RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.1.35-42

Evaluation of tumour associated macrophages in different histopathological types and grades of canine mammary tumours#

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Citation: Sajeev,N.E., Sajitha,I.S., Devi,S.S., Dhanush Krishna,B., Sudheesh,S.N. and Prasanna, K.S. 2023. Evaluation of tumour associated macrophages in different histopathological types and grades of canine mammary tumours. *J. Vet. Anim. Sci.* **54**(1):35-42
DOI: https://doi.org/10.51966/jvas.2023.54.1.35-42

Received: 30.06.2022 *Accepted: 20.12.2023* *Published: 31.03.2023*

Abstract

Canine mammary tumours (CMTs) are the second highest reported tumours in female dogs, following skin tumours. Human breast cancers (HBCs) and CMTs share common clinical and molecular features and hence, CMTs are considered as ideal models to study the different aspects of HBC. The study utilised samples from 25 CMT suspected cases presented to University Veterinary Hospitals in Thrissur district from December 2020 to October 2021. The tumour samples were analysed histopathologically and the lesions were classified. Among the 25 cases, one was identified as ductal hyperplasia, one as a benign myxoma and all the others were found to be malignant neoplasms. Malignant tumours were further categorised into different histotypes. Histological Malignancy Grading (HMG) was also done in 23 malignant CMTs and 21.74 per cent were found to be of grade I, 47.83 per cent were grade II and 30.43 per cent were grade III. Majority of the malignant tumours were simple carcinomas which comprised tubulopapillary, ductal, cribriform, solid and comedocarcinomas. Highly aggressive tumours like cribriform, solid, comedo and inflammatory carcinomas belonged to higher grades, either II or III. Infiltration of tumour associated macrophages (TAMs) was studied in different histotypes and grades of CMTs. It was identified that malignant high grade CMTs had greater TAM infiltration and hence, with further validation TAMs could be effectively used in predicting prognosis and also as a therapeutic target.

Keywords: *Canine mammary tumour, histological classification, grading, tumour associated macrophages*

#Forms part of the M.V. Sc thesis of the first author submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Mammary tumour is a disease condition commonly reported in dogs, cats and human beings. In dogs, mammary tumours are reported to have second highest occurrence, following skin tumours (Rezaie *et al*., 2009). Highest occurrence of canine mammary tumour cases was seen in dogs belonging to age group of 8-10 years, whereas least occurrence was seen in dogs belonging to the age group of 2-4 years (Nayyar, 2002). Mammary tumours may affect either multiple glands or a single gland and majority of the cases were noticed in posterior pairs of glands (Gupta *et al*., 2012). An actual breed predisposition to the condition is difficult to be established owing to the variation in breed preferences in different areas at different time points. A classification scheme for mammary tumours was put forth by Goldschmidt *et al.* (2011) based on the morphology and arrangement of neoplastic cells and myoepithelial cells in the tumour tissue. Histotyping and malignancy grading of CMTs will help in better understanding of the disease and predicting the prognosis. The Tumour Associated Macrophages (TAMs) are important cellular components of tumour microenvironment which act as regulators of anti-tumour immunity and help in immune evasion of cancer (Lin *et al.,* 2019). They secrete various cytokines like CSF1, VEGF, urokinase, MMP-9 *etc*. which help in cancer cell proliferation, angiogenesis, degradation of extracellular matrix and metastasis. This study looks into the relationship between the malignancy grade and the infiltration of TAMs in different subtypes of CMTs. A number of studies focusing on TAMs have been undertaken in human cancers especially breast cancers but it is very limited in the field of veterinary oncology. Hence, this study was undertaken to evaluate the prognostic value of TAMs in CMTs.

Materials and methods

A total of 25 CMT suspected cases presented to University Veterinary Hospitals in Thrissur formed the material for the study. Excisional biopsy samples collected in 10 per cent neutral buffered formalin were subjected to routine histopathological processing and staining. (Suvarna *et al.,* 2019).

