Full Length Research Articles

HISTOPATHOLOGICAL STUDIES ON CANINE MAMMARY TUMORS

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ABSTRACT

Tumors affecting mammary gland are the most common neoplastic condition encountered in dogs. Frequency of occurrence and histopathological features of canine mammary tumors were investigated.A total of 54 tumor samples were collected from 51 clinical cases.All the samples were subjected to routine histopathological evaluation and classified according to WHO nomenclature.Among 54 samples, 12.96% were diagnosed as benign tumors that include cystadenoma, myxoma and mixed mammary tumors. Malignant tumors constitute 87.04% of the samples that comprised of regular adenocarcinomas (42.55%), special carcinomas (27.66%), sarcomas (8.52%), carcinosarcomas(17.01%) and carcinomas arising in mixed tumors (4.26%). Regular carcinomas include tubular, papillary, tubulo-papillaryand ductal carcinomas. Special types were micropapillary adenocarcinoma, solid carcinoma, carcinomas with squamous differentiation and anaplastic carcinoma. Sarcomas include fibrosarcoma, osteosarcoma, combined sarcoma and hemagiosarcoma.The wide versatility of canine mammary neoplasms with respect to their histological features and higher incidence of malignant variety of tumors is very clearly evident in this study.

Keywords: Benign, Canines, Histopathology, Malignant, Mammary tumors

INTRODUCTION

Cancer prevalence has increased tremendously among companion animals, in recent years.This trend could be due to longer survival of pet animals because of the advances in veterinary medicine. Most common malignancies encountered in dogs are mammary gland tumors, skin tumors, osteosarcomas and haemopoietic tumors (Todorova, 2006).

Mammary tumors constitute overall second most frequent neoplasia in dogs, surpassed only by skin tumors. They rank first among bitches especially in sexually intact ones with around 52% occurrence (Brodey*et al.,* 1983). Approximately, one third to half of them is malignant(Misdorp, 2002). Thus, they represent a serious problem and reflected in escalating number of studies in this research area (Gama *et al.,* 2008). Apart from this, because of their epidemiological, morphological,

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clinicopathological and prognostic features, spontaneous canine mammary tumors represent a natural animal model for studying the human breast cancers(Sorenmo, 2003).

Canine mammary tumors can be diagnosed relatively easily, from detailed case history and thorough clinical examination of the animal. Cytological examination of aspirated cells from the suspected growth is the preliminary tool towards classifying neoplastic and non-neoplastic lesions. However, excisional biopsy followed by histopathological examination of suspected tissue mass remains to be a gold standard for accurate diagnosis.

In this back ground, present study was undertaken with the objectives of determining the frequency of occurrence of various canine mammary tumors and their histopathological and histochemical features.

MATERIALS & METHODS

A total of 54 tissue samples, clinically suspected as canine mammary tumors were collected from 51 dogs presented at various Veterinary Hospitals and private clinics in Andhra Pradesh, Pondicherry and Kerala states of Southern India. Representative tissue samples were fixed in 10% neutral buffered formalin (NBF)and then subjected to routine paraffin embedding followed by microtomy sectioning (4-5 µm) and hematoxylin and eosin staining as described by Luna (1968). Stained tissue sections were examined microscopically and classified according to the diagnostic criteria proposed by World Health Organisation (Misdorp, 2002). Wherever necessary, special staining techniques like Van Geison's for collagen fibers, Gordon & Sweet's for reticulinfibersand AFIP procedure for lipofuchsin(Luna, 1968) were employed, to ascertain the actual identity of tissue components.

RESULTS& DISCUSSION

Out of 54 samples, histopathologically forty seven (87.04%) were of found to be malignant type, while seven (12.96%) were benign. Such a higher occurrence of malignant tumors is in agreement with the earlier reports (Millanta*et al.,* 2002). The histological types of mammary tumors and their occurrence are given below inTable 1.

