Tumor Infiltrating Mast Cell Density in Gastric Adenocarcinoma: Association with Clinicopathological Parameters

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Abstract

Background: Tumor-infiltrating mast cells have been reported to play functional roles in the tumor microenvironment. However, controversial evidence exists regarding their impact on different cancers. The present study aimed to determine tumor-infiltrating Mast Cell Density (MCD) in gastric adenocarcinoma and its relationship with clinicopathological parameters.

Materials and methods: Fifty cases of gastric adenocarcinoma were included in this study from the Department of Surgery of Chittagong Medical College Hospital from September 2020 to June 2022. Gastrectomy specimens were sectioned and stained with routine Hematoxylin and Eosin stain. Toluidine blue stain was used to calculate MCD.

Results: The mean age of the patients was 56.9±9.6 years (Range: 30-75), 56% were male, and the mean MCD was 7.13±2.85. Mean MCD was similar in patients aged <60 years and 60 years (p=0.186) and in male and female patients (p=0.654). MCD was statistically significantly increased in poorly differentiated tumors (11.25±1.24) than in moderately differentiated (6.11±1.24) and well-differentiated (3.85±1.18) adenocarcinoma. Increasing MCD was observed with the higher tumor stages (4.93±1.48, 7.17±3.02, and 7.54±2.80 in tumors with pT1, pT2, and pT3 stage, respectively, without any statistical significance (p=0.175).

Conclusion: The study suggests that mast cells play a role in the tumor development and progression in gastric adenocarcinoma. Understanding the role of mast cells in this context can help develop targeted therapies and improve patient outcomes.

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Introduction

Stomach cancer is one of the most commonly diagnosed cancers in humans, and adenocarcinoma accounts for the majority of gastric cancer cases.[1] Several parameters, such as histological type, grade, stage and lymph node involvement, are considered to be prognosticfactors.[2] Problems arise because gastric carcinoma exhibits diverse biological behavior, while some parameters, such as tumor type and histologic grade, are prone to the observer's subjectivity. In this context, the search for more objective prognostic factors is ongoing.[1] Mast Cell Density (MCD) is a widely researched parameter in various human malignancies, including gastric cancer, and has been suggested as a prognostic factor.[3]

Mast Cells (MCs) are a group of innate immune cells that have profound immunomodulatory effects on tumor progression, such as angiogenesis, tumor microenvironment reconstruction and interaction with other immune cells.[4-8] Mast cells secrete proangiogenic factors like Vascular Endothelial Growth Factor (VEGF) Fibroblast growth factor 2, Platelet-derived growth factor, and interleukin-6 and proteases, such as chymase and tryptase, promote angiogenesis. MCs support tumor invasion by secreting matrix metalloproteinase.[9] Mast cells help to modulate blood vessel growth during tumor progression.[10] By secreting immunosuppressants like histamine, tumor necrosis factor-α, transforming growth factor-β and interleukin-10, MCs may contribute to tumor growth.[11]

Due to their role in tumor angiogenesis, MCs targeted therapy might be an attractive strategy for anti-cancer treatment. In the clinical trial, the most commonly used treatments against MCs in cancer are tyrosine kinase inhibitors (Imatinib and masitinib) for c-Kit receptor-targeted action and

MC tryptase inhibitors (Gabexate mesylate, nafamostat mesylate and tranilast).[12] In gastric cancer, MCs have recently been recognized as a novel independent prognostic marker and MCs targeted therapy has been recognized as a potential therapy for this disease.[13] However, the effect of mast cells on tumor progression is contradictory and seemingly dependent on the cancer type.[3,14-16] Moreover, the phenotype, functional regulation, and clinical correlation of MCs in the human gastric adenocarcinoma microenvironment remain a matter of debate.[6]

Therefore, the present study aimed to investigate the relationship between tumor-infiltrating MCD and the context of clinicopathological parameters in gastric adenocarcinoma.

Materials and methods

This analytical cross-sectional study was conducted at the Department of Pathology, Chittagong Medical College, Chattogram, and the Department of Pathology, BSMMU, Dhaka, from September 2020 to June 2022. Before starting this study, ethical clearance was obtained from the Ethical Review Board of Chittagong Medical College (Memo No. CMC/PG/2022/8/0, Date: 07/03/2022). Fifty conveniently selected patients who were histopathologically diagnosed with gastric adenocarcinoma were included in this study. Patients with recurrent cases of gastric adenocarcinoma and those who had received chemo or radiotherapy for gastric adenocarcinoma were excluded.

Demographic, clinical, and histopathological information were collected using a structured case record form containing all the variables of interest. Tumors were graded into well-differentiated, moderately differentiated, and poorly differentiated adenocarcinoma. Tissue processing was done in the laboratory of the Department of Pathology of Chittagong Medical College following standard protocol. The resected gastrectomy specimens were fixed in 10% formalin.The paraffin blocks were sectioned with a rotary manual microtome at 5-micrometer thickness. Two tissue sections were taken from each paraffin block. One section was stained with routine H & E stain in the laboratory of the Department of Pathology of Chittagong Medical College. Another section was stained with 1% toluidine blue stain in the laboratory of the Department of Pathology of BSMMU, Dhaka.

