

Original Research Article

Mast Cell Density and Lymph Node Metastasis in Gastric Carcinoma: Inferring the Role of Mast Cells in the Metastatic CascadeAnand C.D.¹, Shivashekar G.², Muthu S.³, Sundaram A.⁴, John J.J.⁵, Tamaskar S.M.⁶¹Assistant Professor ²Professor & Head ³Associate Professor ⁴Professor & Dean (Medical) ^{5,6}Professor, Department of Pathology, SRM Medical College Hospital & Research Centre, SRM Institute of Science and Technology (SRMIST), Kattankulathur, Tamil Nadu 603203, India.**Abstract**

Introduction: Mast cells in the tumour microenvironment have both pro-tumorigenic and anti-tumorigenic roles and play a major role in determining the progression of the tumour including metastatic potential. The present analysis was undertaken to assess their role in the metastatic potential to regional lymph nodes in gastric carcinoma. *Methods:* Tissue from fifty three cases of gastric carcinoma including fourteen endoscopic biopsies and fourteen gastrectomy specimens were analyzed and routine histological findings were recorded. Mast cells were clearly demonstrated in tissue using Toluidine Blue stain at pH 2.3. Mast cells were counted using an eyepiece grid and expressed as no. of cells/per sq. mm, i.e., mast cell density (MCD). The distribution of mast cells within all the sampled lymph nodes (with and without metastasis) was recorded. *Results:* Mast cell density was not statistically significantly different in regional lymph nodes without microscopic metastatic deposits (n= 71; Mean±SD: 1.5±0.67) compared to lymph nodes showing metastatic deposits (n=74; Mean MCD±SD: 1.2±0.42). *Conclusions:* Our results indicate that the role of mast cells in gastric carcinoma especially metastatic potential to regional lymph nodes is equivocal, in sharp contrast to our previous study in breast carcinoma which indicated a protective role. This could also be explained by the participation of mast cells in the benign inflammatory stages even before the onset of gastric carcinoma, a phenomenon that is not seen in breast carcinoma

Keywords: Mast Cell Density; Gastric Carcinoma; Lymph Node; Mast Cell; Metastasis; Toluidine Blue.

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Introduction

Cancers are one of the leading causes of mortality and morbidity worldwide [1]. Recent advances in diagnosis and treatment of cancers are throwing more light on the tumour microenvironment as

a potential area for identifying new biomarkers for diagnosis and therapeutic targets including targeted therapy and in the era of personalized medicine. The interactions of the tumour with the host immune system are of growing interest to identify therapeutic targets. Inflammatory cells

including cytotoxic T cells, mast cells, macrophages dendritic cells and mast cells have been shown to infiltrate the tumoural stroma. They have been shown to significantly modulate the tumour behavior, growth and progression through complex interactions with the tumour cells [2,3].

The role of mast cells in the tumour environment has been studied extensively and their biological significance has been determined through *in-vitro* and *in-vivo* studies along with clinical correlation. Mast cell derived mediators have both pro-tumorigenic and anti-tumorigenic effects and their net effect on the progression or regression of the tumour is dependent on the stage of the tumour and the site or organ involved. Mast cells also produce mediators necessary for extracellular matrix degradation and facilitate metastasis from the primary malignancies through formation of lymphovascular tumour emboli [4-7].

Gastric carcinomas are the second most common cancer worldwide. The etiopathogenesis of gastric carcinoma is multifactorial including genetic, environmental (including dietary habits) and contributing factors like associated *H. pylori* infection. Over 99% of gastric cancers are adenocarcinomas. Gastric carcinoma is one of the malignancies where the role of mast cells has been found to be difficult to ascertain clearly, though majority of the studies have been found to have a pro-tumorigenic role. Mast cells are found to be increased in gastric tissue associated with benign conditions like inflammation and benign gastric ulcers [9-11]. Chronic inflammation is one of the predisposing factors for gastric carcinoma, hence the role of mast cells in initial stages of the tumour progression associated with angiogenesis, progression of the tumour, metastasis to regional lymph nodes and distant metastasis have been described with varying degrees of association. This unique association with inflammation and malignancy is unique implying complex interaction of the tumour and host immune system in gastric carcinoma [12-14].

Targeted therapy in the form of anti-mast cell therapy and targeted anti-c-KIT therapy is one of the many modalities now being considered in oncological practice [15-18]. Hence, we have endeavoured to analyse the potential pathologic role of mast cells in the metastatic cascade from the primary carcinoma to the regional lymph nodes and compared to the previous studies [19] and also to the authors' previous study [20] on breast carcinoma that showed a protective, anti-metastatic role in the regional lymph nodes.

Subjects and Methods

Study design: The study was conducted at the Department of Pathology after obtaining approval from the Institutional Ethics Committee. Histopathology specimens representing gastric carcinoma received in the laboratory between January 2011 and July 2013 were included in this cross sectional study.

Material: Paraffin-embedded tissue blocks and stained sections from gastric tissue and gastrectomy (total/ subtotal) specimens with lymph node clearance were used. A total of 145 regional lymph nodes were analyzed in this study from 53 cases of gastric carcinoma including 14 gastrectomy specimens.

