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Histopathological and immunophenotypical classification of canine mammary tumours[#]

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Abstract

Canine mammary tumour (CMT) is the most common malignancy reported in intact female dogs. The present study is envisaged to classify the CMTs based on the histopathologic type, grade and hormone receptor status. Twenty-five cases of CMTs were taken as the study material. The excisional biopsy samples from all the cases were collected and processed for histopathology and grading of tumours. The immunohistochemical evaluation of oestrogen receptor (ER) and/or progesterone receptor (PR) or human epidermal growth factor type II receptor (HER2) in CMTs was carried out to classify them. Luminal A had the highest number of cases followed by luminal B. One case of triple negative breast cancer (TNBC) and two cases of HER2 enriched tumours were also noted. All the tumours in HER2 enriched and TNBC were grade 3 with aggressive characters like lymphatic invasion and angiogenesis. The prognosis of the above cases was studied and the data revealed poor prognosis in the HER2 enriched and TNBC compared to luminal A and B. The implementation of targeted therapy in canine mammary tumours based on hormone receptor status may increase the chances of recovery or may prolong the survival period in canine patients.

Keywords: *Canine mammary tumours, ER, PR, HER2, TNBC*

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Human breast cancer (HBC) is classified based on the presence or absence of hormone receptors (HR), depending on which, patients are stratified for targeted and conventional chemotherapy and it has got prognostic significance also (Tsang and Gary, 2020). In majority of HBCs, there is overexpression of ER, PR and HER2. The HBCs are classified into molecular subtypes as luminal A, luminal B, HER2 enriched and TNBC based on this receptor profile. Endocrine targeted therapies which modulate or degrade these receptors, inhibit their synthesis or competitively inhibit them are used for the management of these cancers. Canine mammary tumour has got similarity to HBC in etiological, epidemiological, clinical, molecular, genetic and prognostic factors and thus is considered as the best translational model for breast cancer (Gray *et al.,* 2020). The prognostic evaluation and treatment of CMT is still based on the clinical stage alone and hormone receptor status is not looked into and hence, its management is still a challenge in veterinary health care. Though reports on molecular classification and its prognostic significance in CMT are there, many have yielded contradictory results. This is primarily because of the different systems of classification used to define the receptor status. This study was taken up to classify the CMTs in and around Thrissur, based on the hormone receptor status and see their prognostic significance. Immunohistochemistry (IHC), which uses labelled antibodies to identify the target protein in a tissue segment, is one technique used for molecular characterisation of cancers (Ramos-Vara, 2005) and is considered as the 'gold standard' test to evaluate the expression of HR. Classification of CMTs based on hormone receptors followed by targeted therapies will help in effective management of canine mammary tumours.

Materials and methods

A total of 25 CMT suspected cases collected from TVCC Mannuthy and University Veterinary hospitals, Kokkalai from July 2022 to June 2023 were included in the present study. Tissue samples were fixed in 10 per cent neutral buffered formalin for 48 hours and processed by routine paraffin embedding method (Spencer and Bancroft, 2013). The sections were cut at 5-micron thickness and stained with routine haematoxylin and eosin procedure (Suvarna *et al.,* 2018). The sections were examined under microscope and were graded and histologically classified (Clemente *et al.,* 2010). Immunohistochemistry was done for the proteins ER, PR and HER2 in formalin fixed paraffin embedded tissues according to the procedure described by Ramos-Vara, (2005). The ER, PR and HER2 antibodies were diluted to a concentration of 1:50 using primary antibody dilution buffer. Scoring for ER and PR was done by Allred score and for HER2, Hercep test system was used (Pena *et al*., 2014).

Results and discussion

Of the 25 malignant mammary tumours evaluated, nine (36%) were Grade 2 and sixteen (64%) were Grade 3 and there were no grade 1 tumours. Solid and tubulopapillary carcinomas and carcinosarcoma were the most common histological type of tumours (20%

Table 1. Histological grades of different types of CMT(n=25)

Histological Types	Grade 1	Grade 2	Grade 3
Carcinosarcoma	0	0	5
Comedocarcinoma	0	0	1
Ductal carcinoma	0	3	0
Intraductal papillary carcinoma	0	0	1
Lipid rich carcinoma	0	1	0
Micropapillary carcinoma	0	0	1
Papillary carcinoma	0	2	1
Solid carcinoma	0	1	4
Tubulopapillary carcinoma	0	0	5