I. Benign mammary tumors

(i) Cystadenoma:This is characterised by the acinar proliferation of glandular epithelium along with cystic dilatation of alveoli and ducts. Retention of glandular secretions and flattened lining epithelium were evidentin cystic acini. Distortion of glandular architecture by the proliferating fibrous tissue was observed. Thus, these tumors were classified as intra-canalicular as against to peri-canalicular type where fibrous tissue would just surround the alveoli without distorting their architecture (Cassali*et al.,* 2011).

(ii) Myxoma:It is characterised by the abundant proliferation of myxomatous tissue that was supported by fibrous connective tissue (Fig. 1). Mild proliferation of myoepithelial cell population was also evident at certain areas. Pigment laden macrophages were seen widely distributed within the stroma, ductal walls and in the alveoli. These pigments were confirmed as lipofuchsin, upon special staining by AFIP (Armed Forces Institute of Pathology)method (Fig. 2).

(iii) Benign mixed mammary tumor: Mixed tumors are the most common type of benign mammary cancers in dogs (Cassali*et al.,* 2011). Histologically, extensive proliferation of well differentiated connective tissue was observed with areas of cartilagenous metaplasia and ossification. Glandular component was, however, minimal and showed mild proliferation of the epithelium. Although the histogenesis of mesenchymal elements in these tumors is not clear, the current

consensus however is to assume that all elements originate from myoepithelial or ductal reserve cells, through the expression of Bone Morphogenic Protein – 6 (BMP-6) (Moulton, 1970, Akiyoshi*et al.,* 2004).

II. Malignant mammary tumors

Fourteen different types of malignant mammary tumors were observed that include regular adenocarcinomas (42.55%), special carcinomas (27.66%), sarcomas (8.52%), carcinosarcomas (17.01%) and carcinomas arising in mixed tumors (4.26%). Higher occurrence of carcinomas (both regular and special types)is in accordance with the earlier reports that malignant mammary tumors of epithelial origin are the most common in dogs (Moulton, 1970).

A) Adenocarcinomas: Four different types were observed.

(i) Tubular adenocarcinoma:Histologically, proliferation of glandular epithelium in distinct tubular pattern was the predominant feature. Lining epithelium was either columnar or cuboidal. Sometimes, ducts were dilated and cystic with corpora amylaceae. Myoepithelial cell proliferation was also evident.Generally, there exists a strong tendency of stromal infiltration by neoplastic cells in these tumors(Cassali*et al.,* 2011) that was evident even in the present study.

(ii) Papillary adenocarcinoma:These tumors were characterised by papillary proliferation of glandular epithelium.Epithelial lining is either of single layered or of multiple layers. Some areas revealed proliferation of myoepithelial cells within the connective tissue core of papillary growths.

(iii) Tubulopapillary adenocarcinoma: Proliferation of glandular and ductal epithelium forming both papillary and tubular patterns was observed. Lining epithelium was tall and columnar in papillary growths while short and cuboidal in tubules. Extensive area of necrosis was seen with macrophage and polymorphs infiltration.

(iv) Ductal carcinoma:Extensive proliferation of ductal epithelium forming mild to extensive papillary projections into the lumen was noticed. Sheets of dirty brown pigment laden macrophages and lymphoid cells were seen with in the stroma, especially adjacent to the affected ducts (Fig. 3).Although different varieties like cribriform, papillary, micropapillary, solid and comedo patterns were described earlier with respect to ductal carcinomas (Misdorp, 2002), it was the papillary pattern that was observed in the present study.

B) Special type of carcinomas: Four different types of special carcinomas were recorded that were characterised by the histological features other than regular tubular and papillary patterns.

