

Association of Ki-67 expression in radical cystectomy specimens of infiltrating urothelial carcinoma with histopathological parameters

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Abstract

Urothelial carcinoma is the most common neoplasm of urinary bladder. Antigen Ki-67 is a nuclear protein that is necessary for cellular proliferation. The aim of this study was to determine the association of expression of Ki-67 with histopathological parameters in radical cystectomy specimens of histologically diagnosed cases of infiltrating urothelial carcinoma. This is a cross-sectional study conducted among 36 cases of infiltrating urothelial carcinoma collected from Department of Pathology, Bangabandhu Sheikh Mujib Medical University. Histopathological parameters i.e. morphologic variants, stage (pT), lympho-vascular invasion, perineural invasion and lymph node metastasis were assessed. Ki-67 immunohistochemistry were performed. Mean \pm SD age of the patients was 58.9 \pm 13.8 years. Among the morphological variants, 64% cases were microscopically arranged in papillary pattern. Eighteen (50%) patients underwent surgical intervention at stage pT2. Lympho-vascular invasion, perineural invasion and lymph node metastasis were identified in 12 (33.3%), 9 (25%) and 13 (48.1%) of the cases, respectively. Among the morphological parameters, Ki-67 shows association with pathological stages and lymph node metastasis. The use of Ki-67 immunomarker along with grading and staging may provide significant prognostic information in urothelial carcinoma of the urinary bladder.

Key words: Urothelial carcinoma, Ki-67, lympho-vascular invasion, perineural invasion, lymph node metastasis.

Article Info

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Received: 23 August 2022
Accepted: 15 November 2022
Available Online: 00 November 2022

The publication history and additional supplemental material for these paper are available online. To view these files, please visit: <http://dx.doi.org/10.3329/bsmmuj.v15i3.63302>

ISSN: 2224-7750 (Online)
2074-2908 (Print)

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A Journal of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

Introduction

Bladder cancer is the 10th most common form of cancer worldwide.¹ Urothelial carcinoma is the most common malignant neoplasm of the urinary tract and characterized by a propensity for divergent differentiation. Bladder cancers are more common in industrial areas and the incidence is increased with exposure to cigarette smoking and aryl amines.² Prevalence of bladder

cancer in Bangladesh is 2.08%.¹ Staging is the most important factor to determine patients' outcome at the time of diagnosis in case of muscle-invasive bladder carcinoma (MIBC). Almost all infiltrating carcinoma are high grade and for this reason, grading of infiltrating component is not critical.³

Approximately 70-80% of patients with primarily diagnosed bladder cancer present with non-invasive or

early invasive (non-muscularis propria invasive) disease (NMIBC). With these tumors, recurrence is common (occurring in 50-70% of cases), but progression occurs in only 15-25% of cases.² MIBC is highly aggressive and can rapidly progress and metastasize.² Despite improvement in therapeutic strategies, most patients face death.⁴ Current clinical and pathologic parameters, such as tumor grade, stage, and vascular and lymphatic extension provide important prognostic information.⁵ In addition, some molecular markers have been identified in recent studies.⁶ Analysis of new molecular markers in predicting prognosis of bladder cancer is a recent topic of interest among the pathologists due to lack of specific serum prognostic markers.⁷ Besides, better understanding of the pathological process is expected from the study of these molecular markers.⁸

Antigen Ki-67 is a nuclear protein that is necessary for cellular proliferation. The Ki-67 antigen accumulates in the nuclei of proliferating cells from the G1 phase to mitosis, but not in the nuclei of quiescent or resting cells. Ki-67 is an excellent marker in determining the growth fraction of a given cell population. It represents proliferative subsets of tumour cells indirectly denoting aggressiveness of the tumour. As molecular alteration precedes phenotypic change, immunohistochemical study may be a valuable tool for screening patients and early identification of aggressive cancers.

