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Mast cells as prognostic indicator in spontaneous canine mammary carcinomas#

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Abstract

Tumour micro-environment is essential to nourish the cancer stem cells (CSC) which are said to be the major cause to initiate recurrence/ metastasis. Though there are many players, mast cells are said to play a key role in tumour metastasis. A cohort of twenty canine mammary carcinomas (CMC) was analysed for the presence of intra-tumoral and peri-tumoral mast cells, co-relating it with the progression of the disease and exploring the possibility of finding a prognostic indicator. The mast cell density in the recurrent and non-recurrent CMC cases was examined with toluidine-blue staining. It could be appreciated that there is a significant difference between the intra-tumoral mast cell count/density in malignant recurrent and non-recurrent carcinomas. However, such a difference could not be appreciated with the peri-tumoral tissues in both groups. The malignant recurrent carcinomas had less than 10 mast cells/ hpf, on an average from ten different fields, in the intra-tumoral tissues. Survival analysis carried out revealed that the CMC cases with less than 10 mast cells / hpf had a mean survival time of 24 months. This is the first report where the mast cells were studied for prognostic implications in CMC cases of Kerala. The potential role of these cells in the tumour micro-environment needs further investigation and may open avenues that lead to the identification of anti-oncogenic targets.

Keywords: *Mast cells, canine mammary tumour, prognosis*

Canine mammary tumours (CMT) are common in intact, aged female dogs next to skin tumours. The problem of recurrence and metastasis throws a challenge to the therapeutic regimen. Cancer stem cells, which are chemo and radio-resistant are claimed to contribute to

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tumour recurrence (Walcher *et al*., 2020). The conducive environment for the CSC to participate in recurrence/ metastasis is provided by the tumour microenvironment. Many key factors play a role in tumorigenesis and metastasis. One such factor is the mast cell (MC), which releases the necessary chemokines essential for neoangiogenesis and lymphangiogenesis. Angiogenic factors like vascular endothelial growth factor (VEGF) and enzymes like chymase and tryptase which activate the matrix metalloproteinase (MMP) are released by MCs, thereby aiding angiogenesis and metastasis (Derakhshani *et al*., 2019; Komi and Redegeld., 2020). The MC provide an extraembryonic environment nourishing the CSC thereby enabling them to proliferate and invade (Aller *et al*., 2019). When the presence of MC in different tumour subtypes was investigated in human breast cancers, it was found that denser MC infiltration was associated with lesser grades (Glajcar *et al*., 2017). When such an investigation was carried out in CMT it was found that the lower intra-tumoral mast cell density predicted poor prognosis and metastasis. The MC density of <10 cells/hpf predicts the probability of metastasis (Ariyarathna *et al*., 2020).

Materials and methods

The study was conducted by collecting fresh surgically resected tumour samples of CMT cases from the District Veterinary Hospital Thiruvananthapuram and Kollam, Multi-specialty Hospital Thiruvananthapuram, after obtaining the owner's consent. The tissue samples collected in 10% Neutral Buffered Formalin (NBF) were further processed and sectioned. Twenty carcinoma samples were selected for analysis after histopathological examination of the haematoxylin and eosin-stained sections. The cases were subjected to a periodical follow-up for 36 months to record recurrence/metastasis. The tissue sections were stained with 1% Toluidine blue (TB) to enumerate the mast cells (Suvarna *et al*., 2018). The MC which were present within the carcinomas were taken as intra-tumoural MC, while the cells in the connective tissue peripheral to the carcinomas were taken as peri-tumoral. The cells were counted as the number of cells per high power field (hpf). Ten non-overlapping fields of hpf (0.2mm^2) were observed for the enumeration of the MCs in intra and peritumoral areas and the average was taken for statistical analysis. Statistical analysis was carried out using GraphPad Prism version 8 and Microsoft Excel software. Wilcoxon and Mann-Whitney tests were performed to find the significance of mast cell count in intra and peritumoral tissues in recurrent and non-recurrent cases. A log-rank (Mantel-Cox) test was performed to compare the survival curves. Statistical significance was defined as (*) $p \leq 0.05$ and (**) ≤ 0.01. Error bars were given based on calculated standard deviation (S.D) values.

