

# The role of tumour infiltrating mast cells (TIM) in gastric carcinoma remains an enigma: clinicopathological correlation of mast cell density

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## Abstract

**Background:** Tumour infiltrating mast cells (TIM) may have pro-tumorigenic and anti-tumorigenic roles based on the mediators released and the outcome of that balance at any given stage could determine the net effect on the progression of the cancer. Their pro-tumorigenic role has been documented in many cancers including prostatic cancer and a beneficial role in breast cancer. Their exact role in gastric cancer is still not very clear. Hence the present study was undertaken to make an attempt to infer the role of tumour infiltrating mast cells in gastric carcinoma by measuring mast cell density and correlating with clinicopathological parameters. **Methods:** Tissue from fifty one cases of gastric carcinoma were analysed 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 in the tumour tissue using an eyepiece grid and expressed as no. of cells / per sq. mm, i.e., mast cell density (MCD). Statistical correlation of the mean Mast cell density (MCD) to clinical parameters like age, gender, location of lesion and pathological parameters like histologic type, grade, depth of invasion, lymph node status were analysed for statistical significance. **Results:** MCD was statistically significantly higher (Mean MCD +/- S.D: 3.48 +/- 2.31) in patient group above 60 years of age compared to patient group below 60 years. MCD was statistically significantly increased in well differentiated adenocarcinoma (Mean MCD +/- S.D: 7.2 +/- 3.55) stomach compared to moderately differentiated adenocarcinoma and poorly differentiated adenocarcinoma. However, no significant difference in MCD was observed in primary gastric tumour tissue in cases with metastatic deposits in regional lymph nodes and cases without lymph node metastasis. **Conclusions:** Our results indicate that the role of mast cells in gastric carcinoma could be pro-tumorigenic in the early stages especially during angiogenesis with an increase in MCD in well differentiated tumours and a relative decrease of mast cells in higher histological grades. The absence of any significant difference of MCD in lymph node positive (for metastatic deposit) and lymph node negative group may indirectly indicate no significant role in later stages of the cancer. These results indirectly show that a balance between pro-tumorigenic and anti-tumorigenic factors is involved in the pathogenesis and progression of gastric carcinoma. The role of mast cells in inflammatory and ulcerative gastric lesions which could be precursor lesions to gastric carcinoma also needs to be considered.

**Keywords:** Mast cell, Gastric carcinoma, Adenocarcinoma, Toluidine blue

## Introduction

Cancers are a leading cause of mortality and morbidity in spite of rapid advances in the diagnosis and treatment [1]. Mast cells are potent effector cells of the immune system that infiltrate the tumour stroma and periphery of the tumours along with other inflammatory cells like cytotoxic T cell subsets, macrophages and fibroblasts.

The diverse mediators derived from mast cells are reported to have both pro-tumourigenic and anti-tumourigenic effects. The balance between these opposing mechanisms determines their net effect on the progression or regression of the tumour at any given stage [2-4]. Tumour infiltrating mast cells (TIM) have been shown to have varied prognostic significance in many solid organ and haematolymphoid malignancies. In fact, presence of mast cells is now considered an independent prognostic marker in certain cancers.

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Mast cells are also being considered a predictive marker in certain malignancies where therapeutic approaches targeting mast cells and/or mast cell mediators could be effective [5-7]. In such cancers, mast cell density and distribution could enable identifying patients likely to respond to targeted anti-mast cell therapy. The detrimental or beneficial effects of mast cells in benign gastric lesions and gastric malignancies are still an ongoing debate in the scientific community with the number of studies done in Indian population were very less [8-11]. This study was undertaken to infer and report the clinicopathological significance of the density of mast cells in the tumour compartment in cases of gastric carcinoma.

### Materials and Methods

**Place and Type of Study-** The study was conducted at the Department of Pathology, SRM Medical College Hospital & Research Centre, Kattankulathur, Tamil Nadu, India during the time period June 2011 and July 2013. This cross-sectional study was carried out after obtaining approval from the Institutional Ethics Committee.