Methods: Clinical parameters like age, gender and other information were obtained from the referring departments and from hospital records. Previous biopsy reports were also recorded. Gastrectomy specimens with regional lymph node clearance were routinely sampled. The number and size of lymph nodes dissected along with gross evidence of tumour deposits (if any) was recorded.

(i) Histopathological evaluation (H & E): H & E stained tissue sections were evaluated microscopically (OLYMPUS- CX-21; Field area of 0.196 mm²). Routine microscopic parameters in gastric carcinoma and lymph node involvement were recorded: (1) Histological type, (2) Histological grade, (3) Involvement of surgical margins, (4) Lymph node status - number of lymph nodes with metastatic deposits (if any)

(ii) Demonstration of mast cells in lymph node tissue using Toluidine blue stain: Mast cells were demonstrated histochemically on tissue sections on all 145 regional lymph nodes (with and without metastatic deposits from stomach) by staining with 1% acidified toluidine blue solution [17].

a. Material: "Microscopy-grade Toluidine Blue" (Loba Chemie; CI no: 52040; Lot no: S26701111; Dye content- 80%; Solubility- 0.1%) was used for preparing a water clear solution. An electronic pH meter (Eutech Instruments; Catalog No: 35624-02) was used to control the pH.

b. Mast cell counting: Toluidine blue stained sections were microscopically examined immediately along with the corresponding H & E stained slides. Mast cells were identified on sections due to the violet-purple metachromatic staining of their granules against the blue orthochromatic background.

Mast cells were counted on sections using an eyepiece grid (model WF-18). Each side of the large

square represented one millimeter (mm) on the tissue section and used for counting mast cells and the average density was expressed as:

Mast cell density (MCD) = No. of mast cells/ sq. mm area of the tissue section.

Statistical Analysis: Data Analysis was performed using SPSS (Statistical Package for the Social Sciences, v 17.0) software. Mast cell density (MCD) in lymph node sections was compared between the group of lymph nodes showing tumour deposits and the group without tumour deposits. A p-value of less than 0.05 was considered significant.

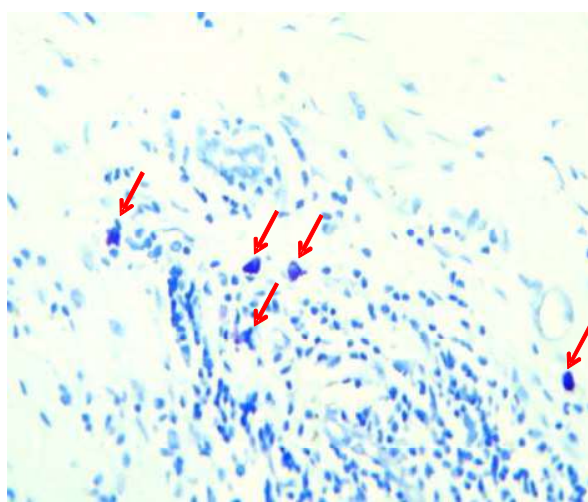


Fig. 1: Mast cells (with violet- purple granules; marked in red arrows) of various shapes seen in regional lymph node in a case of gastric carcinoma that is not involved by tumour (Toluidine Blue stain; X 400)

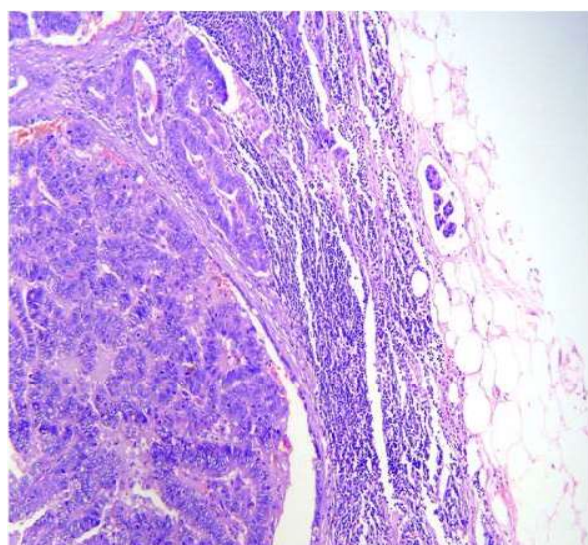


Fig. 2: Metastatic deposits (glandular morphology) in regional lymph node from a case of gastric carcinoma (H &E; X 100)

Results

A total number of 145 lymph nodes were studied from 14 gastrectomy specimens resected for gastric carcinoma with regional lymph node clearance. The mast cells were clearly demonstrated on the lymph node sections by Toluidine blue staining. The mast cell density (MCD) in 74 lymph nodes without metastatic deposit from gastric carcinoma was Mean MCD±SD: 1.2±0.42 (n=74), whereas the MCD in 71 lymph nodes showing metastatic deposits from gastric carcinoma was Mean MCD ±SD = 1.5±0.67 (n =71). The two groups (lymph nodes without metastatic deposits and lymph nodes showing metastatic deposits from gastric carcinoma) did not show a statistically significant difference in the mast cell density (p= 0.2368).

