of showing tubular arrangement of neoplastic cells. HE x 200.

Fig3.Tubulo adenocarcinoma of showing AgNOR counts. AgNOR x 1000

Fig4.Papillary adenocarcinoma of showing papillary acinar growths with delicate collagenous stalks. HE x 200

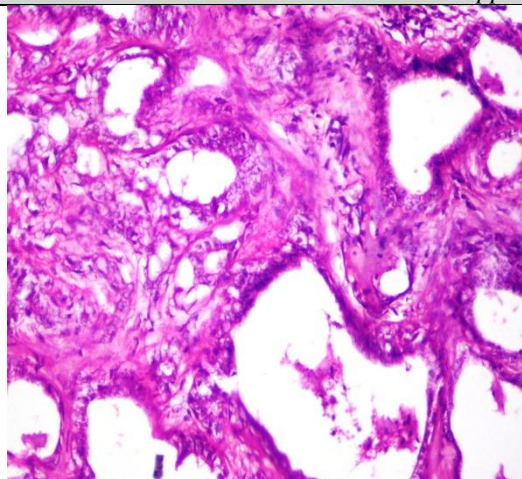


Fig 5. Cystic papillary adenocarcinoma of showing papillary outgrowth of cells in to cystic spaces. HE x 200

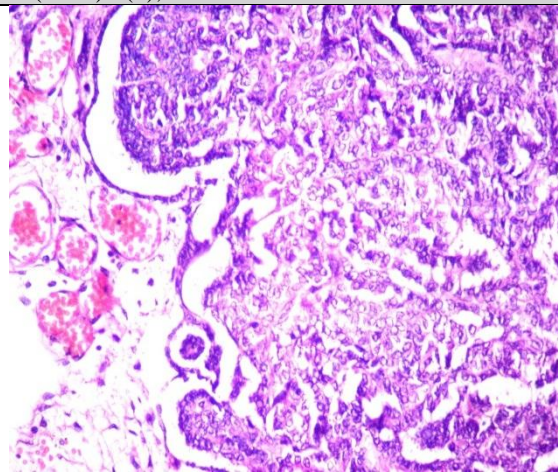


Fig6. Solid carcinoma of mammary gland showing arrangement of neoplastic cells. HE x 200

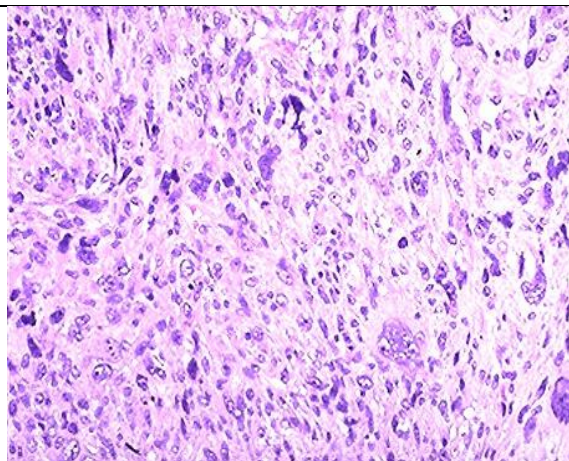


Fig7. Anaplastic carcinoma mammary gland showing pleomorphic neoplastic cells. HE x 200

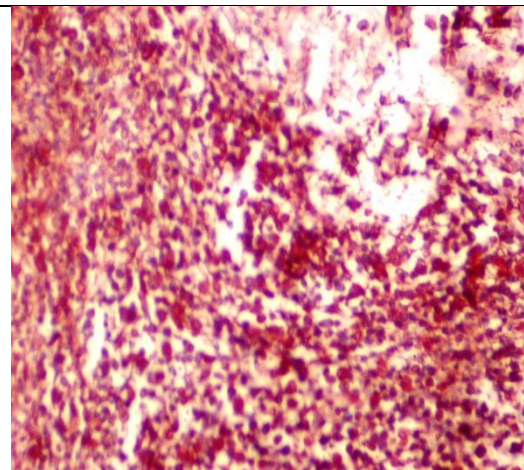


Fig8. Anaplastic carcinoma mammary gland showing positively stained PCNA nuclei. IP- AEC-MH x 200

CONCLUSION

In the present study 25 mammary tumours were collected. Malignant tumours were more than benign tumours. Old dogs are mostly affected. Tumours were seen in caudal mammary glands. The proliferation markers PCNA index and AgNOR counts were more in malignant tumours and the two indices were positively correlated. As the dogs and human display many similar characters, a discovery that could lead to better understanding of cancer progression and prevention could be carried out which might help in diagnosis of cancer at an early stage so that it can be promptly handled and treated efficiently in both humans and dogs.

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