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RESEARCH ARTICLE

Correlation of histological subtypes, grading and lymphovascular invasion in canine mammary carcinomas

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ABSTRACT

Histological diversity of canine mammary tumours (CMTs) provides little prognostic information. Hence, use of a histological grading system may be helpful for prognosis of CMTs. A total 59 canine mammary carcinomas (CMCs) were graded histologically. The carcinomas were classified as grade I, II, or III based on evaluation of the formation of tubular structures, nuclear pleomorphism, and mitotic indices. Microscopic evidence of lymphatic invasion was also examined. Histologically, 26 (26/59; 44.07 %), 23 (38.98%), and 10 (16.95%) CMCs were graded in grade I, II and III respectively. Majority of solid carcinomas, anaplastic carcinomas, comedocarcinoma and squamous cell carcinoma were in histological malignancy Grade III, whereas most tubulopapillary carcinomas, complex carcinomas, carcinoma and malignant myoepithelioma, mixed carcinomas and malignant myoepithelioma were in histological malignancy Grade I. Variable numbers of tubular carcinomas, tubulopapillary carcinomas, solid carcinomas, carcinoma and malignant myoepithelioma, mixed carcinomas and malignant myoepithelioma shown intermediate malignancy Grade II. Lymphovascular invasion was detected in 10 (16.95%) CMCs cases. Of this 10 cases 2 CMCs were in grade I while 4 CMCs were in each grade II and grade III. Tubular carcinoma, micropaillary carcinoma, solid carcinomas, anaplastic carcinoma, carcinoma and malignant myoepithelioma and mixed carcinomas showed lymphovascular invasion. In conclusion, this study validated the Peña method of histopathological grading of CMCs. This grading system of CMCs could be used for future prognostic studies.

Keywords: Canine mammary tumours, histological grading, lymphatic invasion

INTRODUCTION

In domestic animals, mammary gland tumours are commonly reported in dog and cat, whereas other animals are rarely affected[1]. Canine mammary tumours (CMTs) are the most common neoplasms in the female dog with 0.2% prevalence[2]. The annual incidence rate has been estimated at 205/100000[3].

CMTs are diverse group of tumours, composed of either one, two or all three components *viz.*, epithelial, myoepithelial and mesenchyma. Histological diversity of CMTs provides little prognostic information. Hence, use of a histological grading system may be helpful for prognosis of CMTs. Among various suggested grading systems in humans, the most common system used worldwide is the Elston and Ellis numeric method (Nottingham method) for grading human breast cancer[4]. In veterinary science, 2 slightly different systems *viz.*, Misdorp[5] and Peña[6] systems (modifications of the human method) are used for histological grading of the canine mammary carcinomas (CMCs). Recently researchers[6] evaluated Peña system as a prognostic indicator in canine mammary carcinomas. They found that the tumour size, clinical stage, histological diagnosis, presence/absence of myoepithelial proliferation, and regional lymph node metastases were significantly associated with histological grade.

Although CMTs have been well studied in Europe and the United States, the studies on correlation between histological subtypes, histological grading and lymphovascular invasion in CMCs in India are scanty[7]. Therefore, the present study is aimed to establish correlation between histological subtypes, histological grading and lymphovascular invasion in CMCs.

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MATERIALS AND METHODS

A total 97 CMTs samples were obtained from different part of Gujarat, India in a period of three years (2014-2017). All tissues were fixed in 10% neutral-buffered formalin, processed routinely, and embedded in paraffin wax. The 4 to 5 microns thick sections were cut and stained with Haematoxylin and Eosin (H&E). The 97 CMTs were classified as described earlier[8]. Out of 97 CMTs, 59 were CMCs and were included in this study. In 6 CMCs cases, regional lymph nodes were also collected along with primary tumours and were evaluated for metastasis.

A total 59 CMCs were graded histologically as proposed by Peña *et al*.[6]. The carcinomas were classified as grade I, II, or III based on evaluation of the formation of tubular structures, nuclear pleomorphism, and mitotic indices. Microscopic evidence of lymphatic invasion was also examined. In six CMCs cases, regional lymph nodes were evaluated for metastasis by H&E and immunohistochemistry.