**Inclusion criteria-** Cases with histopathological specimens (endoscopic biopsy specimens and gastrectomy specimens) of gastric malignancies between June 2011 and July 2013 with adequate clinical data.

**Exclusion criteria-** Cases with inadequate material and those cases in which the slides/ blocks were not available were also excluded.

**Sample Collection and sampling methods-** A random sampling of histopathology specimens representing gastric malignancies received in the laboratory were included in the study. A total of fifty one cases (thirty seven endoscopic biopsy and fourteen gastrectomy

### Results

**(a) Mast cell density in normal gastric tissue and neoplastic gastric tissue:** The fifty one cases of gastric carcinoma selected for the study included 37 endoscopic mucosal biopsies and 14 gastrectomy specimens. When the mast cell density (MCD) in gastric carcinoma tissues were compared with the normal gastric tissues, the mean MCD was significantly higher in gastric carcinomas compared to normal gastric tissue (Table 1).

**Table-1: Mast Cell Density in Normal and Neoplastic Gastric Tissue**

Tissue Examined	No. of Cases	MeanMCD +/- SD
Control*	10	1.22 +/- 0.42
Gastric carcinoma	51	3.77 +/- 2.76
		<b>p=0.0048**</b>

\*Normal gastric tissue taken 5 cm away from tumour in gastrectomy specimens

\*\* Statistically significant

specimens) were included in the study. Clinical parameters like age, gender and other information were obtained from the referring departments and from hospital records. Paraffin-embedded tissue blocks and histopathological examination of H & E stained section were studied and diagnosis was recorded.

**Demonstration of mast cells in lymph node tissue using Toluidine blue stain:** Mast cells were demonstrated histochemically on tissue sections on all cases by staining with 1 % acidified toluidine blue solution at pH 2.3 [12, 13].

**a. Material:** "Microscopy- grade Toluidine Blue" (LobaChemie; 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 Methods:** Data Analysis was performed using SPSS (Statistical Package for the Social Sciences, v 17.0) software. A p-value of less than 0.05 was considered significant.

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**(b) Gastric Carcinoma– Mast Cell Density & Age-** The mean age of gastric carcinoma patients was 51.3 with a peak incidence in the fifth decade (n= 17; 31. 4 % of cases) (Fig.1).

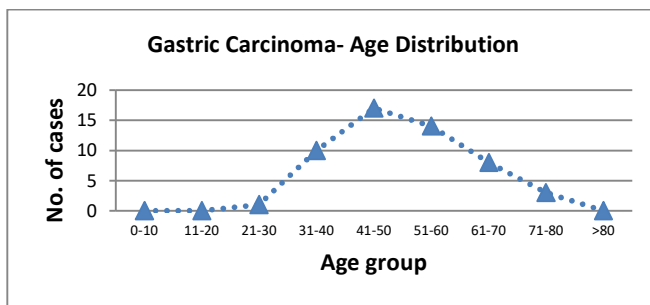


Figure-1: Age distribution of Gastric carcinoma patients

The mean mast cell density in gastric carcinoma patients greater than 60 years age was higher compared to patients in the less than 60 years age group (Table 2). This difference was found to be statistically significant (p-value = 0.0428)

Table-2: Gastric Carcinoma: Mast Cell Density and Patient Age

Age Group	No. of Cases	Mean MCD +/- SD
< 60 years	38	3.48 +/- 2.31
> 60 years	13	5.36 +/- 3.75
		<b>p =0.0428*</b>

\* Statistically significant

**(c) Gastric Carcinoma– Mast Cell Density & Gender-** Among the 51 cases of gastric carcinoma, 33 were male patients (64.8%) and 18 were female patients (33.3%) with a male: female ratio of 1.9: 1 (Table 3)

Table-3: Gastric Carcinoma: Mast Cell Density and Gender

Gender	No. of Cases	Mean MCD +/- SD
Male	33	3.94 +/- 3.17
Female	18	3.41 +/- 1.77
		<b>p=0.5241</b>