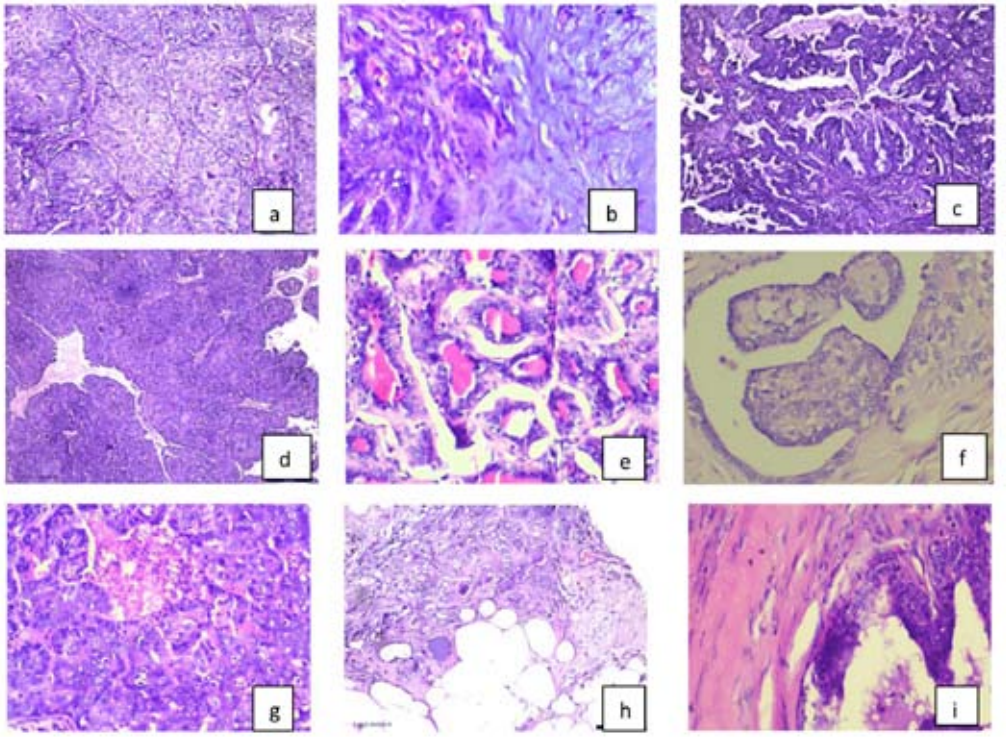

Fig.1. Histological types of canine mammary tumours (H&E)- a. Solid carcinoma (20x), b. Carcinosarcoma (40x), c. Tubulopapillary carcinoma (20x), d. Papillary carcinoma (10x), e. Ductal carcinoma (40x), f. Micropapillary carcinoma (40x), g. Comedo carcinoma (40x), h. Lipid rich carcinoma (10x), i. Intraductal papillary carcinoma (40x)

each), followed by ductal carcinoma (12%) and papillary carcinoma (12%). Intraductal papillary carcinoma, comedocarcinoma, lipid rich carcinoma and micropapillary carcinoma were also present (4% each). Figure 1 shows different histological types of canine mammary tumours and Table 1 depicts histological types and grades of CMTs.

All the cases of carcinosarcoma, comedocarcinoma and tubulopapillary carcinoma had high mitotic indices and were classified as grade 3 tumours. Sixteen per cent of solid carcinoma had grade 3 characters and four per cent of them were of grade 2 type. Ductal carcinoma and lipid rich carcinoma were grade 2 tumours. Papillary carcinoma were of both grade 2 and grade 3 types. Intra ductal papillary and micropapillary carcinoma had one case of grade 3 tumour with high nuclear pleomorphism. In a study conducted by Devi (2022), tubulopapillary carcinomas obtained were grade 2 or grade1 and 66 per cent of carcinosarcoma was grade 3, but all comedo carcinoma was grade 3. The variation in the histological types and grade can be due to the intratumoural heterogeneity and the stage at which tumours are diagnosed.

Expression of ER, PR and HER2 were examined using IHC in all the CMT tissues. Immunostaining was noticed particularly in the nucleus with mild cytoplasmic staining in the case of ER and PR. Immunolabelling of HER2 was less intense compared with other proteins but was specifically bound to the cell membrane.

Twenty two (88%) out of 25 CMTs exhibited positive nuclear immunostaining for ER and were labelled as ER positive tumours, while the other three (12%) tumours displayed negative ER staining. All the grade 2 tumours under the present study were positive for ER (Fig. 2 (j-l) and 3). The results were in accordance with the report of Chang *et al.* (2009), who observed that in 113 dogs with mammary gland tumours, 60 per cent of cases were ER positive. However, Rutteman *et al.* (1988), revealed that expression of the

genes encoding ER and PR was widespread in the non-malignant mammary tissues of adult female dogs. This characteristic may be lost in malignant tumours, particularly in more severe stages of the disease.

