

HISTOLOGICAL CLASSIFICATION AND GRADING OF MALIGNANT MAMMARY NEOPLASMS

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Mammary tumors are the most common neoplasms that affect female dogs. The present study was conducted on 40 cases of malignant canine mammary tumors. Among 40 malignant CMT, 30 (75%) were carcinomas, five (12.5%) carcinosarcomas, four (10%) sarcomas and one other i.e. malignant mast cell tumor (2.5%). The carcinomas further comprised of 13 simple carcinomas (32.5%), 10 complex carcinomas (25%), six solid carcinomas (15%) and one special type i.e. squamous cell carcinoma (2.5%). The study revealed that majority of canine mammary tumors was of simple carcinoma sub-type and of grade II category.

Keywords: Canine, grading, Histological classification, Malignant mammary tumor.

Canine mammary tumors (CMT) are among the most common neoplasms that affect female dogs with very high incidence. Majority of these tumors are malignant (Baba *et al.*, 2016). CMT is of interest to both veterinary and human oncologists because of their similarity in many aspects. During the last 3-4 decades, these tumors have been proposed as model for human breast cancer research (Lavalle *et al.*, 2009, Gupta *et al.*, 2012). Most commonly observed malignant tumor in women is the simple carcinoma, with more than one million cases and nearly 600,000 deaths occurring worldwide annually (Stewart and Kleihues, 2003). Metastasis remains the most threatening aspect of mammary carcinoma in both bitches and women, as most of deaths are recited because of metastatic disease (Yan *et al.*, 2008). Incidence of cancer is three times higher in bitches when compared to dogs (Merlo *et al.*, 2008). Intact bitches are more affected than neutered. Occurrence of CMTs is higher in posterior mammary gland (Gupta *et al.*, 2012) due to higher gland volume and abundant secretion during the lactation period that is approximately 40% of all mammary tumors (Baba and Catoi, 2007). Breeds such as Poodles, English Spaniels, English setters and Terriers exhibit a higher risk for mammary gland tumors, while in Boxers and Chihuahua, the risk is minimal (Gupta *et al.*, 2012). Prognosis of CMTs is directly related

to factors such as tumor size, lymph node involvement, presence of distant metastasis, histologic type, histologic grade, nuclear differentiation (Lavalle *et al.*, 2009). The present study deals with 40 malignant cases of CMT with a view to find their histological classification and grading for determining their prognosis.

Materials and Methods

The present study was conducted on tissue samples from 40 cases of canine mammary tumors, out of which 25 were retrospective cases and 15 prospective cases, collected from Department of Veterinary Pathology and Small Animal Clinics of the Department of Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, GADVASU, Ludhiana, respectively.

The mammary tumors were excised by mastectomy with or without the superficial inguinal lymph nodes. In cases of multiple tumors, the whole chain was resected. Representative tissue pieces were collected from multiple sites from excised tumors and fixed in 10% neutral buffered formalin, given overnight washings under running tap water and dehydrated using ascending grades of ethyl alcohol followed by clearing with acetone and benzene. Thereafter, the tissues were embedded in paraffin wax (56°C). The sections of 4-5µm were cut and stained with routine H&E and Toluidine blue.

The tumors were classified on the basis of latest WHO classification of

mammary tumors of dogs as shown in tables 1 and 2.

Table 1: Grading criteria of canine mammary tumors

Sr. No.	Characteristic	Score		
1	Tubule formation	1	2	3
2	Hyperchromatia and mitoses	1	2	3
3	Irregular size and shape of nuclei	1	2	3

Table 2: Grading scores of canine mammary tumors

Tubule formation	1 point if the section has well-marked tubule formation and 3 points if there are very few or no tubules.
Hyperchromatia and mitoses	1 point if only an occasional hyperchromatic or mitotic figure per high-power field is seen, 2 points if there are two or three such figures and 3 points if the number is higher.
Irregular size and shape of nuclei	1 point if the nuclei are fairly uniform in size, shape, and staining and 3 points if pleomorphism is marked.
Interpretation	
Total score	Grading of Malignancy
3-5	I
6-7	II
8-9	III

Results and Discussion

All the tumors were classified into various histological sub-types and the results have been depicted in Table-3.

Table 3: Histological classification of CMT (n= 40)

Behaviouristic type	Main type	Sub type	No. of Cases (%)
Malignant	Carcinomas	Simple Carcinoma	13/40 (32.5%)
		Complex Carcinoma	10/40 (25%)
		Solid Carcinoma	6/40 (15%)
	Carcinoma special type	Squamous cell Carcinoma	1/40 (2.5%)
	Sarcomas	Fibrosarcoma	4/40 (10%)
	Carcinosarcomas	Carcinosarcomas	5/40 (12.5%)
	Others	Mast cell tumor	1/40 (2.5%)

Carcinomas:

In the present study, 30 cases of carcinoma were recorded, which were further classified as simple carcinoma (n=13), complex carcinoma (n=10), solid carcinoma (n=6), and one case of squamous cell carcinoma.