Histopathological evaluation

Tumour sections were examined microscopically and the lesions observed were initially classified into hyperplastic changes, benign and malignant neoplasms. The malignant tumours were further categorised into different subtypes as per Goldschmidt *et al.* (2011). The infiltration of TAMs was evaluated and the number of TAMs per 10 high power field were noted.

Grading of canine mammary tumours

The malignancy grading of different tumour samples was carried out as per Clemente *et al*. (2010), which is a modification of Elston and Ellis method of histological malignancy grading (HMG) in HBC. The grades were assessed depending on the extent of tubule formation, nuclear pleomorphism and mitotic counts. Each feature was analysed semi-quantitatively and an individual score of 1 to 3 points was given.

When 75 per cent of the tumour area or more was composed of well-defined tubules a score of 1 was given. If 10-75 per cent of the area had well-formed tubules, 2 points were given and three points were given when such tubules were present only in less than 10 per cent of the area.

Minimum anisokaryosis with uniform chromatin if noticed, was given a score of 1. For moderate anisokaryosis, a score of 2 was assigned and a score of 3 was given for marked anisokaryosis with prominent nucleoli.

Mitoses per 10 high power field (HPF) was analysed and if there were only less than 10 mitoses per 10 HPF, a score of 1 was given. For 10-19 mitotic figures per 10 HPF, a score of 2 was given and if it was more than 20 per 10 HPF, a score of 3 was given.

The individual scores were then added to get the final score which was a number between three and nine. Grade was allocated as: -

HMG I (low grade) - three to five points

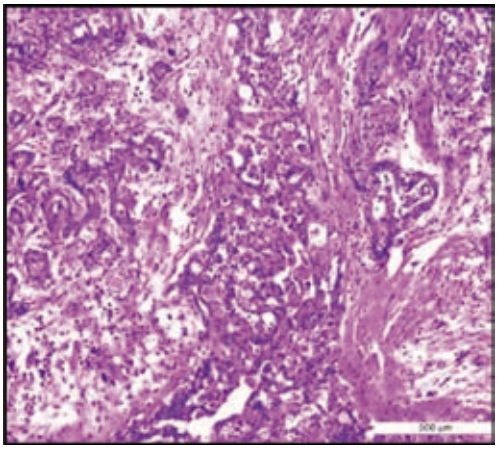

Fig.1. Ductal carcinoma - proliferating neoplastic cells within duct lumen (H&Ex400)

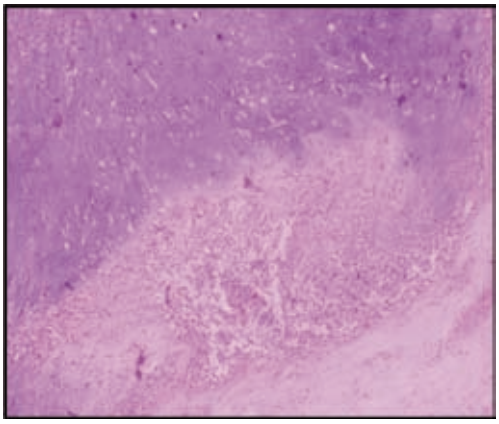

Fig.2. Carcinosarcoma - both epithelial and mesenchymal portions with neoplastic changes (H&Ex200)

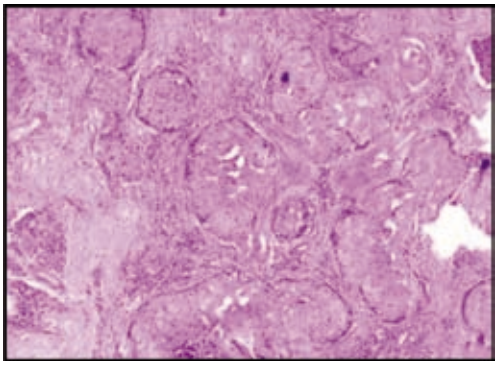

Fig.3. Solid carcinoma – neoplastic cells seen as solid sheets inside duct without any lumen (H&Ex100)