(i)Micropapillary adenocarcinoma: Micropapillary carcinomas are actually a rare type of canine mammary neoplasms. However, they have been well described in human breast cancers and found to be associated with an unfavorable prognosis (Kim *et al.,* 2005). Histologically, they were characterised by bunch of neoplastic cells within the alveolar lumen surrounded by a clear lacunar space (Fig. 4). This was in agreement with the earlier reports (Cassali*et al.,* 2011). Each alveolus was lined by single layer of spindle shaped myoepithelial cells. Desmoplasia was very minimal. In dogs also, these tumors were found to exhibit similar type of biological behaviour as in humans with metastatic nature and grave prognosis (Gama *et al.,* 2008).

(ii) Solid carcinoma:Proliferation of glandular epithelium completely filling the alveolar lumen giving solid appearance was the predominant feature (Fig. 5). Each alveolus was lined by a single layer of myoepithelial cells.Desmoplasia was minimal. Squamous metaplasia was the regular

finding, either focally or in between the solid cords or within the center of each solid alveolus. Extensive areas of necrosis and subsequent infiltration with macrophages and polymorphs were common findings.Solid carcinomas were described as the most common type of malignant mammary cancers in dogs and hypothesized as probably the more advanced as other types (Cassali*et al.,* 2011).

(iii) Carcinoma with squamous differentiation:Mammary squamous cell carcinomas are uncommon in dogs, however, they are found more frequently in dog than in man (Chandrashekaraiah *et al.,* 2011). Histologically, two different patterns were observed: squamous cell carcinoma with exclusive squamous differentiation of the tumor and adeno-squamous cell carcinoma characterised by predominant squamous differentiation along with significant proliferation of glandular epithelium(Fig. 6). Squamous differentiation was characterised by multiple layers of epithelial cells and complete differentiation to keratin pearls. The actual origin of squamous component within the mammary tumors was postulated to be mediated by BMPs signaling through BMPR-IB and BMPR-II, as is the case with heterotopic cartilage and bone formation in mixed tumors.

(iv) Anaplastic carcinoma:Anaplastic carcinomas have been reported to be most aggressive mammary tumors with grave prognosis, because of their tendency to metastasize and to re-occur early after surgical removal (Losco, 1986). Histologically, they were characterised by extensively proliferating and diffusely infiltrating neoplastic cells with no glandular architecture (Fig. 7). These cells were seen arranged as chords over the fine connective tissue strands. Cells were polyhedral with large, bizarre nucleus and scanty eosinophilic cytoplasm. Mitotic figures were abundant with certain cells undergoing atypical mitosis. Connective tissue proliferation amidst the neoplastic cells was noticed. Accordingly, these tumors in canines were referred to as predominantly scirrhous type, unlike the human anaplastic carcinomas (Scarff and Torloni, 1968).Inflammatory reaction was very characteristic with diffusely infiltrating macrophages and polymorphs.

C) Sarcomas: Four different types of sarcomas were observed.

(i) Fibrosarcoma:Fibrosarcomas are reported rather as uncommon mammary gland neoplasms that can arise either within a pre-existing mammary gland neoplasm or from its interstitial stroma (Goldschmidt *et al.,* 2011).Histologically, it was characterised by the proliferation of spindle shaped fibroblasts, arranged as distinct whorls and bundles. Fine capillaries were noticed amidst the fibroblast bundles, giving the hemangiopericytoma-like appearance, as described by Misdorp (2002).Gordon & Sweet's staining revealed silver impregnated reticulin fibers in between the individual fibroblasts and surrounding the blood capillaries (Fig. 8), thus confirming it as fibrosarcoma, in contrast to spindle cell carcinomas where groups of cells will be wrapped by reticulin fibers (Carter *et al.,* 2006).