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For immunohistochemistry, 4-5 micrometer sections of formalin fixed paraffin embedded specimens were cut and mounted on poly-L-lysine coated slides. The tissue sections were deparaffinized, and rehydrated in graded ethanol. Antigen retrieval was done in pressure cooker containing boiled citrate antigen retriever buffer, pH 6.0. The cooled slides were washed in distilled water and placed in Tris-buffered saline (TBS) pH 7.4 for 20 min. Endogenous peroxidase was blocked by Peroxidase Suppressor (Thermo Scientific, USA) for 30 min. After pretreatment, the sections were incubated (1 hour) at room temperature with pancytokeratin (AE1-AE3 /IgG1, Kappa, Dako, Denmark) at 1:400 dilutions. Following primary antibody treatment tissue section were washed in TBS. After which, the sections were incubated (30 minutes) at room temperature with EnVision rabbit/mouse reagent conjugated to peroxidase (Dako REAL™ Envision™ HRP; Dako, Denmark). The sections were subsequently washed 4 times in TBS and developed with 3,3'-diaminobenzidine (DAB) as chromogen. The sections were then counterstained with Mayer's hematoxylin, dehydrated, cleared and mounted. In addition, immunohistochemistry was performed, with appropriate positive control.

RESULTS

In present study, a total 59 CMCs comprised of tubular carcinoma (n=5), tubulopapillary carcinoma (n=8), cystic papillary carcinoma (n=1), micropapillary carcinoma (n=1), solid carcinoma (n=7), comedocarcinoma (n=1), anaplastic carcinoma (n=2), complex carcinoma (n=3), carcinoma and malignant myoepithelioma (n=7), mixed carcinoma (n=12), ductal carcinoma (n=5), intraductal papillary carcinoma (n=1), squamous cell carcinoma (n=1), adenosquamous carcinoma (n=3) and malignant myoepithelioma (n=2) were included in study.

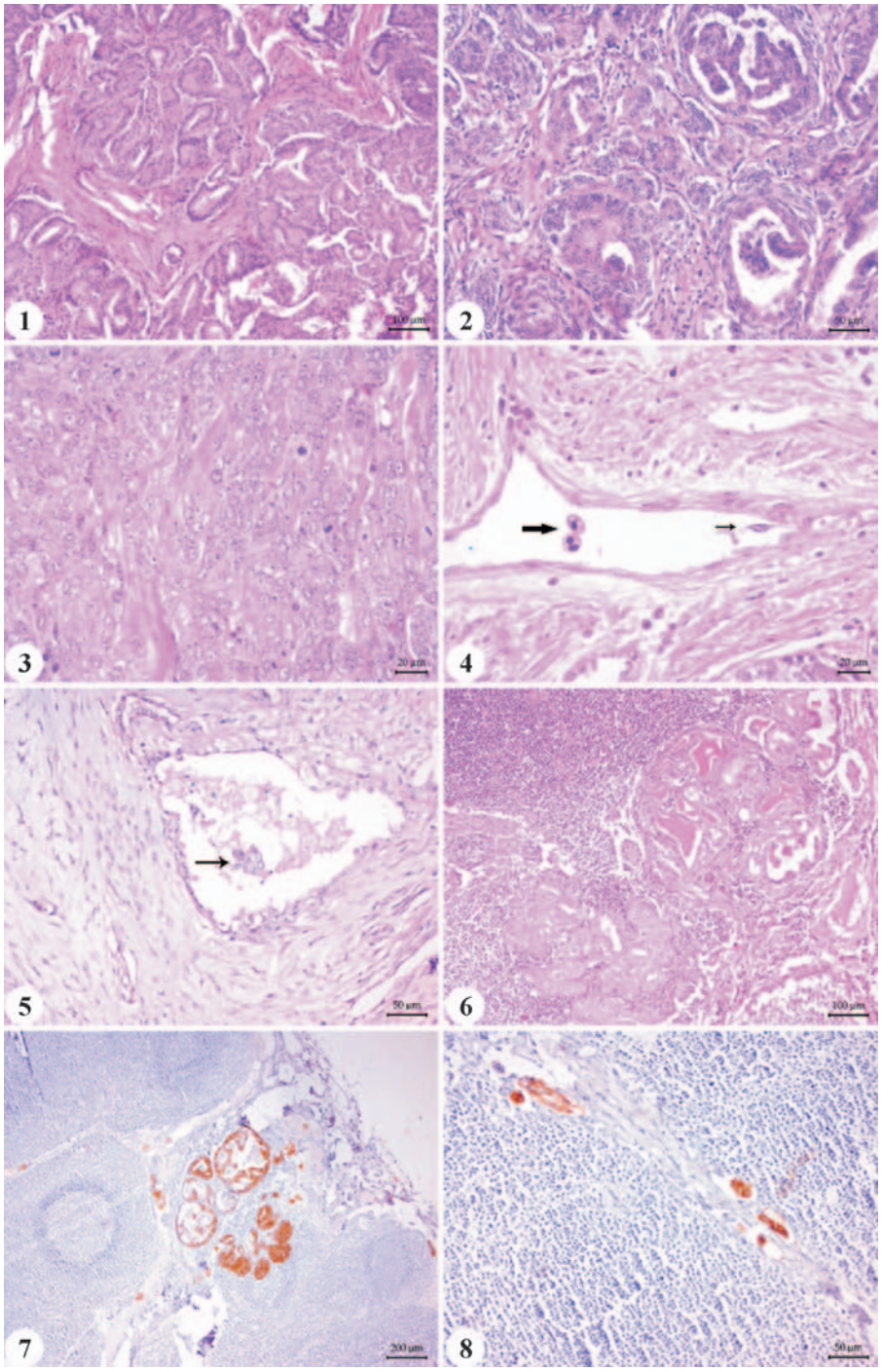
Relationships between histological subtypes and histological grade of CMCs is shown in Table 1. Histologically, 26 (26/59; 44.07%), 23 (38.98%), and 10 (16.95%) CMCs were graded in grade I, II and III, respectively.