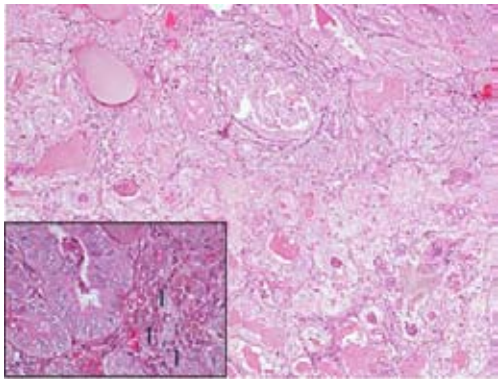


Fig. 1. CMC- Tubular adenocarcinoma- Neoplastic cells arranged as tubules-H&E-100X- (Inset) Fewer mast cells in the intra-tumoral area (blue arrow)-Toluidine blue-H&E-400X. MC marked by arrows.

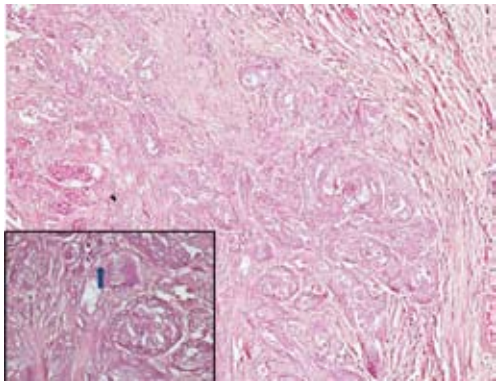


Fig. 2. CMC-Tubulo-papillary adenocarcinoma-Neoplastic cells arranged as tubules and papillae-H&E-100X -(Inset) More number of mast cells in the intra-tumoral area (blue arrows) along with lymphocytes and plasma cells - H&E-400X

Results and discussion

A proper tumour microenvironment (TME) is essential for the CSC to survive and initiate recurrence/ metastasis. Though there are many players, mast cells are said to play a key role in tumour metastasis. The tissue samples subjected to histopathological examination showed characteristics of carcinoma. The representative images of tubular and tubulo-papillary adenocarcinoma with neoplastic cells exhibiting anisocytosis, anisokaryosis, single to multiple nucleoli and eosinophilic cytoplasm arranged in tubular (Fig. 1) and tubulo-papillary (Fig. 2) are given below. The MC were noticed in the intra-tumoral and peri-tumoral areas. Hence, an analysis of the cells using toluidine-blue staining was performed and the average number of cells in ten high-power fields was enumerated. It could be observed that the cells were less in the intra-tumoral areas in metastatic/recurrent cases (Fig.3) when compared to the intra-tumoral areas (Fig. 4) in non-recurrent cases. There was statistical significance in the cell density when the intra and peri-tumoral mast cells were examined (p=0.0020) in metastatic/recurrent carcinomas. The mast cell density was higher in peri-tumoral than intra-tumoral areas in metastatic/recurrent carcinomas (Fig. 5).

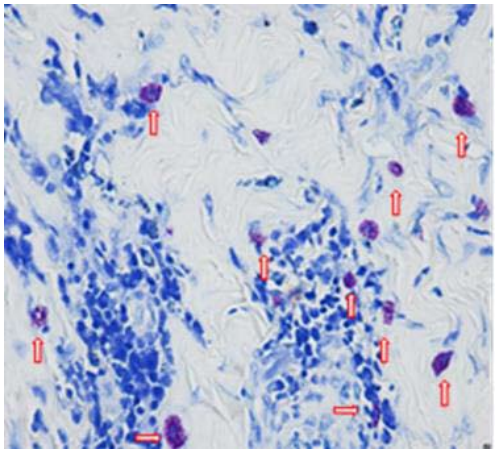


Fig. 3. CMC- Recurrent- Mast cell- Intra-tumoral- Toluidine blue-400X. MC marked by arrows (red).

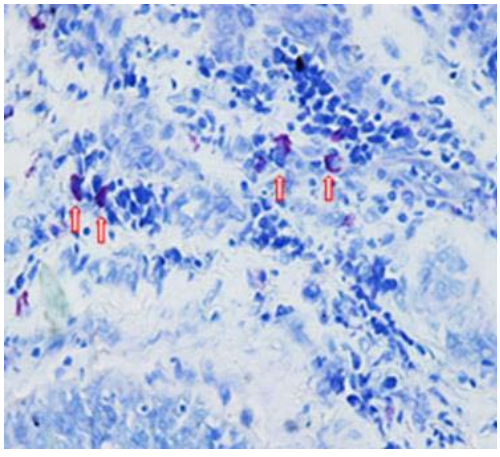


Fig. 4. CMC- Non-recurrent- Mast cell- Intra-tumoral- Toluidine blue-400X. MC marked by arrows (red).