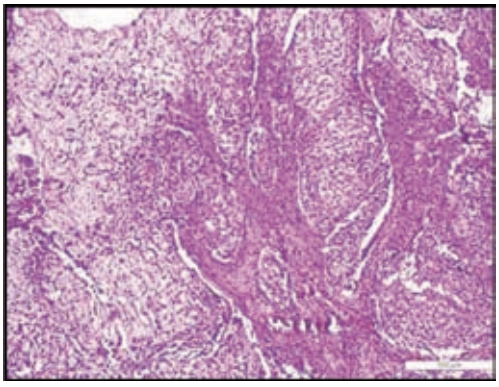

Fig.4. Myxosarcoma - resembled mesenchyme and contained undifferentiated cells (H&Ex100)

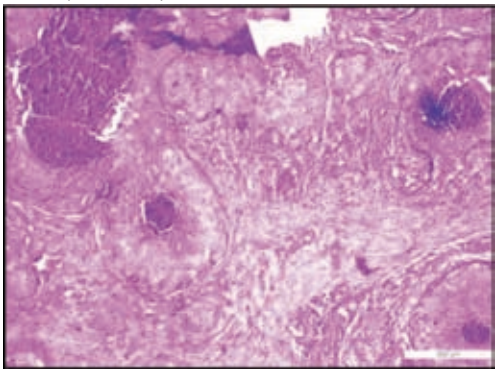

Fig.5. Comedocarcinoma - presence of necrotic areas within the centre of the neoplastic cell aggregates (H&Ex200)

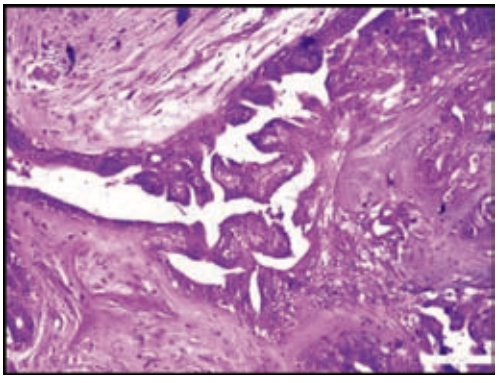

Fig.6. Tubulopapillary carcinoma – pedunculated or sessile papillae extending into lumen (H&Ex100)

HMG II (intermediate grade) - six or seven points

HMG III (high grade) - eight or nine points

Results and discussion

This study utilised canine mammary gland tissues obtained from 25 cases that were presented to the University Veterinary Hospitals in Thrissur during December 2020 to October

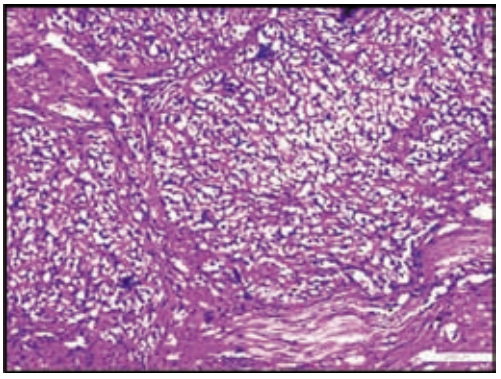

Fig.7. Lipid rich carcinoma - numerous small vacuoles or a single large vacuole (H&Ex200)

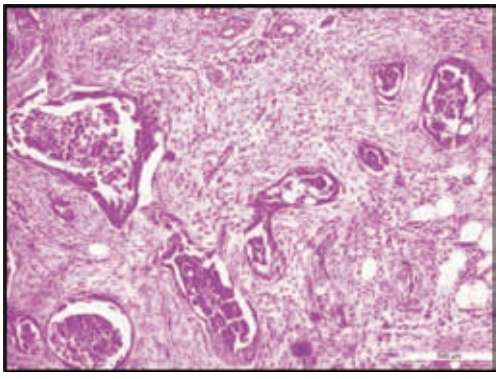

Fig.9. Anaplastic carcinoma - neoplastic cells often seen in small nests - round, oval or polygonal in shape (H&Ex100)