In present study, most tubulopapillary carcinomas, complex carcinomas, carcinoma and malignant myoepithelioma, mixed carcinomas and malignant myoepithelioma were in histological malignancy Grade I (Fig. 1). Variable numbers of tubular carcinomas, tubulopapillary carcinomas, solid carcinomas, carcinoma and malignant myoepithelioma, mixed carcinomas and malignant myoepithelioma shown intermediate malignancy Grade II (Fig. 2). Most solid carcinomas, anaplastic carcinomas, comedocarcinoma and squamous cell carcinoma were in histological malignancy Grade III (Fig. 3). Out of 5 ductal carcinoma cases, 1 case each in grade I and III and 3 cases were in grade II.

Lymphovascular invasion was detected in 10 (16.95 %) cases. Of these, 2 CMCs were in grade I, while 4 CMCs were in each grade II and grade III.

Table 1. Relationships between histological classification and histological grade.

Histological Diagnosis	n	Histological Malignancy Grade					
		Grade I		Grade II		Grade III	
		n[a]	%[b]	n[a]	%[b]	n[a]	%[b]
Carcinoma - Tubular	5	1	20.00	4	80.00	0	0.00
Carcinoma - Tubulopapillary	8	5	62.50	3	37.50	0	0.00
Carcinoma - Cystic Papillary	1	1	100.00	0	0.00	0	0.00
Carcinoma–Micropapillary Invasive	1	0	0.00	1	100.00	0	0.00
Carcinoma - Solid	7	0	0.00	3	42.86	4	57.14
Comedocarcinoma	1	0	0.00	0	0.00	1	100.00
Carcinoma - Anaplastic	2	0	0.00	0	0.00	2	100.00
Carcinoma–complex type	3	3	100.00	0	0.00	0	0.00
Carcinoma and Malignant Myoepithelioma	7	4	57.14	3	42.86	0	0.00
Carcinoma - Mixed Type	12	8	66.67	4	33.33	0	0.00
Ductal Carcinoma	5	1	20.00	3	60.00	1	20.00
Intraductal papillary carcinoma	1	1	100.00	0	0.00	0	0.00
Squamous Cell Carcinoma	1	0	0.00	0	0.00	1	100.00
Adenosquamous Carcinoma	3	1	33.33	1	33.33	1	33.33



Tubular carcinoma, micropaillary carcinoma, solid carcinomas, anaplastic carcinoma, carcinoma and malignant myoepithelioma and mixed carcinomas showed lymphovascular invasion. Among 5 cases of tubular carcinoma, lymphovascular invasion was detected in 1 case of grade II neoplasm. Micropaillary carcinoma grade II showed lymphovascular invasion. In 2 cases of grade III solid carcinomas, lymphovascular invasion was evident. Both anaplastic carcinoma showed lymphovascular invasion. Three cases of carcinoma and malignant myoepithelioma showed lymphovascular invasion; of which one was in grade I while two were in grade II. In grade I carcinoma and malignant myoepithelioma, myoepithelial component was found as neoplastic emboli. In two cases of grade II, one showed epithelial component as neoplastic emboli and one case showed both myoepithelial and epithelial component as neoplastic emboli (Fig. 4). One case of each grade I and II of mixed carcinomas showed lymphovascular invasion (Fig. 5).