No significant difference in mast cell density was observed between male and female patients

**(d) Gastric Carcinoma– Mast Cell Density & Gross Morphology-** We observed the gross morphology in cases with gastrectomy specimen (n=14). Majority of the 14 resection specimens available to assess gross morphology were of polypoidal type (n= 9;64%). Four cases were of ulcerated type (n=4; 28%) and one case showed diffuse infiltrative pattern (8%). No significant difference in mast cell density was observed between tumours with polypoidal morphology and ulcerated morphology. One case showing a diffuse infiltrative growth pattern had a mast cell density of 12 which was higher than the mean MCD of both polypoidal and ulcerated morphology groups.

**(e) Gastric Carcinoma– Mast Cell Density & Histological type-** Histologically most cases of gastric carcinoma were tubular adenocarcinomas (n=45; 88.6%). Mucinous carcinoma and signet ring cell carcinoma were three cases each (5.7% each) (Table 4).

Table-4: Gastric Carcinoma: Mast Cell Density And Histological Type

Histological Type	No. of Cases	Mean MCD +/- SD
Adenocarcinoma- Tubular	45	3.79 +/- 2.9
Mucinous Carcinoma	3	4.0 +/- 1.73
Signet ring cell carcinoma	3	3.33 +/- 1.15
		<b>p=0.758</b>

No statistically significant difference in mast cell density was observed between the three histological types

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**(f) Gastric Carcinoma– Mast Cell Density & Histological Grade-** Histological grading was done in all the gastric tubular adenocarcinoma cases (n=47). Most cases were moderately differentiated adenocarcinomas (n=21; 44.7%).

**Table-5: Mast Cell Density and Histological Grade (Gastric Adenocarcinoma–Tubular)**

Histological Grade	No. of Cases	Mean MCD +/- SD
Well differentiated	7	7.2 +/- 3.55
Moderately differentiated	21	3.14 +/- 2.03
Poorly differentiated	19	2.82 +/- 1.59

The mean MCD in well differentiated adenocarcinomas was more than 2-fold higher compared to mean MCD in moderately differentiated and poorly differentiated adenocarcinomas

**(g) Gastric Carcinoma– Group-wise comparison of Histological grade and Mast Cell Density-** Results of Group-wise comparison of Histological grade and Mast Cell Density are represented in Table 6.

**Table-6: Group-wise Comparison of Mast Cell Density and Histological Grade of Gastric Adenocarcinoma (tubular)**

Histological Grades Compared	p-value
Well differentiated Vs Moderately Differentiated	<b>0.0003 *</b>
Well differentiated Vs Poorly Differentiated	<b>&lt; 0.0001*</b>
Moderately differentiated Vs Poorly differentiated	<b>0.562</b>

\* Statistically significant

1. The difference in MCD between “well differentiated” group and “poorly differentiated” group was of statistical significance (p <0.0001).
2. The difference in MCD between “well differentiated” and “moderately differentiated” groups was also statistically significant (p=0.0003).
3. No statistically significant difference in MCD was observed between moderately and poorly differentiated groups

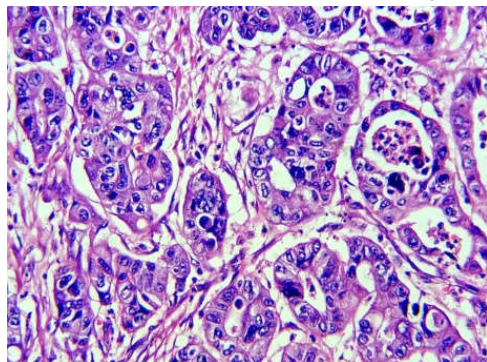
**(f) Gastric Carcinoma– Mast Cell Density & Depth of Invasion by tumour (pT)-** Depth of invasion of the tumour was accessed in the gastrectomy specimens. Most cases (n=6) were under the T4 group, followed by T2 (n=4), T3 (n=3) and T1 (n=1) (Table 7).