Immunolabelling for PR revealed 16 samples as positive while, immunostaining was absent in 9 CMTs (Fig.2. (m-o)). All Grade 2 tumours exhibited IHC staining but positive expression was seen only in some of the grade 3 tumours (56.3%) (Fig. 3) and the results were in agreement with the results of Geraldes *et al.* (2000), who stated that progesterone receptor-negative malignant tumours proliferated more quickly than progesterone receptor-positive tumours, indicating that the development of malignancy in spontaneous mammary tumours is associated with a decline in hormonal steroid dependence. Thuroczy *et al.* (2007) stated that malignant mammary tumours had downregulated PR expression and only 17.8 per cent of cells in malignant tumours exhibited PR positivity. Fisher's Exact test was used to statistically analyse the association between the expression of receptors and grade of the tumours. Statistical significance was observed with the test wherein, the expression of PR was found to decrease with increase in grade of tumours ($P<0.01$).

Nine CMT cases in the present study were positive for HER2 and sixteen cases showed negative immunostaining (Fig.2. (p-r)). High grade tumours showed higher HER 2 expression in the present study. Positive immunostaining for HER 2 was mostly seen in grade 3 tumours (43.8%) and in grade 2 tumours, only 22.2 per cent cases were positive (Figure 3). These results were in accordance with the study results by Dutra *et al.* (2004), overexpression of HER 2 was linked to high histological grade, nuclear and cellular pleomorphism and more aggressive mammary neoplasms. It was also

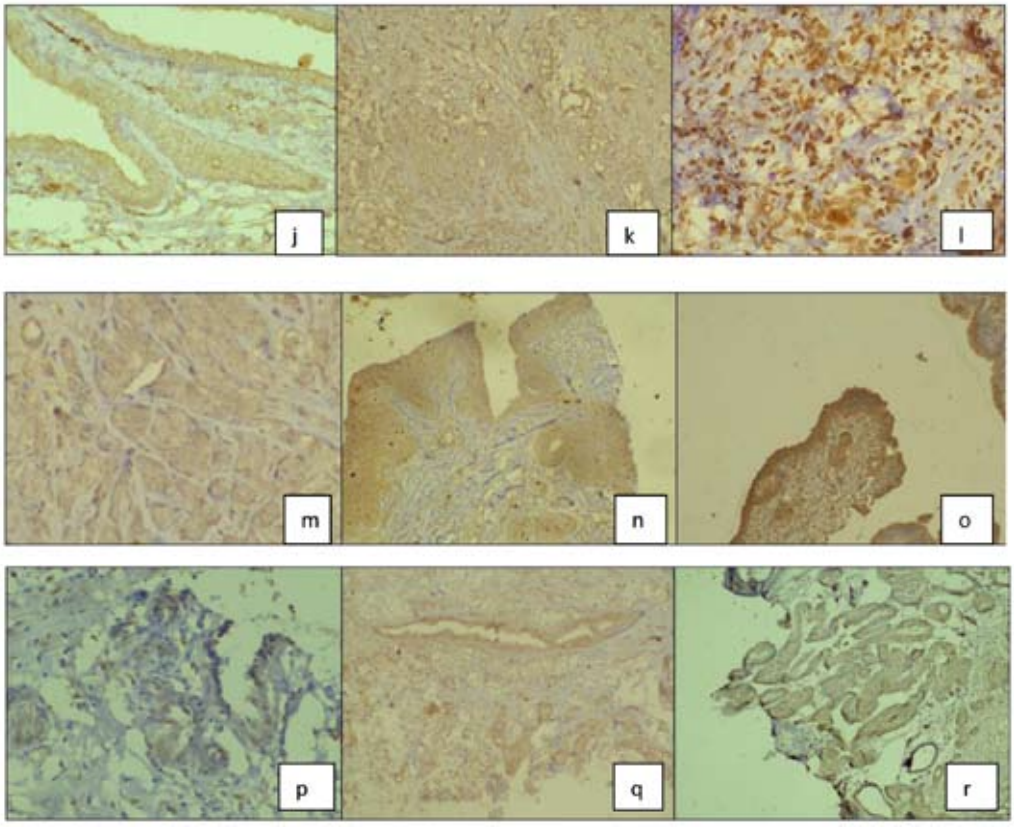

Fig. 2. Immunohistochemistry of canine mammary tumours: Immunostaining of ER (j-l) j. weak (10x), k. moderate (10x), l. strong (40x), immunostaining of PR (m-o), m. weak (40x), n. moderate (10x), o. strong (10x) and immunostaining of HER2 (p-r) p. weak (40 x), q.moderate (10x), r. strong (10x).