Out of six lymph nodes examined, two lymph nodes showed metastasis. In first case, grade I mixed carcinoma was metastasized to regional lymph node. The neoplastic cells were arranged in irregular tubules and found in subcapsular area (Fig. 6). Tubules were filled with eosinophilic secretory material. Neoplastic cells showed strong immunoreactivity to

Fig.1. Carcinoma-tubular, grade I. Total score 1+1+2=4 points. Tubule formation = 1 point (>75% of tubule formation); nuclear grade = 1 point and 12 mitotic figures in 10 high power fields (HPF)=2 points. H&E ×100; **Fig.2.** Carcinoma-tubulopapillary, grade II. Total score 2+2+2=6 points. Tubule formation = 2 point (10%–75% of tubule formation); nuclear grade = 2 points and 16 mitotic figures in 10 HPF =2 points. H&E ×200; **Fig.3.** Carcinoma solid, grade III. Total score 3 + 3 + 3=9 points. Tubule formation = 3 point (<10% of tubule formation); nuclear grade = 3 points and 38 mitotic figures in 10 HPF =3 points. H&E ×400; **Fig.4.** Carcinoma and malignant myoepithelioma. Epithelial cells (thick arrow) and myoepithelial cell (thin arrow) emboli present in blood vessels. H&E ×400; **Fig.5.** Carcinoma mixed type. Neoplastic cells (arrow) showing lymphovascular invasion. H&E ×200; **Fig.6.** Carcinoma - mixed type. Metastasis of epithelial component of neoplasm to regional lymph node. In subcapsular area, foci of neoplastic cells arranged in irregular tubules. Few tubules contained eosinophilic secretory material. HE ×100; **Fig.7.** Lymph node: Metastasis of epithelial component of mixed carcinoma in lymph node. Neoplastic cells showing strong immunoreactivity to pancytokeratin. Immunoperoxidase staining, DAB chromogen, Mayer's hematoxylin counterstain ×50; **Fig.8.** Lymph node: Metastasis of neoplastic cells of solid carcinoma in lymph node. Neoplastic cells showing strong immunoreactivity to pancytokeratin. Immunoperoxidase staining, DAB chromogen, Mayer's hematoxylin counterstain. ×200.

pancytokeratin (Fig. 7). Lymph node was enlarged due to metastasis and prominent secondary follicles. Deep cortical unit and medulla was filled with plasma cells, sinus histiocytes and lymphocytes. Many histiocytes showed erythrophagocytosis or pigmentation. In second case, grade III solid carcinoma showed neoplastic emboli in lymph node. Neoplastic cells showed strong immunoreactivity to pancytokeratin (Fig. 8).

DISCUSSION

Lymph node status, tumour size, histological type, histological grade, distance metastasis, and lymphovascular invasion are the factors to predict the disease-free or overall survival time after CMTs surgery. In the present study, we used only histological grading system and lymphovascular invasion as prognostic factors. Among collected CMTs samples, very few cases had contact details of owner, hence detailed history (survival of patient) was not obtained. Due to this limitation no attempted were made to correlate the histological type, histological grade with survival of patient. In the present study, 44.07% CMCs were in grade I, 38.98% were in grade II and 16.95 were in grade III. In past, few authors conducted the histological grading of CMCs by Elston and Ellis grading method[9-12]. In present investigation, most of the neoplasms were in grade I or II, and 16.95% neoplasm were in grade III. Almost similar findings were reported recently[13], and the authors graded 340 CMCs cases histologically, of which 44 (12.9%) were in grade III. However in other studies, higher percentage of grade III neoplasms have been recorded *viz.*, 35.3%[9], 35.06%[10] and 47.78%[11].