(ii) Osteosarcoma:Osteosarcomas arising in mammary tumors were common in dogs (Cassali *et al.,* 2011). Their biological behaviour was reported to be similar to the osteosarcomas at other sites, with metastasis *via* the haematogenous route, mainly to the lungs (Goldschmidt *et al.,* 2011).Grossly, the mass was completely solid with bony consistency. Histologically, complete differentiation of glandular tissue to osseous tissue was evident. Predominant picture waswell formed bony trabeculae that were seen as eosinophilic osteoid in decalcified sections. There was no evidence of bone marrow formation. Mature osteocytes were seen enclosed in lacunar spaces, while the osteoblasts were seen lining the trabeculae with bluish cytoplasmic contents. Thebasophilia might be due to the aggregates of rough endoplasmic reticulum that was active with

increased protein (osteoid) synthesis as described earlier (Leeson*et al.,* 1981).

(iii) Combined sarcoma:Microscopically, diffuse proliferation of both chondroblasts and osteoblasts was observed with minimal/no glandular component. Focal areas of cartilage were in the process of intrachondral ossification. Since both the osteoblasts and chondrocytes were seen proliferating, it was diagnosed as combined sarcoma, rather than simple chondrosarcoma. Extensive areas of cartilage undergoing necrosis were seen along with subsequent infiltration by macrophages.

(iv) Hemangiosarcoma:They were reported to bea very rare type of canine mammary tumors (Misdorp, 2002), and their histological features were reported to be identical to those found in spleen and subcutis (Goldschmidt *et al.,* 2011). Histopathologically, numerous thin walled blood vessels lined by plump endothelial cells were seen in the connective tissue wall of the cystic tumor mass. Neoplastic cells with large oval to round nuclei were also seen invading the connective tissue stroma in between the proliferating blood vessels. Central cystic contents showed extensive liquifactive necrosis.

D) Carcinosarcoma: Histologically, they were characterised by neoplastic proliferation of both glandular as well as connective tissue components. Epithelial proliferation was either of papillary or tubular or tubulopapillary or solid forms. Mesenchymal component was characterised by both differentiated fibrocytes and immature fibroblasts. Focal areas of cartilagenous differentiation and well differentiated bone formation were evident. Clear zones of metaplasia were seen between proliferating fibroblasts and chondroblasts. Tumors of this type were rare in dogs(Hellmén*et al.,* 1993) and reported to have grave prognosis, with most dogs developing metastasis within the first year after surgery (Benjamin *et al.,* 1999).

E) Carcinomas arising in benign mixed tumor: They werecharacterised by proliferating glandular epithelium along with connective tissue proliferation, the latter being undergone all phases of transition from hyalinization, myxomatous changes, chondroid differentiation to final ossification. Certain larger ducts had also shown moderate papillary ingrowths into the lumen. Ductal contents revealed degenerating polymorphs within the eosinophilic contents.As previously reported, it was difficult in the present study also, in defining these neoplasms (Gama *et al.,* 2008). These tumors might have started as benign type histologically, with subsequent malignant transformation of glandular component.

Thus, with such a huge variation in their clinical and histological features, canine mammary tumors are always the most important pathological condition that needs careful examination and evaluation in order to arrive at precise diagnosis. It also highlights the need of research for the development of other reliable molecular tools that aid in this regard.

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TABLE1

Histological types and occurrence of canine mammary tumors

S. No		Type of Tumor	Occurrence
I.	**Benign Tumors**		**7 (12.96%)**
		Cystadenoma	3 (42.86%)
		Myxoma	2 (28.57%)
		Benign mixed mammary tumor	2 (28.57%)
II.	**Malignant Tumors**		**47 (87.04%)**
	1. Carcinomas **(42.55%)**	Tubular Adenocarcinoma	7 (14.89%)
		Papillary Adenocarcinoma	6 (12.77%)
		Tubulo-papillary Adenocarcinoma	5 (10.64%)
		Ductal Carcinoma	2 (4.26%)
	2. Special Carcinomas **(27.66%)**	Micropapillary Adenocarcinoma	4 (8.51%)
		Solid Carcinoma	3 (6.38%)
		Carcinoma with squamous differentiation	3 (6.38%)
		Anaplastic Carcinoma	3 (6.38%)
	3. Sarcomas **(8.52%)**	Fibrosarcoma	1 (2.13%)
		Osteosarcoma	1 (2.13%)
		Combined sarcoma	1 (2.13%)
		Hemangiosarcoma	1 (2.13%)
	4. Carcinosarcoma		8 (17.01%)
	5. Carcinomas arising in benign mixed tumors		2 (4.26%)