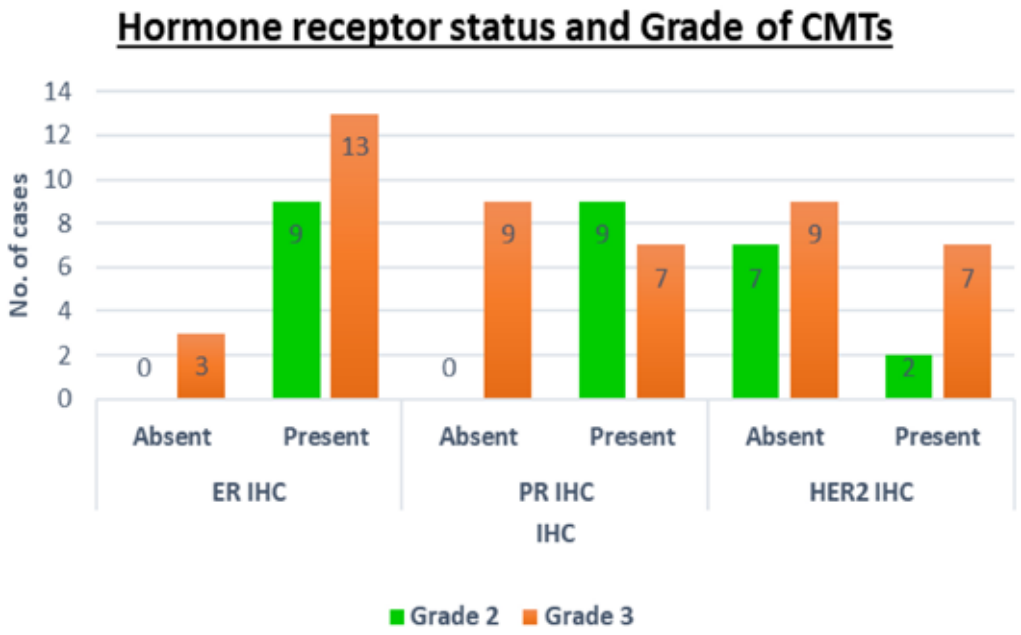

Fig. 3. Hormone receptor status and grade of canine mammary tumours

reported that HER2 over expression was associated with decreased disease free and overall survival in canine mammary tumour patients, similar to that in human breast cancer (Muhammadnejad *et al.,* 2012). The HER 2 pathway activate many transcription factors which regulate the genes involved in cellular proliferation, survival, apoptosis, angiogenesis, invasion and metastasis, which together lead to faster progression of the disease and poor prognosis.

Based on the immunostaining of proteins, CMTs were classified into luminal A (ER^{+ve}, $PR^{+ve/-ve}$, HER 2^{-ve}), Luminal B (ER^{+ve}, $PR^{+ve/-ve}$, HER 2^{+ve}), HER 2 enriched and TNBC. Luminal A had 15 number of cases of which 7 were grade 2 tumours and 8 were grade 3 tumours. In luminal B group, majority of the cases were grade 3 (71%). One case of TNBC and two cases of HER2 enriched tumours were also observed. All the cases in HER2 enriched and TNBC groups were grade 3 with the presence of numerous blood vessels and lymphatic invasion. Luminal B breast cancer had a higher recurrence rate and a poor post relapse prognosis when compared to luminal A type (Ellis *et al*., 2008). Paster *et al.* (2020) reported that triple-negative tumours (ER/PR/HER2) were more frequently detected in advanced clinical stages and were connected to vascular and peritumoral invasion. According to the study of Kim *et al*. (2013), among 241 samples collected 18 per cent of CMTs were triple negative which was much higher than observed in the present study, which could be due to limited sample size and the difference in grading system used to define the receptor positivity.

Conclusion

The analysis of CMTs based on hormone receptors revealed higher occurrence of ER positive tumours followed by PR positive and HER2 positive cases. Only one case of TNBC could be observed. The receptor status was also compared with the grade of the tumours. Studies on prognosis showed poor prognosis in HER2 enriched and TNBC CMTs. In canines, systematic study on expression of these biomarkers is limited and conventional chemotherapy is still used for therapeutic management of tumours in dogs. Hormone receptor targeted therapy after surgery is effectively practiced in human breast cancer cases. Such a treatment modality can be also practised in canine cases so that the survival period of the animals can be surely prolonged. In human breast cancer patients,

receptor status is used not only for providing therapeutic guidelines but also for predicting prognosis. Though some reports are there on the usefulness of these molecular markers for predicting prognosis, they are not routinely analysed in canines as a prognostic factor. The evaluation of receptor status must be carried out in a large sample size so that they can be utilised for therapeutic management and predicting prognosis in CMTs.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Chang, C.C., Tsai, M.H., Liao, J.W., Chan, J.P.W., Wong, M.L. and Chang, S.C., 2009. Evaluation of hormone receptor expression for use in predicting survival of female dogs with malignant mammary gland tumours. *J. Am. Vet. Med. Assoc*. **235**: 391-396.