A large number of studies define Ki-67 as an independent prognostic marker of bladder cancer progression and recurrence in multivariate analysis.⁶ An elevated tumour proliferation index measured by Ki-67 has been consistently shown to predict worse outcome.⁹ The study is conducted to observe Ki-67 immunostaining of paraffin-embedded bladder tumour tissue in radical cystectomy specimens and to assess their association with histopathological markers of prognosis, including morphological variant, stage, lympho-vascular invasion (LVI), perineural invasion (PNI) and lymph node metastasis.

Methods

This was a cross sectional observational study. It was conducted among 36 patients of infiltrating urothelial carcinoma in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University

(BSMMU) during 2019-2020 as per ethical clearance No. BSMMU/2019/13216 dated 01 Dec 2019. The radical cystectomy specimens received at the centers were grossed, processed and reported out according to the ideal protocol. Then paraffin blocks and Hematoxylin and Eosin-stained (H&E) slides of Radical cystectomy specimens histologically diagnosed as infiltrating urothelial carcinoma were collected from archives. The cases were selected for the study on the basis of inclusion and exclusion criteria. The inclusion criteria included patients of any age and gender with histologically diagnosed infiltrating urothelial carcinoma and radical cystectomy specimens. Patients having history of chemotherapy or radiotherapy, extensive necrosis in paraffin blocks were excluded. The slides of the cases were evaluated elaborately and histological parameters including morphological type, tumor grading, staging, LVI, PNI and lymph nodes metastasis were assessed. WHO 2016 classification of urothelial carcinoma and International Society of

Pathological stage (pT) denoted pathological depth of invasion.

T1	Tumor invades subepithelial connective tissue
T2	Tumor invades muscle coat
T3	Tumor invades perivesical fatty tissue
T4	Tumor invades adjacent organs

Urological Pathology (ISUP), 1997 grading system were used for grading of tumours.

Lympho-vascular invasion (LVI) was defined as the unequivocal presence of nests of neoplastic cells within an endothelium-lined space, with no underlying muscular wall. It was assessed with light microscopic examination of H&E stained sections.¹⁰ Perineural invasion (PNI) was defined as 'cancer cells within nerves or surrounding or pass-through nerves, tumor cells closely contacting the nerve and surrounding at least 33% of the nerve periphery or tumor cells invading any of the three layers of the neurilemmal structure'.¹¹ Representative sections from each paraffin block were selected for immunohistochemical stain with ki-67. Formalin fixed paraffin-embedded tissues sections were stained with ki-67 antibody using standard protocol compatible for DAKO Envision™ FLEX + detection system for immunohistochemistry.¹²

Scoring system applied for Ki-67 in this study

For Ki-67, at least 1000 tumor cells at 400x magnification from the most immunopositive region of each slide was visually counted and the percentage of positive cells (Labeling index, LI) were calculated. Immunoreactivity was considered altered when samples show more than 20% nuclear reactivity.^{13, 14} Reactive lymph node with prominent germinal centers was taken as a control. To avoid differential assessment of participants potentially resulting in bias, labelling of the participants' paraffin blocks were coded by unidentifiable numbers.

Statistical Analysis

The statistical analysis was carried out using the Statistical Package for Social Sciences version 20.0 for Windows (SPSS Inc., Chicago, USA). The frequencies of different entities were expressed as percentage. Fisher's

Exact or Chi-square test was used to analyze the association between different variables. A *p* value <0.05 was considered statistically significant.

Results

In the present study, the total number of cases were 36 radical cystectomy specimens which were histologically diagnosed as infiltrating urothelial carcinoma. Among these, 27 specimens had resected lymph nodes. Statistical analysis showed, the mean age of the patients was 58.94±13.83 (SD) years. Majority Patients were in age group 61-70 years (30.6%). About 75% of the patients were male (n=27) with a male to female ratio of 3:1 (Table I).