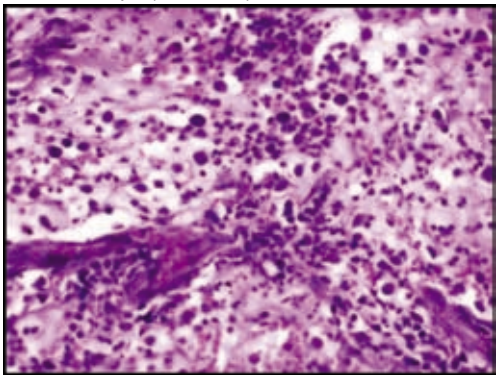

Fig.11. Inflammatory solid carcinoma – high infiltration of Tumour Associated Macrophages (H&Ex400)

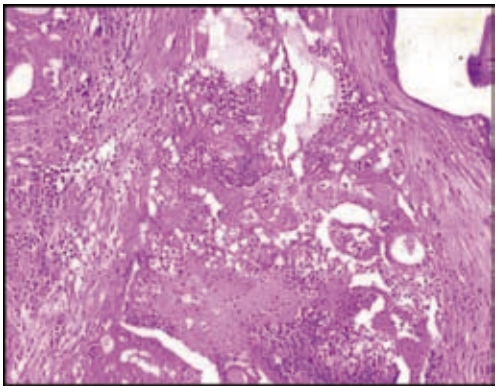

Fig.8. Cribriform carcinoma - proliferation of neoplastic epithelial cells forming a sieve-like arrangement (H&Ex100)

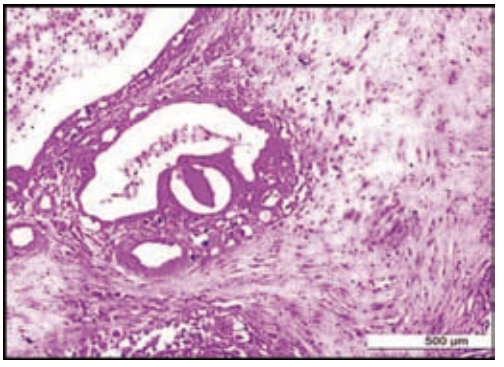

Fig.10. Cystic papillary carcinoma - papillae extended into marked dilated and cystic tubular lumina (H&Ex100)

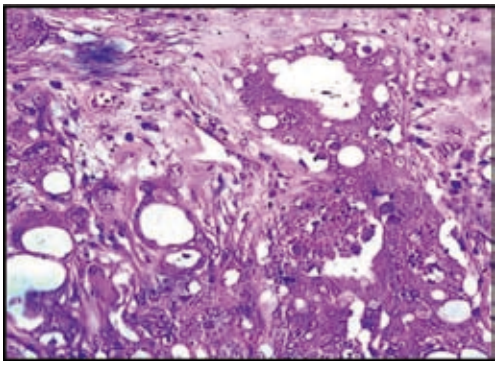

Fig.12. Carcinosarcoma – moderate infiltration of Tumour Associated Macrophages (H&Ex200)

2021. Out of all the cases, one was identified as hyperplasia, one as a benign myxoma and the remaining 23 cases were malignant neoplasms. Similar findings were recorded by Devi *et al*. (2022). Different histotypes observed were ductal carcinoma (Fig.1), carcinosarcoma (Fig.2), solid carcinoma (Fig.3), myxo-sarcoma (Fig.4), comedocarcinoma (Fig.5), tubulopapillary carcinoma (Fig.6), lipid-rich carcinoma (Fig.7), cribriform carcinoma (Fig.8), anaplastic carcinoma (Fig.9), cystic papillary carcinoma (Fig.10) and inflammatory solid carcinoma (Fig.11).