Simple carcinoma:

Thirty cases of simple carcinoma were reported, which were microscopically characterized by proliferation of only one cell

type (Fig.1), resembling either luminal or basal epithelial cells with minimal stromal component. Based on the cellular pattern and the degree of differentiation. Simple carcinomas were further sub-classified as tubulopapillary (n=6) (Fig.2), tubuloacinar (n=2) with or without papillary projections at times giving rise to cystic appearance (n=1) (Fig.3). In addition, two cases of early simple carcinoma were also noted. In the present study, among 13 carcinoma cases, mild to moderate inflammation was noticed in nine

cases (69.24%), connective tissue invasion in 10 cases (76.93%), tumor infiltrating lymphocytes (TIL) in five cases (38.47%), epithelial mesenchymal transition (EMT) in eight cases (61.54%), necrosis in six cases (46.16%) and tumor thrombo-embolism in seven cases (53.85%) were observed as additional histological features.

Complex Carcinoma:

Ten cases of complex carcinoma were recorded, which were microscopically characterised by proliferation of both epithelial and myoepithelial components. The myoepithelial cells were arranged either in stellate or reticulate pattern (Fig.4). In the present study, EMT occurred in all cases of complex carcinoma, while necrosis in four cases only.

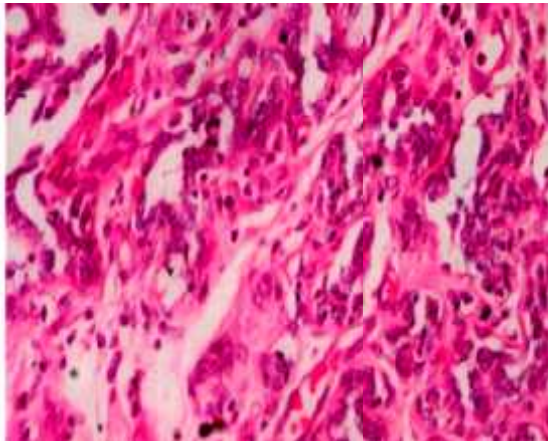


Fig.1. Simple Carcinoma- Showing mainly luminal epithelial cells. H&E x 400X.

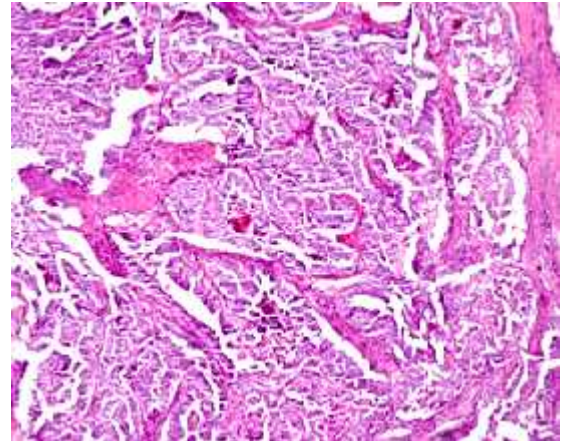


Fig.2. Tubulopapillary Carcinoma – Neoplastic tubules are arranged in a pedunculated papillary fashion. H&E x 100X.

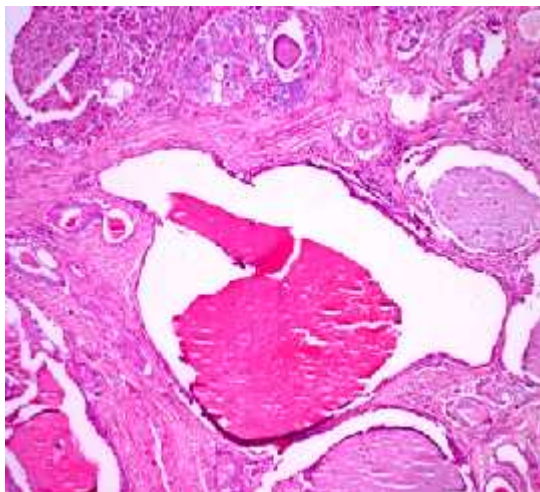


Fig.3. Cystic Carcinoma. original magnification H. E x 400X.