Among the study population, 64% cases were microscopically arranged in papillary pattern and rest of the cases were infiltrating urothelial carcinoma with divergent differentiation. These variants presented at

TABLE 1 Association of Ki-67 expression with clinico-pathologic characteristics of infiltrating urothelial carcinoma (n = 36)

Variables	Total Number (%)	Ki-67 expression		P*
		Ki-67 score Normal (Labelling index ≤20%) (number)	Ki-67 score Over expressed (Labelling index >20%) (Number)	
Age, yrs	(n)	(n)		
21-30	1 (2.8)	0	1	
31-40	2 (5.6)	2	0	
41-50	8 (22.2)	2	6	
51-60	8 (22.2)	4	3	
61-70	11 (30.5)	6	6	0.76
71-80	4 (11.1)	2	1	
>80	2 (5.6)	1	2	
Sex				
Male	27 (75)	15	12	0.08
Female	9 (25)	2	7	
Morphological variant				
Infiltrating urothelial carcinoma- papillary type	23 (64)	12	11	0.63
Infiltrating urothelial carcinoma with divergent differentiation / Non-papillary type	13 (36)	5	8	
Pathological stage (pT)				
pT1	4 (11)	2	2	0.001
pT2	18 (50)	14	4	
pT3	10 (28)	1	9	
pT4	4 (11)	0	4	
Lympho-vascular invasion				
Present	23 (64)	12	11	0.63
Absent	13 (36)	5	8	
Perineural invasion				
Present	27 (75)	14	13	0.99
Absent	9 (25)	3	6	
Lymph node metastasis (n=27)†				
Present	13 (48)	3	10	0.01
Absent	14 (52)	11	3	

* p value was determined by Fisher's Exact or Chi square test. †Resected lymph nodes were present in 27 radical cystectomy specimens.

higher stage in comparison to papillary pattern. When stratified according to pathological stage (pT) or depth of invasion, most of the patients underwent radical cystectomy at stage pT2 (18, 50%) (Table I). Other stages (pT1, pT3 and pT4) collectively constituted rest of the cases. Four patients were at stage pT1. Three of them

Discussion

Infiltrating urothelial carcinoma accounts for about 90% bladder cancers in industrialized countries.⁹ Radical cystectomy remains the specific treatment modality for patients with MIBC or NMIBC which is refractory to

