

Determination of Mast Cell Density in Urothelial Carcinoma of Urinary Bladder and its Relation with Grading and Invasiveness

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Abstract

Background: Urothelial carcinoma (UC) has a high incidence rate and related mortality in developing countries like Bangladesh which is increasing day by day. Mast cells (MCs) in UC are possibly involved in tumor angiogenesis, tissue remodeling, immunomodulation, and most importantly with a favorable or unfavorable prognosis. Mast cell targeted strategies in cancer therapy showed success in many tumors. Our study was designed to determine mast cell density in urothelial carcinoma of the urinary bladder and to evaluate its relation with histologic grading and invasiveness.

Methods: This cross-sectional study was carried out at the Department of Pathology, Rajshahi Medical College from September 2019 to August 2021. A total of 51 case of urothelial carcinoma were included in this study. The continuous variable (age of patient) was expressed as frequency, percentage, mean $\pm$ SD, and range. MCD were expressed as mean $\pm$ SD and median. An unpaired t-test was done to see the differences of MCD of the patients according to grade and invasion.

Results: Most of the cases were found in between 61 to 70 years. Male to female ratio was found to be 2.4:1. Most frequent tumor location was a lateral wall of the urinary bladder (37.3%), 56.9% of cases were high grade & 43.1% were low-grade urothelial carcinoma. Muscle-invasive bladder carcinoma (MIBC) was found in 51.0% of patients and 49.0% of patients were found with non-muscle invasive bladder carcinoma (NMIBC). It was observed that with Toluidine blue staining mean mast cell density was 48.48 ± 18.66 in high-grade urothelial carcinoma and 18.86 ± 10.59 in low-grade urothelial carcinoma. So mast cell density (MCD) was increased with an increase in the grade of tumor. The mean ($\pm$ SD) MCD of MIBC and NMIBC were $48.96 (\pm 18.92)$ and $21.92 (\pm 14.15)$ respectively. So mast cell density was observed to increase in muscle-invasive bladder tumors. Determination of the relation of MCD in urothelial carcinoma with grade and invasiveness was done in this study. An unpaired t-test was done to measure the level of significance. The p-value is highly significant ($p < 0.05$).

Conclusion: Mast cell density (MCD) positively correlates with invasion and grading of urothelial carcinoma of urinary bladder.

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Introduction

Bladder cancer is the 10th most common form of cancer worldwide.¹ It is more common in men than women, sixth most common cancer, and the ninth leading cause of death in men. White people are more commonly affected than black people.¹ Prevalence of bladder cancer in Bangladesh is 2.08%.¹ About 90% of urinary bladder tumors are of epithelial origin, the remainder being mesenchymal origin. Most epithelial tumors are urothelial (transitional cell) type and are thus interchangeably called urothelial or transitional tumors, but squamous and glandular carcinomas also occur.² In 1998, the International Society of Urological Pathology, Canadian Academy of Pathology, and World Health Organization (WHO) proposed the classification of urothelial tumors into papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), low-grade urothelial carcinoma and high-grade urothelial carcinoma.² Tumors that invade the detrusor muscle are considered Muscle-Invasive Bladder Cancer (MIBC) and are more likely to metastasize to lymph nodes or other organs. Approximately 75% of newly diagnosed patients have Non-Muscle-Invasive Bladder Cancer (NMIBC) and 25% have MIBC or metastatic disease.³

Mast cells were previously known for their function in allergies and anaphylaxis, however, in recent years, evidence indicates that mast cells may take part in angiogenesis, tissue remodeling, and immunomodulation in various tumors.⁴ Mast cells have been shown to accumulate around several types of tumor and are generally the first inflammatory cells to infiltrate developing tumors such as urinary bladder carcinoma, malignant melanoma, breast carcinoma, etc.⁵ Mast cell density correlates with angiogenesis and poor outcome in a study by Saxena et al found that aggregation of mast cells were high in high grade than low-grade urothelial carcinoma of

the urinary bladder.⁴ The contribution of mast cells to the promotion of urinary bladder cancer includes their action on immunosuppression, the release of proangiogenic and mitogenic factors, and degradation of the extracellular matrix (ECM). Mast cell produces and releases potent proangiogenic mediators including mast cell-specific tryptase, histamine, angiopoietin-1, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), transforming growth factor beta (TGF β), tumor necrotic factor alpha (TNF α), interleukin – 18 (IL-18) etc.⁶ Mast cells also help in tumor metastasis by stimulating matrix metalloproteinase-9 (MMP-9) which degrades extracellular matrix.⁴ Patients with low mast cell density (MCD) tumor tissue had significantly longer survival than those with high mast cell density tumor biopsy.⁴

The prevalence rate of bladder cancer in Bangladesh is 3.4%.⁷ It is quite high. The present study will evaluate the relation of mast cell density in urothelial carcinoma of the urinary bladder with grading and invasion among patients with histologically confirmed urinary bladder carcinoma at the pathology department of Rajshahi Medical College (RMC) and admitted in Rajshahi Medical College Hospital (RMCH). Thus this study may have a significant impact on the assessment of biologic behavior, prognosis, and treatment protocol of urothelial cancer.