The histological subtypes were reported to be associated with prognosis and

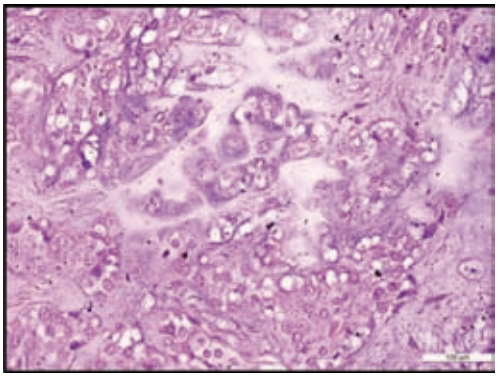

Fig.13. Lipid rich carcinoma – low infiltration of Tumour Associated Macrophages (H&Ex400)

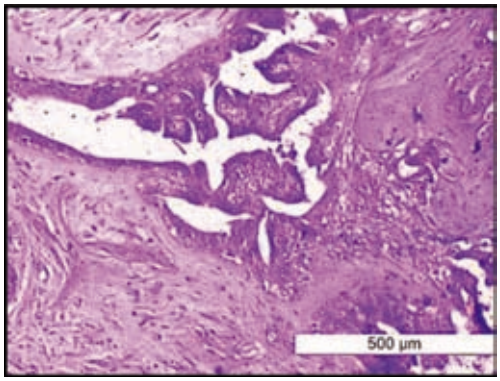

Fig.14. Tubulopapillary carcinoma – low infiltration of Tumour Associated Macrophages (H&Ex100)

survivability in CMT. In a study conducted by Rasotto *et al* .(2017) it was concluded that solid carcinomas and anaplastic carcinomas were highly aggressive. Comedocarcinomas and carcinosarcomas were found to have poor survivability rates by Canadas *et al.* (2019). In our study also similar association was found between aggressive histological subtypes and grades of the tumours. Solid carcinomas were identified primarily by the presence of cells arranged as a solid sheet without a central lumen. The cells had poorly demarcated cell margin and scant cytoplasm. Anaplastic carcinoma was characterised by the presence of diffuse invasion of interlobular connective tissue and lymphatic vessels by the neoplastic cells. The cells were large and highly pleomorphic with round to oval nuclei. Neoplastic cells were often seen in small nests. Carcinosarcomas were identified by the presence of cells resembling the epithelial phenotype as well as cells resembling connective tissue component, both of which were malignant. Necrotic cell areas in the centre of neoplastic cell aggregates were characteristic to comedocarcinoma.

Ductal carcinomas were characterised by proliferating cell population arranged in cords and tubules that surrounded slit like lumen. According to Rezaie *et al*. (2009) 70 per cent of the dogs diagnosed with mammary tumour had tubulopapillary carcinoma. Tubulopapillary carcinoma was characterised by the presence of proliferating epithelial cells in a pedunculated and papillary manner. These papillae were supported by fine fibrovascular connective tissue stalks which were found extending into the tubular lumen. Cribriform carcinoma consisted of neoplastic cell aggregates which formed a sieve-like arrangement. Neoplastic cells appeared columnar to polygonal with scant, eosinophilic cytoplasm. Cystic papillary carcinoma was characterised by the presence of papillae which extended into a cystic or markedly dilated lumen. Lipid rich carcinomas were primarily identified by the presence of vacuoles within the epithelial cell cytoplasm. The vacuoles were either small and numerous or large and single. In case of a single large vacuole the nucleus was observed to be pushed to the periphery. Myxosarcoma was characterised by the presence of high number of undifferentiated cells. Cells were stellate, round or spindle shaped with vesicular nuclei and scant cytoplasm. Inflammatory solid carcinoma was characterised by extensive infiltration of macrophages into the tumour stroma.

The histopathological appearance of myxoma was similar to myxosarcoma except for reduced cellularity. Abundant perivascular lymphocytic infiltration was observed in myxoma. Ductal hyperplasia was characterised by the presence of large number of ducts in the tissue. The ducts were lined by cells with small nuclei which showed very less or no anisokaryosis.