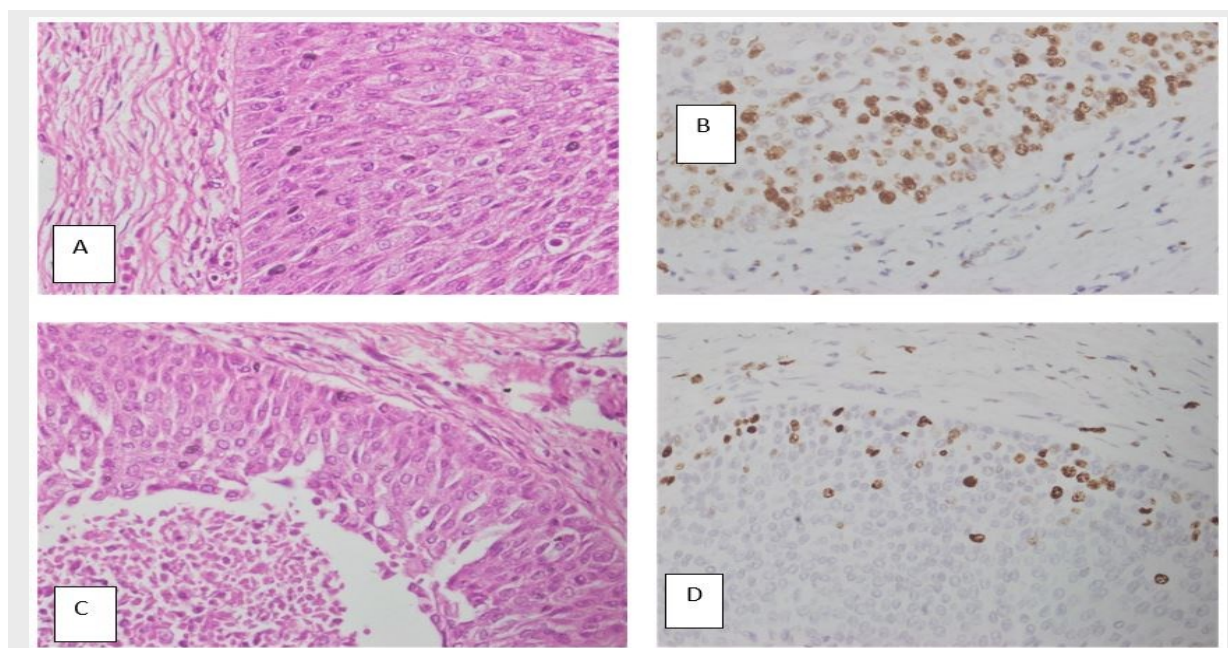


FIGURE 1 showing Ki-67 immunohistochemistry of infiltrating urothelial carcinoma. A and C shows Hematoxyline and Eosin staining of infiltrating urothelial carcinoma (400x) and B shows Ki-67 overexpression (400x). Figure D shows normal nuclear expression of Ki-67(400x).

underwent radical cystectomy due to failure of BCG therapy and recurrences of infiltrating urothelial carcinoma. One patient presented with rupture of urinary bladder during Transurethral Resection of Bladder Tumour (TURBT). Stage pT4 were found in 11% cases.

Among the 36 cases, LVI and PNI were identified in 12 (33.3%) cases and 9 (25%) cases respectively. Resected lymph nodes were present in 27 cases. Among these, 13 (48.1%) cases showed metastatic urothelial carcinoma.

Regarding evaluation of Ki-67, percentage of tumour cells showing nuclear staining was calculated visually with a light microscope and a cut off value of 20% was considered overexpressed (**Figure 1**). Ki-67 was overexpressed in most of the cases (19, 52.8%). In this study, association between pathological stage (pT) and Ki-67 expression was statistically significant. Among the other parameters, overexpression of Ki-67 was associated with lymph node metastasis.

Trans-urethral resection or intra-vesical BCG therapy. Molecular markers might be useful for choosing patients best appropriate for early surgical intervention and for post-surgical radiotherapy.¹³

Many studies showed prognostic significance of molecular marker Ki-67. Regarding evaluation of Ki-67, a cut off value of 20% was considered overexpressed as described by other authors such as Margulis et al.¹² and Thakur et al.^{13,14} Among 36 cases, Ki-67 was overexpressed in 19 (52.8%) cases. There was no significant association between morphological variants and Ki-67 expression pattern which corresponded with other research.⁷

In this study, association between pathological stage (pT) and Ki-67 expression was found statistically significant. This could be explained by increased proliferative capacity of the tumour cells leading to

increased aggressiveness. The tumour cells having greater proliferative potential can invade the underlying stroma and can metastasize to lymph nodes and distant sites. Similar results were obtained in other studies as well.^{7, 13, 14, 15}

Among the other parameters, overexpression of Ki-67 was associated with lymph node metastasis. This finding was observed by a number of researchers.^{7, 13, 15} No significant association was found between Lymphovascular and perineural invasion and Ki-67 expression. The data regarding these variables varied among authors. Some showed significant association while others failed to establish any correlation.¹³ Large multicentre study with greater sample size can yield better result. Small sample size was a limitation of the present study. Assessment of LVI would be more effective if it was done by immunohistochemical markers.

Conclusion

Grading and pathological (pT) staging are the most important histological prognostic factors of infiltrating urothelial carcinoma. As antigen Ki-67 showed statistically significant association with pathologic staging, routine use of Ki-67 in conjunction with histopathological grading and staging in TURBT and radical cystectomy specimens may provide prognostic information for categorizing high risk patients in infiltrating urothelial carcinoma of the urinary bladder.

Acknowledgments

Authors would like to express heartfelt gratitude to Professor Dr. Ferdousy Begum, Chairman, Department of Pathology, BSMMU for her support in conducting the research work.

Author Contribution

Rahman P conceived and designed the original idea, carried out study, collected data, performed data analysis and write the manuscript. Khan K H supervised the study and critically revised the manuscript. Rahman M M contributed supervise the study and critically revised the manuscript. Afroz S contributed data analysis and revised the manuscript. Rashid J S contributed manuscript writing and revised the manuscript. Emita U contributed data collection and revised the manuscript

Conflict of Interest

No conflict of interest

Funding

BSMMU research grant, self

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