Rationale

Bladder cancer has the highest recurrence rate of any malignancy (70% within 5 years).¹ Although most patients with bladder cancer can be treated initially with organ-sparing therapy, most experience either recurrence or progression.² Divergent and interconnected molecular pathways are responsible for the development of noninvasive and invasive bladder tumors.² Mast cells (MCs) were initially disregarded but gradually emerged as

an important influencer in cancer treatment. They have either pro-tumorigenic or anti-tumorigenic functions based on the specific cancer type, the level of tumor advancement, and the positioning of these immune cells within the tumor mass. However, the precise function of MCs in urothelial carcinoma remains unclear due to contradictory findings in previous publications. A more thorough understanding on the determination of mast cell density in urothelial carcinoma and their relation with tumor grade and invasiveness are still required before they can be used as prognostic/predictive markers or even as targets for innovative therapeutic approaches. This research can contribute to enhancing knowledge in this regard.

Objectives

To determine the histological grading, muscle invasion, and mast cell density of urothelial carcinoma and to observe the relation of mast cell density with muscle invasion and grading of tumor.

Methods

It was a descriptive type of cross-sectional study from September 2019 to August 2021. The study was carried out at the Department of Pathology, Rajshahi Medical College RMC, Rajshahi. Routine Hematoxylin and Eosin stain and Toluidine Blue stain were. Fifty-one blocks of urothelial carcinoma of the urinary bladder were collected from the Department of Pathology, RMC. Fifteen blocks of non-malignant bladder tissue were taken as control from there also. The study included the following variables: Age, sex, smoking habit, location of the tumor, occupation, presence of hematuria, tumor grade, tumor stage and mast cell density with the toluidine blue staining method. sample size was 51. A consecutive sampling technique was followed.

All the patients with histopathologically confirmed urothelial carcinoma of the urinary bladder were included in the study. Patients with urothelial carcinoma were recently exposed to chemotherapy and patients with urothelial carcinoma but absence of muscularis propria in the sample were excluded from the study.

Tumor grading was done according to WHO recommendations and invasiveness was evaluated by -Muscle-Invasive Bladder Cancer (MIBC) - Tumors that invade the muscularis propria (detrusor muscle) microscopically.

Non-muscle Invasive Bladder Cancer (NMIBC) - Cases with no evidence of muscularis propria invasion.⁸

Interpretation of Special Stain for Mast Cell

Mast cell cytoplasm contains granules composed of heparin and histamine etc. These granules are metachromatic with toluidine blue stain. Mast cells are stained red-purple and the background is stained blue.⁹

Observation of Mast Cell Number for Determination of Mast Cell Density

At first, the stained section was screened at low power (10x) to identify the areas of the 'hot spots' (Areas with the highest number of mast cells). Ten 'hot spots' were identified from each slide. The total count of the ten fields was recorded as the mast cell count per 10 HPF.⁹

Data Collection Procedure

Filling up of the data sheet was performed in all cases either from patient or patient attendant statements and clinical and investigation files.

Statistical Analysis

Data were entered into an Excel worksheet to generate a master sheet. Then they were

transferred into the computer-based software Statistical Package for Social Sciences, version 23 (SPSS Inc., Chicago, IL) for processing and analysis. Results are shown as the table & figure.

Ethical Consideration

The research protocol was submitted to the ethical committee of the IRB of Rajshahi Medical College, Rajshahi for approval. All data was secured with the confidentiality of the study population. There was no disclosure of the information that may be harmful to the patient.

Results

Maximum (47.1%) patients age was between 61 to 70 years, followed by 25.5% patients age were between 51 to 60 years, 15.7% patients age were between 45 to 50 years and rest (11.8%) of the patients age were between 71 to 80 years. The mean ($\pm$ SD) age of the patients was 62.29 ($\pm$ 8.48) years with minimum and maximum age were 45 and 80 years respectively. Most (70.6%) of the patients were male and only 29.4% patients were female. Only 11.8% patients were industrial worker. More than 50% patients used tobacco. Maximum (88.2%) patients had history of hematuria. Most (37.3%) of the patient's location of tumor involved in lateral wall, followed by 19.6% patients had involved multiple sites, 15.7% patients had involved both lateral wall & anterior wall. In this study, among 51 patients, 56.9% patients were high grade & 43.1% patients were low grade. In this study, among 51 cases, 51.0%

patients were found MIBC and 49.0% patients were found NMIBC.