**Table-7: Correlation of Mast Cell Density with Depth of Invasion (pT) in Gastric Carcinoma (gastrectomy specimens)**

Depth of invasion (T) of Gastric carcinoma	No. of cases	Mean MCD +/-SD
T1	1	4
T2	4	4.75 +/- 2.63
T3	3	7.0 +/- 4.36
T4	6	4.75 +/- 2.63
		<b>p=0.758</b>

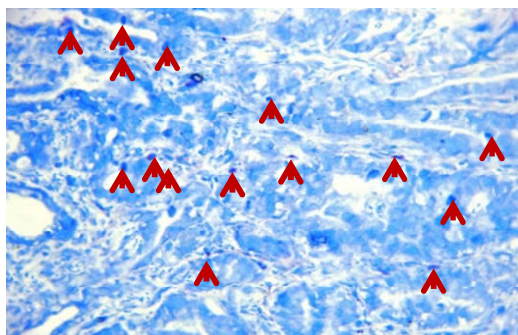
No statistically significant difference in MCD was observed between gastric carcinomas with different depths of microscopic invasion.

**(g) Gastric Carcinoma– Mast Cell Density & Status of lymph node metastasis-** Among the 14 cases of gastric carcinoma (gastrectomy specimens) in which lymph node metastasis stasis were exactly known, 10 cases showed metastasis. No statistically significant difference in the MCD of primary tumours was observed between node negative and node positive groups. The ten cases which showed lymph node metastasis were further grouped as N1, N2, N3 based on the number of lymph nodes involved. No statistically significant difference in the MCD of primary tumours was observed between N1, N2 and N3 groups.



**Figure-2: moderately differentiated gastric adenocarcinoma**

**Tumour composed of neoplastic glands lined by malignant cells and infiltration into stroma (H &E; x 400)**



**Figure-3: Mast cells within tumoral area in gastric adenocarcinoma (moderately differentiated)**

**Mast cells (arrow) visualized by violet-purple granules (metachromatic staining) against a blue background (Toluidine Blue stain; x 400)**

## Discussion

Mast cells were clearly demonstrated in tissue sections using toluidine blue staining method and mast cell density was found to be significantly higher in neoplastic tissue of stomach compared to normal tissue, the difference being statistically significant (Table 1). This highlights their potential interaction with tumour cells and the stroma in the tumour microenvironment as observed in other studies.

### Correlation of mast cell density (MCD) with clinicopathological parameters

**Patient Age:** Gastric carcinoma is generally a disease with higher incidence in the older age group. Jiyang *et al* [14] compared mast cell density in gastric carcinoma between age group less than 60 years and age group greater than 60 yrs. A higher MCD was found in patients over 60 years of age and the association was found to be statistically significant. In the present study, the same grouping method was followed and the mean MCD in age group greater 60 years was significantly higher than in age group less than 60 years, the difference between these groups being statistically significant (Table 2). This correlates with the previous report.

**Gender:** A male: female ratio of 1.8: 1 was observed in the present study similar to reports from literature [14, 15]. No significant difference in the mast cell density between male and female patients was noted (Table 3).

**Morphologic appearance of the tumour:** Grossly, a polypoidal growth pattern has been found to have a better prognosis compared to a diffuse growth pattern. The present study did not find significant correlation of mast cell density with the various gross morphologic appearances of the tumour.

However the present study had only one case presenting with diffuse gastric wall involvement out of 14 gastrectomy specimens. Previous studies had not included morphologic appearance as a parameter for correlation with MCD [14-16].

**Histological type:** Previous reports (Jiyang *et al*, Yano *et al*) did not find significant association of MCD with specific histologic types of gastric carcinoma. The present study also did not show significant association between mast cell density and different histological types of gastric carcinoma (Table 4).

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**Histological grade:** The present study revealed a statistically significant correlation between mast cell density and histological grade of gastric carcinoma. The mean MCD in well-differentiated adenocarcinoma of stomach was significantly higher compared to moderately or poorly differentiated adenocarcinoma (Table 5). The differences between these groups were statistically significant (Table 6). This is consistent with previous studies.