Figures

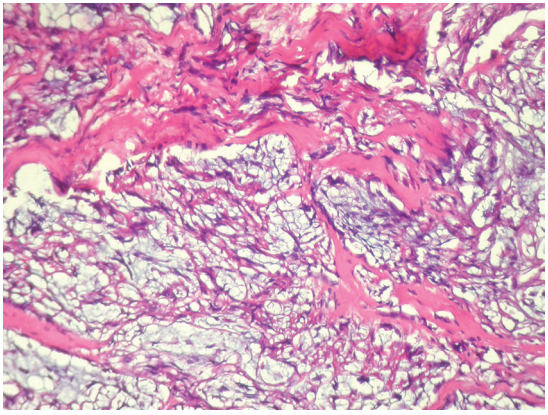

Fig. 1: Myxoma- Proliferation of myxomatous tissue with stellate cells in basophilic matrix. H&E x200

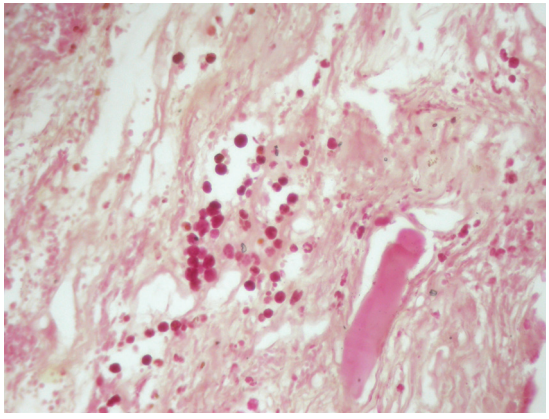

Fig. 2: Fibromyxoma- Macrophages with bright red lipofuchsin pigments within the connective tissue stroma. AFIP method x200

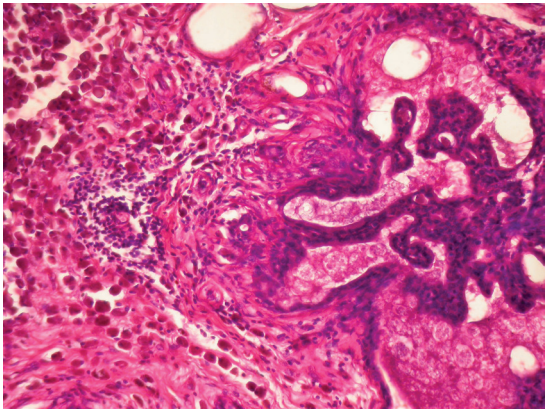

Fig. 3: Ductal carcinoma- Sheets of pigment laden macrophages and lymphoid aggregates adjacent to the proliferating duct. H&E x200

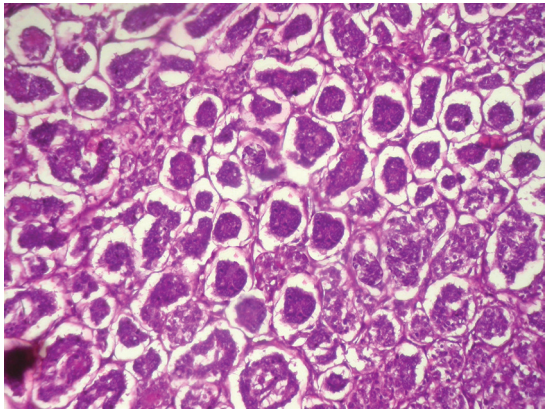

Fig. 2: Fibromyxoma- Macrophages with bright red lipofuchsin pigments within the connective tissue stroma. AFIP method x200