It was found that the metastatic/ recurrent cases had a lower intra-tumoral MC count, against the non-recurrent carcinomas, which showed statistical significance (p=0.0433). However, such a difference could not be appreciated within the intra and peri-tumoral tissues in non-recurrent CMC as well as within the peri-tumoral tissues in both groups (Fig. 6). The malignant metastatic/recurrent carcinomas had <10 mast cells/ hpf on an average from ten different fields in the intra-tumoral tissues. The same has been observed by Glajcar *et al*. (2017), in human breast cancers (HBC) and Ariyarathna *et al*. (2020) in CMC cases. However, Sakalauskaite *et al*. (2022), have reported that higher intra-tumoral MC density has resulted in shorter survival time in CMC cases.

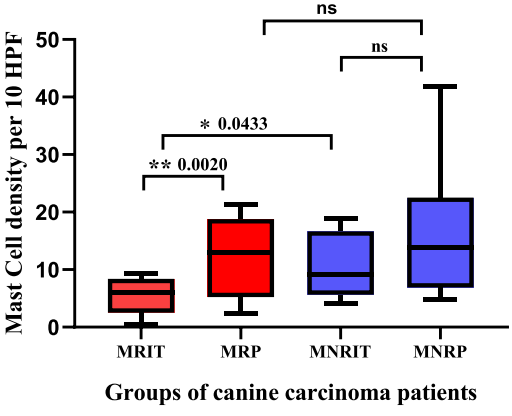
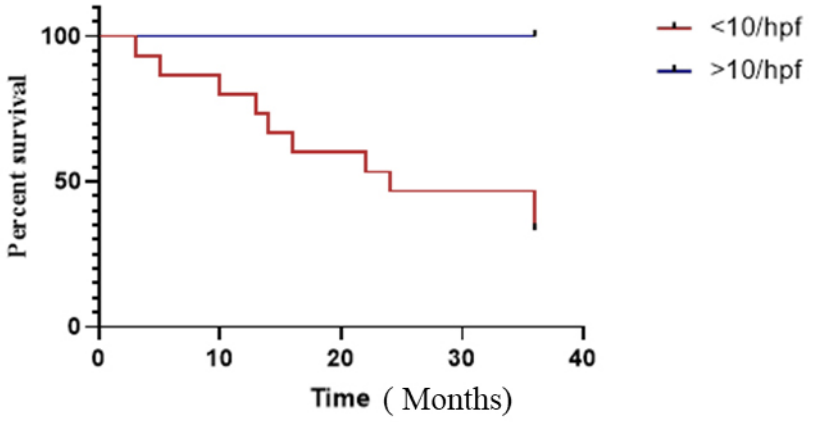


Fig. 5. Analysis of MC density in MRIT- Metastatic/ recurrent intra-tumoral (10 nos.), MRP- Metastatic/ recurrent peri-tumoral (10 nos.), MNRIT- Malignant non-recurrent intra-tumoral (10 nos.), MNRP- Malignant non-recurrent peri-tumoral (10 nos.). (*) p ≤ 0.05 and (**) ≤ 0.01.



Median survival	
<10/hpf	24.00 Months
>10/hpf	Undefined

Fig. 6. Survival curve analysis using Log-rank (Mantel-Cox) test with cases (n = 20) having intra-tumoural MC density <10 cells/ hpf and >10 cells/ hpf.

The decreased density may indicate that degranulation of the MC has taken place as the cells with granules are only stained. The degranulation releases the chemokines and other factors essential for recurrence and metastasis; hence, a lower density is encountered. The release of chemokines which augment neoangiogenesis and lymphangiogenesis facilitates the dissemination process. Keser *et al*. (2017), have reported the possible role of MC in lymphangiogenesis of HBC cases. Apart from releasing the chemokines, the MC provide an extraembryonic niche for the CSCs helping them to proliferate and invade (Aller *et al*., 2019). The potential role of these cells in TME needs further investigation and may open avenues that lead to the identification of anti-oncogenic targets. Survival curve analysis showed that the CMC cases with <10 cells/ hpf have a mean survival time of 24 months (Fig.4). It is noteworthy that all cases which died due to metastasis/ recurrence fell into the group which had MC density of <10 cells/ hpf. This is in contrast to what was observed by Sakalauskaite *et al*. (2022). The different staining procedures using various stains and defining the intra and peri-tumoral areas could lead to variation in observations by different researchers. Hence, research with defined guidelines with a bigger cohort can throw more light on this area.

Conclusion

The MC density in the intra-tumoral region can act as predictor of prognosis, thereby enabling the practitioner to decide the treatment regimen. Similar studies with an increased number of CMC patients and probing the molecular mechanisms and pathways might help in getting more insights into the behaviour and role of MC in the TME.

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Conflict of interest

There is no conflict of interest involved.

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