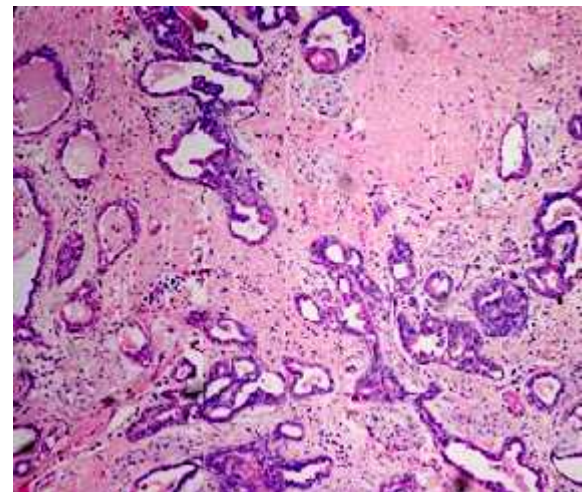


Fig.4. Complex Carcinoma – Showing presence of two cell populations in nearly equal proportion ie; epithelial cells and spindle shaped cells (myoepithelial cells) supported by a fibrovascular stroma, Grade I. H&E x 400X.

Solid Carcinoma:

In the present study, 6 solid carcinoma cases were observed. On microscopic observation, the neoplastic cells were predominantly arranged in solid sheets, cords,

or masses, without lumina to form dense, irregularly sized lobules supported by a fine fibrovascular stroma (Fig.5). Mild to moderate inflammation was found in all the

cases. Tumor embolism was recorded in two cases.

Special type of Carcinoma (Squamous cell carcinoma):

In the present study, one case of squamous cell carcinoma was found. In which the neoplastic cells were arranged in this tumor in cords, sheets and trabaculae with varying degree of squamous differentiation along with extensive thrombo-embolism.

Sarcomas:

Microscopically, all the four sarcomas were fibrosarcomas. It was observed that the neoplastic fibroblasts were arranged haphazardly and in concentric arrangement around the proliferating blood vessels and resembled somewhat the histological appearance of perivascular fibrosarcoma (Fig.6), as described by earlier workers Misdorp (2002) and Gupta (2011). In addition, there was necrosis, tumor embolism and infiltration of neutrophils with abundant mitotic figures.

Carcinosarcoma:

In the present study five cases of carcinosarcoma were diagnosed. On microscopic observation it was observed that the tumor contained cells resembling both malignant epithelial and connective tissue components in varying proportions. Appearance of carcinoma varied from early

lesions to tubulo-acinar, cystic papillary and solid to adeno-squamous types. The sarcomatous pattern comprised of varying degree of malignant connective tissue, mucin, chondroid and osteoid components (Fig.7). In the present study, among 5 carcinosarcoma cases, inflammation and connective tissue invasion were recorded, EMT was observed in all cases and tumor embolism in 4 cases only.

Other tumor: Malignant Mast cell tumor:

One case of other type tumor included the malignant mast cell tumor. The mast cells were scattered randomly below the epidermis and dermis. There were also significantly large number of eosinophils intermingled with tumor cells, particularly in perivascular location. Mast cell tumor was confirmed by observing meta-chromatic granules using toluidine blue stain (Fig.8).

In the present study, the highest incidence of carcinomas was observed, followed by carcinosarcoma and sarcoma, which is in accordance with previous findings of Ezerskyte *et al.* (2011) and Baba *et al.* (2016). On the contrary, Gupta *et al.* (2012) reported a higher incidence of carcinosarcoma as compared to carcinoma in dogs. In the present study, all malignant tumors had greater tumor angiogenesis, which, in turn, facilitates hematogenous dissemination of neoplastic cells as reported earlier by Luong *et al.* (2006).

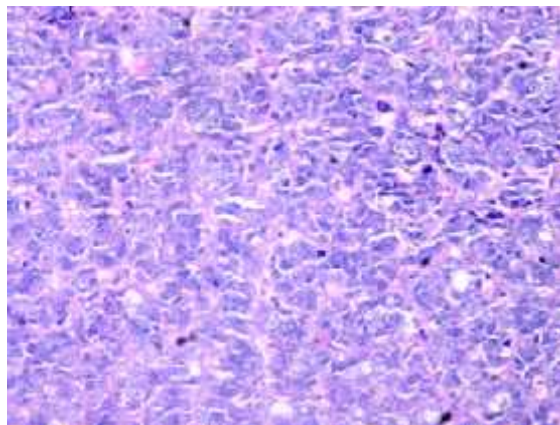


Fig.5. Solid Carcinoma – Cells are arranged in sheets/masses without tubular lumen, vacuolated cytoplasm, Grade II. H&E x 400X.