Table I: Distribution of the patients according to socio-demographic variables (n=51)

Socio-demographic variables	Frequency	Percent
Age (years)		
45-50	8	15.7
51-60	13	25.5
61-70	24	47.1
71-80	6	11.8
Mean $\pm$ SD (Min-Max)	62.29 $\pm$ 8.48 (45-80)	
Gender		
Male	36	70.6
Female	15	29.4
Occupation		
Industrial	6	11.8
Non-industrial	45	88.2

Table II: Distribution of the patients according to use of tobacco (n=51)

Use of Tobacco	Frequency	Percent
Yes	27	52.94
No	24	47.06
Total	51	100.0

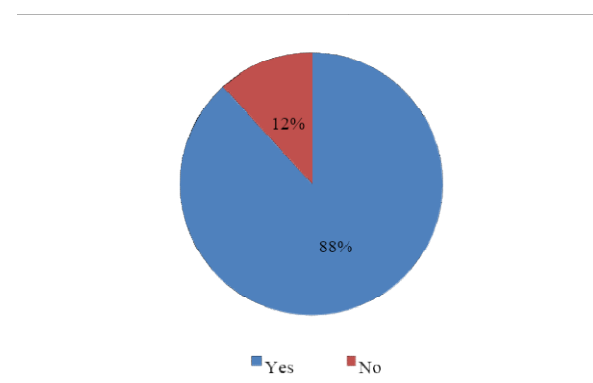


Figure 1. Pie chart of the patients according to hematuria (n=51)

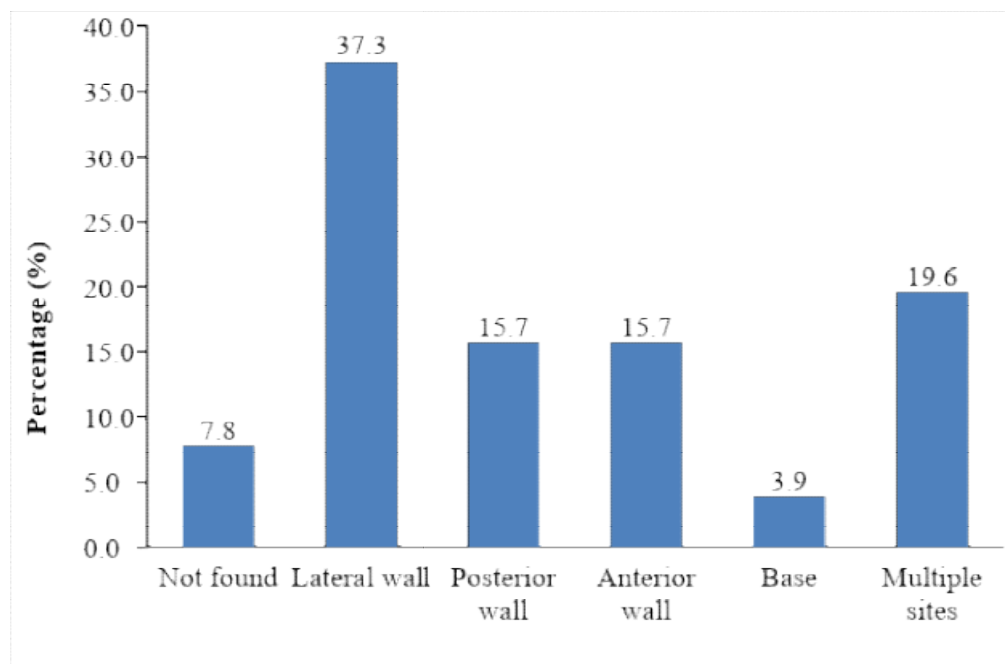


Figure 2. Bar diagram of the patients according to the location of the tumor (n=51)



Figure 3. TURBT specimen

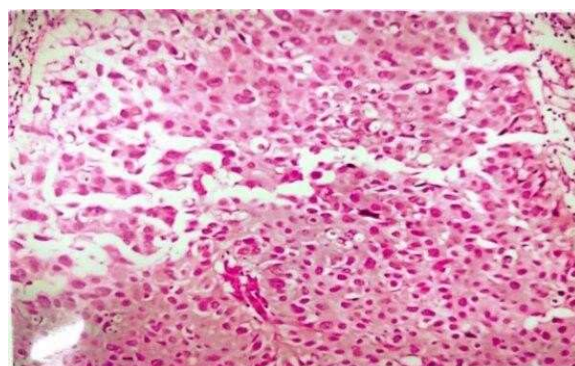


Figure 5. Urothelial carcinoma, Highgrade, MIBC, H&E (x20).