There was no significant difference of MCD between moderately and poorly differentiated adenocarcinoma. A study by Mukherjee *et al* [10] that included 22 cases of gastric carcinoma in endoscopic biopsy tissues found similar differences between well differentiated adenocarcinoma and other grades. Their studies had also shown a significant difference in mean MCD between moderate and poorly differentiated adenocarcinoma. However the present study did not reveal a significant difference between these groups, although the sample size was twice than studied in the previous report.

**Depth of invasion of primary tumour (T):** Ribatti *et al* [16] had shown that patients with T4 level of invasion had a higher MCD compared to those with T2 and T3. However the present study showed no significant correlation of MCD with these parameters (Table 7).

**Lymph node status:** Regional lymph node metastasis has been shown to a poor prognostic factor in gastric carcinomas. Ribatti *et al* showed significantly higher number of mast cells in gastric tissue in the lymph node positive group. In the present study the mean mast cell density in the primary gastric tumour did not have significant difference between lymph node negative group and lymph node positive group

**Number of lymph nodes with metastasis:** The present study did not show significant difference in mast cell density of the primary gastric tumour between patients with N1 (1-2 lymph nodes involved), N2 (3-6 lymph nodes involved) and N3 (>7 lymph nodes involved) stage. Previous reports [16, 17] have shown that mast cell density in primary gastric tumour was higher in N3 patients compared to N2 in whom MCD was higher compared to N1.

### Conclusion

The present study has shown the utility of Toluidine Blue staining as a simple and economical method to assess mast cell density in the tumoural tissue of gastric carcinoma and to infer the clinicopathological significance of those results. Our results indicate that

the role of mast cells in gastric carcinoma could be pro-tumorigenic in the early stages especially during angiogenesis with an increase in MCD in well differentiated tumours and a relative decrease of mast cells in higher histological grades. The absence of any significant difference of MCD in lymph node positive (for metastatic deposit) and lymph node negative group may indirectly indicate minimal or no significant role in later stages of the cancer.

These results indirectly show that a balance between both pro-tumorigenic and anti-tumorigenic factors released by tumour infiltrating mast cells (TIM) is involved in the pathogenesis and progression of gastric carcinoma. The role of mast cells in inflammatory and ulcerative gastric lesions which could be precursor lesions to gastric carcinoma also needs to be considered.

A more precise evaluation of the role of mast cells in gastric carcinoma can be further ascertained by larger patient groups and *in-vitro* studies for assessing mast cell function. Evaluation of specialized mast cell subsets in these cases and inferring their specific functions can be more accurately assessed using immunohistochemical (IHC) methods.

**Contribution by different authors-** Dr. C.D. Anand contributed to conception of the study, study designing, literature search and review, experiment optimizing, performing the experiments, data acquisition and analysis, statistical analysis, manuscript preparation and editing; Dr. G. Shivashekar contributed to study designing, data analysis, manuscript preparation and editing; Dr. S. Muthu contributed to data analysis, statistical analysis, manuscript preparation and editing; Dr. Kalaivani Amitkumar contributed to histological data generation and data analysis; Dr. A. Sundaram contributed to enabling mast cell counting methodology, data analysis, manuscript preparation and editing; Dr. S.M. Tamaskar contributed to study designing, review of literature and data analysis.

**Additional knowledge gained from the study-** The data from the present study adds new insights to existing knowledge in this area, especially in Indian population by indicating that the role of mast cells in gastric carcinoma is more of a balance between both pro-tumorigenic and anti-tumorigenic role depending on the stage of the tumour, rather than just a pro-tumorigenic role. The present study has analysed more number of cases of gastric carcinoma and also resection specimens compared to previous studies done in India involving gastric carcinoma and mast cell density.

**Findings:** Nil; **Conflict of Interest:** None initiated  
**Permission from IRB:** Yes

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