For the determination of MCD, toluidine blue stained sections were screened at low power (100X) to identify the areas of the hotspots (areas with the most significant number of mast cells). MCs counting was performed at high power (400X) magnification in three randomly chosen fields in the hot spot areas. The MCs count was expressed as the number of MCs per high power field (n/hpf). The average figure obtained in the hot spot fields was considered the MCD for a given case.[17] For control, skin was used. MCD= number of mast cells/high power field (n/hpf). Patients with MCs counts below the cutoff value were classified as “Low MCD” and patients with counts above this cutoff value were classified as “High MCD”.[18]

After collection, data were entered into an Excel sheet to generate a master sheet. Further data processing and analyses were conducted using SPSS (Statistical Package for Social Sciences) version 28.0 for Windows. Quantitative data were reported as the means ± SD and categorical data were reported as counts and percentages. Unpaired t-test and one-way Analysis of Variance (ANOVA) test were used to evaluate the association between MCD and the clinicopathological parameters. p value <0.05 was considered statistically significant.

Results

The age ranged between 30 and 75 years in the present study. Out of a total of 50 cases, most of the cases 29 (58%) belonged to the age group of 40-60 years, with a mean age of 56.98 ± 9.55 years and 28 (56%) patients were male (Table I). More than half (60%) of the tumors were moderately differentiated (Grade II) tumors, followed by poorly differentiated (26%) and well-differentiated tumors (14%). Regarding pathological stage, 26(52%) patients were at stage pT3. Stage pT2 comprised 19(38%) cases and stage pT1 were found in 5(10%) cases. Only 13(26%) cases had lymphovascular invasion.

Table I Tumor characteristics of the 50 cases of gastric adenocarcinoma

Characteristics	Count	Percent (%)
Age groups		
<40 years	4	8.0
40-60 years	29	58.0
>60 years	17	34.0
Sex		
Male	28	56.0
Female	22	44.0
Tumor grade		
Well- differentiated	7	14.0
Moderately differentiated	30	60.0
Poorly differentiated	13	26.0
Tumour stage		
pT1	5	10.0
pT2	19	38.0
pT3	26	52.0
Lymphovascular invasion		
Present	13	26.0
Absent	37	74.0

Table II shows that among all the patients of the present study, the mean MCD was (7.13 ± 2.85). The cutoff value of MCD was 6.66 (the median value of mast cell counts among 50 cases). High MCD was found in 33 (66%) cases and low MCD was found in 17 (34%) cases.

Table II MCD in 50 cases with gastric adenocarcinoma

MCD	Count	Percent (%)
Low MCD	17	34.0
High MCD	33	66.0
Mean±SD MCD	7.13±2.85	
Median MCD	6.66	
Range (Min-Max)	2.00-12.66	

Table III shows a significant association between MCD and grades of tumor, and the mean MCD increased with higher grades (Figure 1-3). A similar significant association was found between MCD and lymphovascular invasion, with higher MCD in tumors with lymphovascular invasion than their counterpart. There was no association of MCD with age, sex, and stage of the tumor.

Table III Clinicopathological factors associated with MCD in the hot spots of gastric adenocarcinoma (n=50)

Variables	MCD (Mean±SD)	p value
Age groups		
≤60 years	7.64 ±3.15	0.186*
>60 years	6.56 ± 2.39	
Sex		
Male	7.31 ± 2.64	0.654*
Female	6.94 ± 3.14	
Tumor grade		
Well- differentiated	3.85 ± 1.18	<0.001†
Moderately differentiated	6.11 ± 1.24	
Poorly differentiated	11.25 ± 1.24	
Tumor stage		
pT1	4.93 ± 1.48	0.175†
pT2	7.17 ± 3.02	
pT3	7.54 ± 2.80	
Lymphovascular invasion		
Present	8.67 ± 2.75	0.024*
Absent	6.61 ± 2.72	

*Unpaired t test, †ANOVA test

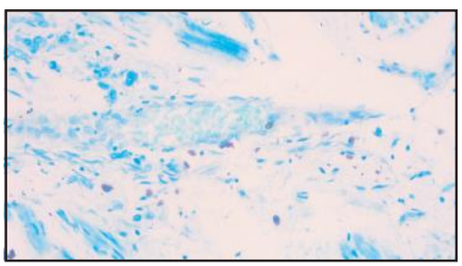

Figure 1 Photomicrograph showing MCs in well-differentiated (Grade I) gastric adenocarcinoma (toluidine blue, 400x)