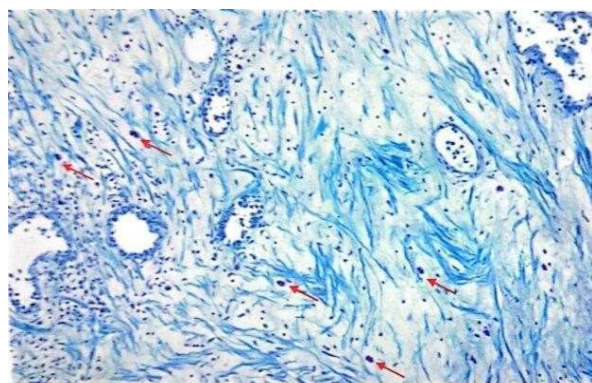


Figure 4. Urothelial carcinoma, High grade, MIBC, TB(x20). Red arrows indicate mast cells.

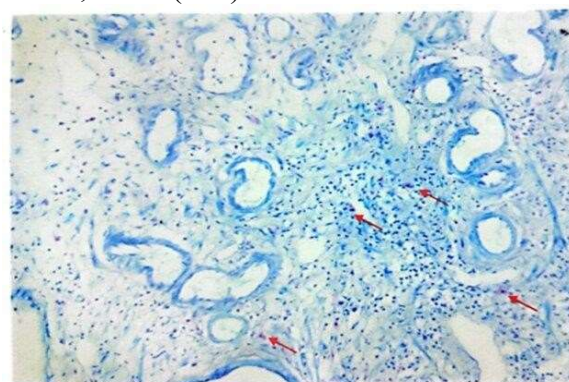


Figure 6. Urothelial carcinoma, Highgrade, NMIBC, TB(x20). The red arrow indicates the mast cell.

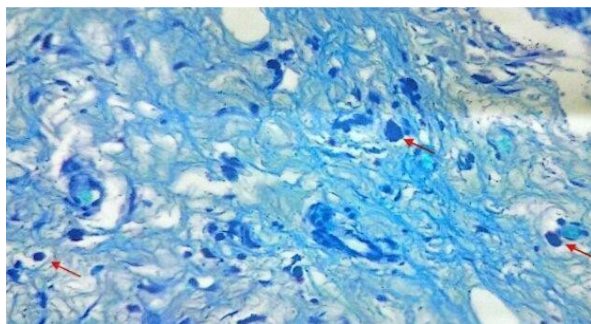


Figure 5. Urothelial carcinoma, Low grade, NMIBC, TB(x40). Red arrows indicate mast cells

Table III: Distribution of the patients according to grade (n=51)

Grade	Frequency	Percent
Low	22	43.1
High	29	56.9
Total	51	100.0

Table IV: Distribution of the patients according to invasiveness (n=51)

Invasiveness	Frequency	Percent
MIBC	26	51.0
NMIBC	25	49.0
Total	51	100.0

Differences in MCD of the Patients According to Grade

In this study, the mean ($\pm$ SD) MCD of low and high grades were 18.86 ($\pm$ 10.59) and 48.48 ($\pm$ 18.66) respectively. The mean ($\pm$ SD) MVD of low and high grades were 23.31 ($\pm$ 6.28) and 31.45 ($\pm$ 6.82) respectively. There were statistically significant ($p < 0.05$) differences in MCD between low and high grades.

Table V: Differences in MCD of the patients according to grade (n=51)

	Grade		p-value
	Low	High	
MCD	18.86 $\pm$ 10.59	48.48 $\pm$ 18.66	<0.001 ^a

^aUnpaired t-test was done to measure the level of significance.

Data was expressed as Mean $\pm$ SD.

Differences in MCD of the Patients According to Invasiveness

In this study, the mean ($\pm$ SD) MCD of MIBC and NMIBC were 48.96 ($\pm$ 18.92) and 21.92 ($\pm$ 14.15) respectively. There were statistically significant ($p < 0.05$) difference of MCD between MIBC and NMIBC.