Clemente, M., Perez-Alenza, M.D., Illera, J.C. and Pena, L. 2010.Histological, immunohistological, and ultrastructural description of vasculogenic mimicry in canine mammary cancer. *Vet. Pathol*.**47**: 265-274.

Devi, S.S., George, A.J., Dhanushkrishna, B., Prasanna, K.S., Radhika, G. and John Martin, K.D. 2022. Histomorphological stratification of stromal types associated with canine mammary tumour. *J. Vet. Anim. Sci*. **53**(4): 688-693.

Dutra, A.P., Granja, N.V.M., Schmitt, F.C. and Cassali, G.D. 2004. c-erbB-2 expression and nuclear pleomorphism in canine mammary tumors. *Braz. J. Med. Biol. Res.* **37**: 1673-1681.

Ellis, M.J., Tao, Y., Luo, J., A'hern, R., Evans, D.B., Bhatnagar, A.S., Chaudri Ross, H.A., Von Kameke, A., Miller, W.R., Smith, I. and Eiermann, W., 2008. Outcome prediction for estrogen receptor–positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst*. **100**:1380-1388.

Geraldes, M., Gärtner, F. and Schmitt, F., 2000. Immunohistochemical study of hormonal receptors and cell proliferation in normal canine mammary glands and spontaneous mammary tumours. *Vet. Rec.* **146**: 403-406.

Gray, M., Meehan, J., Martínez-Pérez, C., Kay, C., Turnbull, A.K., Morrison, L.R., Pang, L.Y. and Argyle, D., 2020. Naturally-occurring canine mammary tumors as a translational model for human breast cancer. *Front. Oncol*. **10**: 617.

Kim, N.H., Lim, H.Y., Im, K.S., Kim, J.H. and Sur, J.H. 2013. Identification of triple negative and basal-like canine mammary carcinomas using four basal markers. *J. Comp. Pathol.* **148**: 298-306.

Muhammadnejad, A., Keyhani, E., Mortazavi, P., Behjati, F. and Haghdoost, I.S., 2012. Overexpression of her-2/neu in malignant mammary tumors; translation of clinicopathological features from dog to human. *Asian Pac J Cancer Prev*. **12**: 6415-21

Pena, L., Gama, A., Goldschmidt, M.H., Abadie, J., Benazzi, C., Castagnaro, M., Díez, L., Gärtner, F., Hellmén, E., Kiupel, M. and Millán, Y. 2014. Canine mammary tumors: a review and consensus of standard guidelines on epithelial and myoepithelial phenotype markers, HER2, and hormone receptor assessment using immunohistochemistry. *Vet. Pathol.* **51**: 127-145.

Ramos-Vara, J.A. 2005.Technical aspects of immunohistochemistry. *Vet. Pathol.***42**: 405-426.

Rutteman, G.R., Misdorp, W., Blankenstein, M.A. and Van den Brom, W.E. 1988. Oestrogen (ER) and progestin receptors (PR) in mammary tissue of the female dog: different receptor profile in non-malignant and malignant states. *Br. J. Can*. **58**: 594-599.

Spencer, L.T. and Bancroft, J.D. 2013. Tissue processing. In Bancroft, J. D., Layton, C. and Suvarna, S.K.(eds), *Theory and Practice of Histological Techniques.*(7th Ed.). Churchill Livingstone, Edinburgh, pp. 114-123.

Suvarna, K., Layton, C. and Bancroft, J. D. 2018*. Bancroft's Theory and Practice of Histological Techniques.* (8th Ed.). Elsevier Health Sciences, Churchill, 584p.

Thuroczy, J., Reisvaag, G.J.K., Perge, E., Tibold, A., Szilágyi, J. and Balogh, L., 2007. Immunohistochemical detection of progesterone and cellular proliferation in canine mammary tumours. *J. Comp. Pathol*. **137**: 122-129.

Tsang, J.Y. and Gary, M.T., 2020. Molecular classification of breast cancer. *Adv Anat Pathol.* **27**:27-35. ■