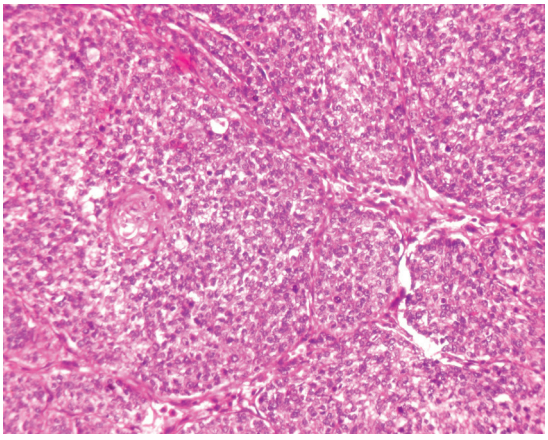

Fig. 5: Solid carcinoma- Neoplastic cells completely filling the alveolar lumen along with focal area of squamous differentiation. H&E x400

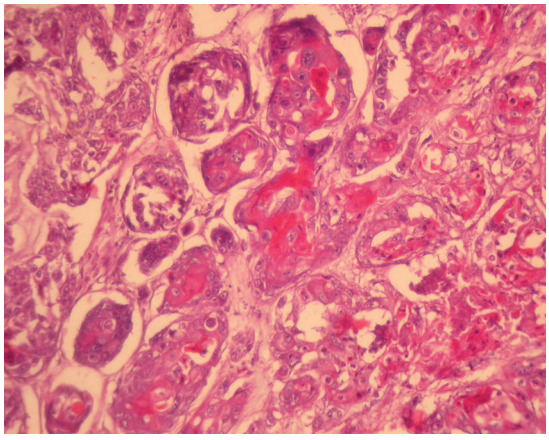

Fig.6: Adenosquamous cell carcinoma- Squamous differentiation along with glandular epithelial proliferation. H&E x400

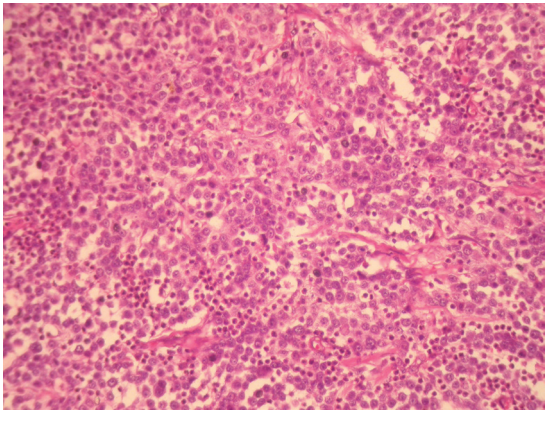

Fig.7: Anaplastic carcinoma- Diffusely infiltrating neoplastic cells with no glandular architecture. H&E x400

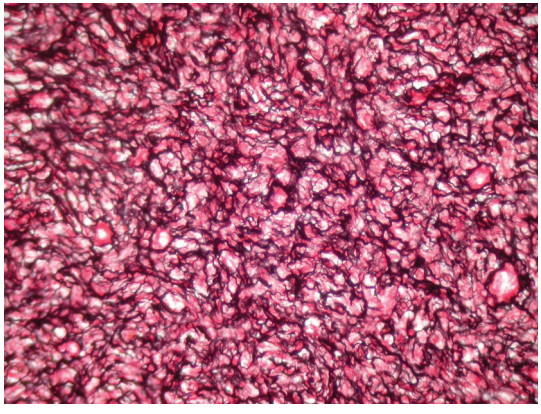

Fig 8: Fibrosarcoma- Silver stained reticulin fibers surrounding the individual fibroblasts. Gordon & Sweet's x100