Table VI: Differences of MCD of the patients according to invasiveness (n=51)

	Invasiveness MIBC	NMIBC	p value
MCD	48.96 $\pm$ 18.92	21.92 $\pm$ 14.15	<0.001 ^a

^aUnpaired t test was done to measure the level of significance.

Data was expressed as Mean $\pm$ SD.

Discussion

This cross-sectional study was carried out with the aim to evaluate the mast cell density and its relation with invasion and grading of urothelial carcinoma (UC) of the urinary bladder among patients with histologically confirmed UC and admitted to RMCH.

In the present study, it was observed that the maximum (47.1%) patient age was between 61 to 70 years, followed by 25.5% of patients aged between 51 to 60 years. The mean age of the study population was 62.29 ($\pm$ 8.48) years, ranging from 45 to 80 years. A study by Jawad et al showed that 80% patients of with urinary bladder carcinoma fall between the ages of 50 to 80 years.¹⁰

In this study out of 51 study patients, 36 (76.5%) cases were male, and 15 (24.5%) cases were female. The male-to-female ratio was found 2.4:1. Mumtaz et al also showed that 81.1% of patients were male and 18.9% were female with a male-to-female ratio of 4.2.¹¹ In the present study 52.94% of patients had a habit of smoking. Most of the male patients in this study had a habit of cigarette

smoking. Chinnasamy et al revealed in their study that most bladder cancer patients (71.2%) had a smoking habit which is consistent with this study result.¹² Regarding clinical findings 88.2% of the cases of this study presented with gross hematuria. Similar finding was observed by Chinnasamy et al.¹² The Most frequent tumor location among 51 cases was the lateral wall of the urinary bladder. It was found in 37.3% of cases. A similar finding was reported by Jeelani et al. The location was assessed through sonographic and cystoscopic examination. In our country, the probable cause may be due to poor economic conditions, lack of knowledge, lack of urological treatment facilities as well as social and religious restrictions, especially for female patients which prevent them from utilizing hospital facilities. The tumors in this study were graded according to the WHO 2004 grading system for urinary bladder carcinoma. It was observed that 56.9% of patients were diagnosed as high-grade urothelial carcinoma (HGUC) and 43.1% of patients were diagnosed as low-grade urothelial carcinoma (LGUC). Similarly, Chou et al in a study found 56.8% high-grade and 43.2% low-grade tumors.¹³ Incidence of high-grade urothelial carcinoma was more in this study. All our cases were collected from RMCH, which is a tertiary level hospital and so patients are admitted with advanced stages of disease or referred to as complicated cases. Another study by Chinnasamy et al in India observed 63.5% high-grade and 36.5% low-grade carcinoma which were almost similar to our study.¹²

In the present study, it was also observed that there were 26 (51%) cases of muscle-invasive urothelial carcinoma and 25(49%) cases of nonmuscle-invasive urothelial carcinoma. Chou et al also found a high incidence of MIBC, revealing 40.5% NMIBC and 59.5% MIBC. Among MIBC most of them were found over 60 years (16 cases, 53.3%).¹³

In the present study, the MCD were evaluated for different grades of tumor. An unpaired t-test was done to measure the level of significance. The mean ($\pm$ SD) MCD of low and high grades were 18.86 ($\pm$ 10.59) and 48.48 ($\pm$ 18.66) respectively. There were statistically significant ($p < 0.05$) differences in MCD between low and high-grade UC. Similarly in Turkey, a group of authors found that the median MC count was lower in low-grade UC than high-grade UC ($p < 0.001$) in low-grade UC than high-grade UC ($p < 0.001$).¹⁴ Similarly Kim et al. also observed a highly statistically significant difference of MCD in low and high-grade urothelial carcinoma.⁵ In the present study, the MCD was evaluated in MIBC and NMIBC. An unpaired t-test was done to measure the level of significance. The mean ($\pm$ SD) MCD of MIBC and NMIBC were 48.96 ($\pm$ 18.92) and 21.92 ($\pm$ 14.15) respectively. There was a statistically significant ($p < 0.05$) difference in MCD between MIBC and NMIBC in the present study. A group of authors also found a statistically significant difference of MCD between MIBC and NMIBC.¹⁴

Conclusion

It can be concluded that Mast cell density (MCD) positively correlates with the invasion and grading of urothelial carcinoma of the urinary bladder.

Recommendation

Long-term follow-up of the patients to correlate MCD with the progression and recurrence of disease and survival of the patients would be beneficial to establish the prognostic role of mast cell density.

Limitations

Identifying muscularis propria by Masson's trichrome stain could not be possible.

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