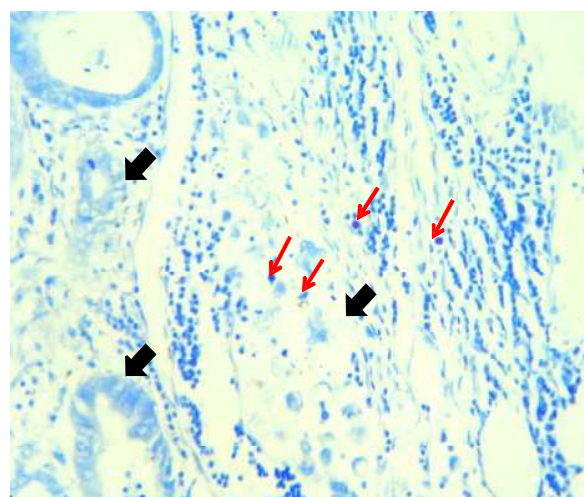


Fig. 3: Mast cells (red arrows; violet-purple granules) in above lymph node with metastatic tumour deposits (black, block arrows) from gastric carcinoma. Mast cells seen both within the tumor area and at the periphery (Toluidine Blue stain; X 400).

Table 1: Comparison of mast cell density in lymph node tissues with and without metastasis from gastric carcinoma

Lymph node status	No. of Lymph nodes	Mean MCD +/- SD
Metastasis present	74	1.2 +/- 0.42
Metastasis absent	71	1.5 +/- 0.67
		p= 0.2368

Discussion

Regional lymph node metastasis is an important and often independent prognostic factor in malignancies including gastric carcinoma and the number of positive nodes also determines the staging (TNM) which is a crucial parameter in further management of the patient.

The metastatic cascade is a sequence of events within the tumour and its interaction with the cellular, soluble and structural factors within the microenvironment, many of those factors being derived from mast cells. Pro-tumorigenic or pro-metastatic contribution of mast cells in this include enzymes like tryptase, chymase are involved in tissue damage by digestion of extracellular matrix allowing tumour cells to migrate to adjacent lymphatic and vascular channels and subsequent formation of tumour embolization. One of the potent anti-tumorigenic or anti-metastatic functions of mast cells has been explained by chondroitin sulfate which acts a decoy and prevents metastases [4-7].

Many studies on MCD in gastric carcinoma suggest a pro-tumorigenic role of mast cells in gastric carcinoma through measurement of MCD in primary gastric tumour tissue. The specific sequence of events in the metastasis and its influence by mast cell derived mediators warranted a specific analysis of MCD in lymph node tissue as a separate parameter [9-12,18,19]. The correlation of MCD within regional lymph nodes (without metastasis and with metastatic deposit) of gastric carcinoma could vary from the correlation MCD in the primary gastric tumours and its clinicopathological correlation due to its complex inflammation associated etiopathogenesis.

The results from the present study of 175 regional lymph nodes from gastric carcinoma cases do not show a statistically significant difference in MCD between the lymph nodes without metastasis (n=74) and lymph nodes with metastasis (n=71), irrespective of the microscopic extent of metastasis within the lymph node. If we were to follow the hypothesis of the pro-tumorigenic of mast cells in gastric carcinoma as per literature, an expectation of difference in MCD in lymph node tissue among between the two groups could have been expected. But the values in both groups were almost identical with minimal Standard Deviation (SD)

The present study suggests that the mechanisms involved in regional lymph node metastasis in gastric carcinoma could be an independent factor irrespective of other clinico-pathological criteria like histological grade and depth of invasion. The near equal MCD in both lymph nodes with and without metastasis is suggestive of an equivocal role or the possibility of a balance of both pro-tumorigenic and anti-tumorigenic role of mast cell derived mediators.

However, our earlier study [20] evaluating mast cell density in axillary lymph nodes in carcinoma

breast showed a clear protective role with significantly higher MCD in lymph nodes without metastasis compare to those with metastatic deposit, irrespective of the microscopic extent of the metastatic deposits which was also aligned with the results of Naik et al.

Conclusion

Regional lymph node metastasis is an important prognostic factor in many solid organ malignancies including gastric carcinoma. The understanding of mast cell function in the tumour progression and pathology is very important, especially in the context of using anti-mast cell therapy against its pro-tumorigenic role or conversely immunotherapy or immunomodulation to enhance the anti-tumorigenic role. The results of this study is suggestive of an equivocal or balancing role for mast cells and its mediators and possibly other unknown independent factors in lymph node metastasis from gastric carcinoma unlike the clear anti-tumorigenic ascertained in our earlier study in breast carcinoma. Hence, the inferred role of tumour associated mast cells is dependent on local factors and the switch between pro-tumorigenic and anti-tumorigenic roles and its balance seems to depend on the stage of tumour progression and could also be based on the site/organ involved. *In-vivo* studies in animal models are required for elucidating the exact function of mast cells in particular stages of tumours.

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Key Messages

Mast cells, through their mediators play an important role in outcome of tumour progression, metastasis and are now considered therapeutic targets. Their potential role in lymph node metastases from primary gastric carcinoma has been evaluated

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