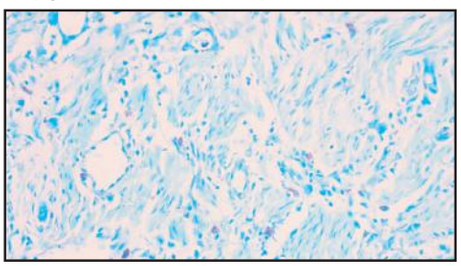

Figure 2 Photomicrograph showing MCs in moderately differentiated (Grade II) gastric adenocarcinoma (Toluidine blue, 400x)

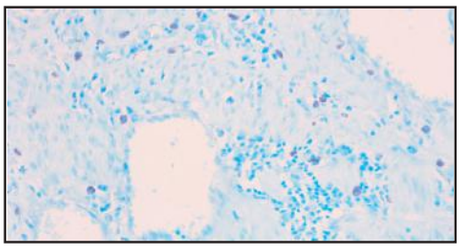

Figure 3 Photomicrograph showing MCs in poorly differentiated (Grade III) gastric adenocarcinoma (Toluidine blue, 400x)

Discussion

The detrimental or beneficial effects of MCs in gastric carcinomas are still an on going debate in the scientific community with the number of studies done in Bangladeshi population were lacking. MCDs varied in different cancers and were heterogeneous within the same tumor. Therefore, areas of the greatest MCD were counted as "Hot Spots." In this study, the average MCD was 7.13 ± 2.85, and with the cutoff value of median MCD (6.66) high MCD was found in 66% of cases. MCs were first identified in small groups of patients with stomach cancer more than 50 years ago in Italy.[19] MCD was also found to increase in gastric cancer patients compared to microscopically normal tissue in Japan.20 MCs in gastric cancer were found to be chymase+, and it was suggested that patients with a high number of MCs had a poor prognosis.[20,21]

Most of the patients with gastric adenocarcinoma in the present study were males in the older age group, who had been predominately diagnosed with gastric cancer in the advanced stage with moderate or poor differentiated grades and with lymphatic vascular invasion in more than a quarter of patients. MCD had no relation with the age and sex of the present studied patients, which was consistent with previous studies.[18,22]

The present study revealed a statistically significant correlation between MCD and the histological grade of gastric adenocarcinoma. The mean MCD in poorly differentiated gastric adenocarcinoma was significantly higher compared to moderately or well-differentiated adenocarcinoma. The differences between these groups were statistically significant. Regarding tumor stages, the highest MCD was observed in patients with pT3 and the lowest in patients with pT1. Still, probably due to a small sample in the pT1 stage (n=5) the association failed to reach statistical significance. It has been found that within gastric carcinoma, MCs could play a positive role in promoting tumor progression. Lv et al. found that the percentage of tumor-infiltrating MCs was significantly increased at advanced stages of the tumor, with a high percentage of MCs positively correlating with poor overall survival of patients with stomach cancer.[13] The study of Nam et al. demonstrated increased MCD in positive VEGF-expressed gastric tumors and observed an increasing MCD with a higher histologic grade of tumor.[15] The results of the study of Ribatti et al. showed that stage IV gastric carcinoma has a higher degree of vascularization than other stages and that MCD increases in parallel with malignancy grade and is highly correlated with the extent of angiogenesis.[14] In contrast, two previous studies by Anand et al. and Mukherjee et al. found that MCD MCD is increased in well-differentiated gastric cancers.[22,23] In this study, in the poorly differentiated cancer group, the MC-mediated anti-tumor response was lacking, with no clear explanation.[22,23]

In the present study, higher MCD was observed with the presence of lymphatic vascular invasion. Similar observation was noted by Yodavudh et al. in Thai patients with in colorectal carcinoma.[18] Lymphatic vascular invasion is considered a poor prognostic indicator in gastric adenocarcinoma. The presence of lymphatic vascular invasion is associated with more aggressive tumor characteristics and a higher likelihood of recurrence.[24]

Limitations

Only 50 samples were studied and study samples were taken from a single institute, which may not reflect the exact scenario of the overall population. Also, a traditional histochemical stain, toluidine blue, was used to identify MCs. More specific methods like immunohistochemistry could increase the accuracy of the findings.

Conclusion

From histopathological and special stain findings, the MCD in gastric adenocarcinoma was much higher in poorly differentiated carcinoma than in moderately differentiated and well-differentiated gastric adenocarcinoma. This study would help to understand the significance of MCD in tumor grading and staging.

Recommendations

MCD can be used as a tool to predict the prognosis of patients with gastric adenocarcinoma. Therapeutic strategies against MC mediators might help control the progression of gastric adenocarcinoma. However, further study with a larger sample size is needed to get a clear picture regarding the influence of MCD in patients with gastric adenocarcinoma.

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Authors contribution

MFA-Conception, data acquisition and analysis, manuscript preparation and final approval.

MHM-Data acquisition, analysis, drafting and final approval.

UKAA-Acquisition of data, study designing, interpretation of data, critical revision and final approval.

RM-Data analysis, manuscript preparation and editing, critical revision and final approval.

MSUA-Design, critical revision and final approval.

Disclosure
All the authors declared no conflict of interest.

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