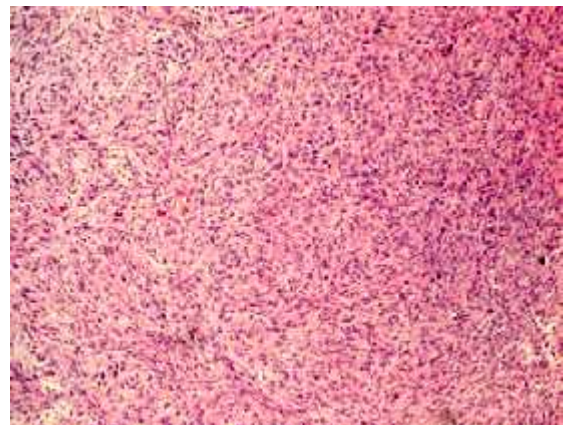


Fig.6. Fibrosarcoma – Showing spindle cells with solid various amounts of intercellular fibers, grade II, H&E x100X.

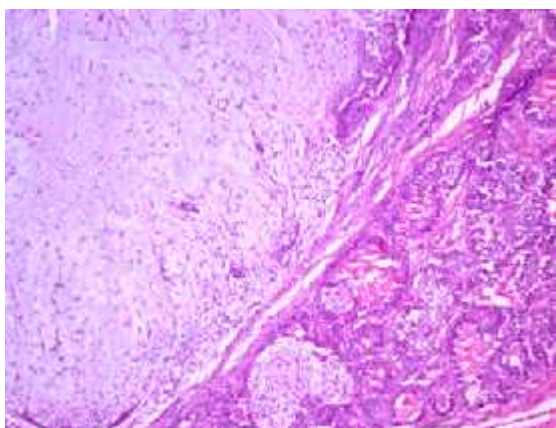


Fig.7. Carcinosarcoma - Partly resembling the epithelial component and partly of connective tissue elements, Grade I. H&E x 400 X.

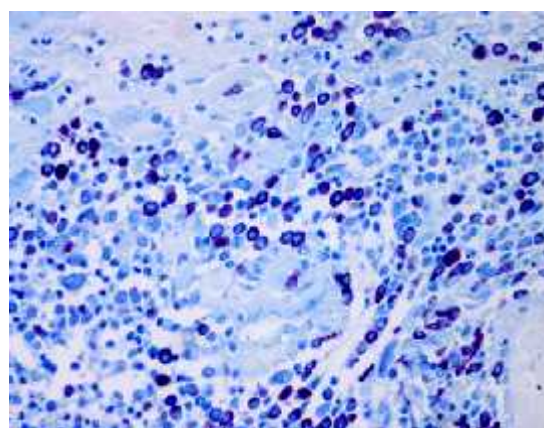


Fig.8. Malignant Mastocytoma- Showing meta-chromatic granules. Toluidine blue staining x 400 X.

Grading of carcinomas:

All the 40 malignant CMT were graded into grade I, grade II and grade III as per Goldschmidt *et al.* 2011. Among 13 simple carcinoma cases, grade II was observed in nine cases, whereas, the remaining four cases were of grade I. Among six solid carcinomas, grade I was observed in one case, grade II was observed in three cases, while grade III was observed in two cases, while in complex carcinoma, grade I, II

and grade III were observed in five, four and one case respectively. All the four cases of fibrosarcoma belonged to Grade II category. Whereas, both squamous cell carcinoma and malignant mast cell tumor were of grade III type. The results of histological grading are depicted in table 4. Most of the CMT cases in the present study fell in grade I and II categories. Grade II and III mammary tumors are usually associated with poor prognosis.

Table 4: Grading of histological sub-types of CMT (n=40)

Histological type	Number and percentage of cases			Total
	Grade I	Grade II	Grade III	
Simple carcinoma	4 (30.76%)	9 (69.24%)	-	13
Solid carcinoma	1 (16.66%)	3(50%)	2 (33.33%)	6
Complex carcinoma	5 (50%)	4 (40%)	1 (10%)	10
Squamous cell carcinoma	-	-	1 (100%)	1
Fibrosarcoma	-	4 (100%)	-	4
Carcinosarcoma	2 (40%)	3 (60%)	-	5
Malignant Mastocytoma	-	1 (100%)	-	1
Total	12 (30%)	24 (60%)	4 (10%)	40 (100%)

Conclusions

From the present study it was concluded that all the cases of CMT were malignant in nature and majority of them fall in grade II category, as the owners were ignorant about the disease and its outcome and they generally present their animals at a later stage to the veterinary clinics.

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