In the present study, out of the 23 malignant tumours graded, five (21.74 per cent) were grade I, eleven (47.83 per cent) were grade II and seven (30.43 per cent) were grade III. This finding was in accordance with Mathew *et al.* (2019) and Christy *et al*. (2022). It also corroborated the findings of Misdorp *et al.* (1999) which stated that the carcinomas

Table 1. Histopathological subtypes and their respective histological malignancy grading

Tumour subtype	Grade I	Grade II	Grade III	Total
Tubulopapillary carcinoma	1	4	1	6
Ductal carcinoma	3	1		4
Solid carcinoma			2	2
Comedocarcinoma			1	1
Inflammatory carcinoma			1	1
Cribriform carcinoma		1		1
Carcinosarcoma	1	2	1	4
Lipid rich carcinoma		1		1
Cystic papillary carcinoma		1		1
Anaplastic carcinoma		1		1
Myxosarcoma			1	1
Total	**5**	**11**	**7**	**23**

Table 2. Histopathological subtypes and infiltration of TAMs

Subtype of tumour	TAMs/ 10HPF
Inflammatory solid carcinoma	667
Myxosarcoma	558
Comedocarcinoma	342
Solid carcinoma	225
Cribriform carcinoma	157
Anaplastic carcinoma	112
Carcinosarcoma	107.5
Ductal carcinoma	90.2
Tubulopapillary carcinoma	77.67
Cystic papillary carcinoma	70
Lipid rich carcinoma	6
Myxoma	6

Table 3. Malignancy grading and infiltration of TAMs

GRADE	TAMs/10HPF
Grade I	43.4
Grade II	99.7
Grade III	304

with most favourable outcomes were grade I whereas those which were malignant with least favourable outcome were either grade II or III. The different histotypes and their malignancy grades are given in table 1.

Various cytokines secreted by TAMs help in immune evasion and metastasis of the cancer cells and these M2 macrophages are hence considered to be pro-tumourigenic unlike the classically activated M1 phenotype macrophages, which are anti-tumourigenic (Murray and Wynn, 2011). Study conducted by Monteiro *et al.* (2018) discovered that higher number of TAMs was associated with higher proliferation rate of tumours and nodal metastases in CMTs. It was also concluded that a shift from normal macrophages to TAMs occurred in malignant CMTs. According to Sousa *et al.* (2015) various factors secreted by tumour stroma influenced the macrophage polarisation resulting in formation of more TAMs in HBCs. Presence of TAMs correlated positively with larger size and higher grades of HBCs (Medrek *et al.,* 2012). The role of TAMs in the six malignancy traits of tumours namely invasion, angiogenesis, matrix remodelling, inflammation and seeding was elucidated by Condeelis and Pollard (2006) and it was suggested that TAMs were excellent targets for drug therapy in HBCs. It was determined by Leek *et al.* (1996) that macrophage infiltration trumped nodal status as a better predictor of survival in HBCs. In CMTs it was proven that the number of TAMs were low in benign tumours when compared to malignant ones. There was also a significant association of high infiltration of TAMs with ulceration of the tumours (Raposo *et al.,* 2014).

In the present study also, significant association was found between higher grades and aggressive histotypes with the infiltration of TAMs in the tumour stroma in CMT. The presence of TAMs was evaluated histopathologically (Fig.11-14). The number of TAMs were counted under high power of microscope and was later correlated with the histological subtype (Table 2) as well as malignancy grade of tumours

(Table 3). This showed that more aggressive tumours with inflammatory stroma like solid carcinoma and comedocarcinoma showed higher rate of TAM infiltration than those with a non-inflammatory stroma. Higher the grade of the tumour, higher was the infiltration of TAMs. Hence, with further validation TAMs could be effectively used in predicting prognosis and as a therapeutic target in CMTs.

Conclusion

In the present study histological subtyping and malignancy grading was carried out in CMT cases in and around Thrissur district. The findings revealed that majority of CMTs were malignant and were of higher grades. The highly aggressive subtypes observed were inflammatory, solid, comedo and cribriform carcinomas. The malignancy grades correlated well with their histological subtypes. Infiltration of tumour associated macrophages was greater in high grade tumours and in more aggressive tumours with predominantly inflammatory stroma. Considering the role of TAMs in tumour proliferation, angiogenesis and metastasis, together with the results of the present study, extensive research is warranted to establish the significance of TAMs in predicting prognosis and as a therapeutic target in CMTs.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, Thrissur for providing facilities to carry out this research work.

Conflict of interest

The authors declare that they have no conflict of interest.

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