In the present study, 4 cases of solid carcinoma, 2 cases of anaplastic carcinoma, and one case of each comedocarcinoma, ductal carcinoma, squamous cell carcinoma, and adenosquamous carcinoma were classified in grade III. In one recent study[6] all comedocarcinoma and anaplastic carcinoma, 66% of solid carcinoma and 36% of adenosquamous carcinoma were in grade III. Almost similar findings had been reported by some earlier authors[13,14]. Lymphovascular invasion was found in 2 cases of grade III solid carcinomas and 2 cases of grade III anaplastic carcinoma. In one retrospective study, 100% grade III solid carcinoma, 100% of anaplastic carcinoma and 89% comedocarcinoma shown either lymphovascular invasion or lymphnode metastasis[14]. Similarly recent study[13] documented that 100% of carcinoma-anaplastic, comedocarcinoma, squamous cell carcinoma and 26% of solid carcinoma shown lymphovascular invasion.

In the present study, 80% tubular carcinoma, 37.5% tubulopapillary carcinoma, 100% of micropapillary carcinoma, 42.68% solid carcinoma, 42.86% carcinoma and malignant myoepithelioma, 33.33% mixed carcinoma, 60% ductal carcinoma, 33% adenosquamous carcinoma, and 50% malignant myoepithelioma were in grade II. Peña *et. al*[6] reported that, 33.3% solid carcinoma, 100% carcinoma and malignant myoepithelioma, 50% ductal carcinoma, and 18.2% adenosquamous carcinoma, were in grade II. In the present study, among grade II neoplasms, 1 case of tubular carcinoma, 2 case of carcinoma and malignant myoepithelioma and 1 case of mixed carcinoma revealed lymphovascular invasion. In one retrospective study, 74/100 (74.0%) grade II neoplasm shown lymphovascular invasion[14]. They also reported that, most comedocarcinoma, tubular carcinoma, solid carcinoma and carcinoma and malignant myoepithelioma shown lymphovascular invasion.

In the present study, 47.07% (n=26) neoplasms were in grade I. Almost similar findings had been reported before[6]. They reported that out of 65 CMCs, 46.6% were in grade I. Similarly in one more study[14] 50.00% neoplasm was graded in grade II. In one recent study 65.3% neoplasms were in grade I[13]. In the present study, among grade I neoplasms, one case of carcinoma and malignant myoepithelioma and one case of mixed carcinoma shown lymphovascular invasion. In one retrospective study, 20.0% (6/30) grade I neoplasm showed lymphovascular invasion[14].

In most of the previous studies, Elston and Ellis method was used for histological grading of CMCs, where myoepithelial component was not evaluated. However in Peña[6] method, myoepithelial component was also evaluated microscopically for grading of CMCs. In a recent study[15], the subtype-specific median survival times (MST) and local recurrence/distant metastasis rates of CMTs was determined. They reported that lowest MST was noted in anaplastic carcinoma (MST= 3 months) while solid carcinoma (MST = 8 months), comedocarcinoma (MST = 14 months), adenosquamous carcinoma (MST = 18 months) also have worse prognosis. In present study, all anaplastic carcinoma, comedocarcinoma and most solid carcinoma were in grade III. These findings indicate grade III neoplasms have poor prognosis and low MSTs. A recent study[15] reported that complex carcinoma and simple tubular carcinoma had prolonged survival while tubulopapillary carcinoma, intraductal papillary carcinoma, and carcinoma and malignant myoepithelioma had a more than 10-fold higher risk of tumour-related death. In present study, all complex carcinoma and intraductal papillary carcinoma were in grade I, while tubulopapillary carcinoma, and carcinoma and malignant myoepithelioma where in grade I or II. These findings indicate grade I or II neoplasms have good MSTs as compared to grade III.

In present study, in many cases of CMCs, surgical margins were not submitted or available for

histopathological evaluation. Hence, lymphovascular invasion was detected in only 10 (16.95%) cases. One recent study[13] has recorded relatively lower number of CMCs cases (40/340; 11.8%) with lymphovascular invasion, whereas, yet another report[14] found 190 (77.5%) cases out of 245 CMCs with lymphovascular invasion.

In conclusion, Peña method of CMCs histopathological grading is simple and practical method for prognosis of CMCs. This method facilitates the clinical interpretation of complicated histological diagnoses, and offers pathologists uniform criteria for the assessment of grading myoepithelial proliferation areas and mixed tumours.

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