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Original Article

Expression of Ki-67 and p63 in Carcinoma of Urinary Bladder

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Abstract

Background: *For urinary bladder carcinoma, a specific tumor marker to distinguish the progression of disease is very much needful to ensure early diagnosis and prevent further morbidity. This study was designed to evaluate the expression of Ki-67 and p63 in patients with carcinoma of urinary bladder.* ***Methods:*** *This cross-sectional observational study was conducted at the department of Pathology in Dhaka Medical College Hospital for one year of period following approval of this protocol. A total 36 cases of urinary bladder carcinoma who underwent surgical intervention were enrolled in this study. Written informed consent was ensured from each of the participants. Data collection was done through a structured questionnaire. After collection of all the required data, analysis was done by SPSS 23.0.* ***Results:*** *Mean age of the patients was 56.94±13.46 (SD) years with clear male dominance (88.90% VS 11.10%). High grade tumor was observed in 80.6% of patients and low grade tumor in 19.4% of patients. Higher stage of muscle involvement was observed statistically in high grade tumor. Mean Ki-67 was statistically higher in patients with high grade tumour. But mean p63 was statistically similar in both high and low grade tumors.* ***Conclusion:*** *This study observed significant relation between Ki-67 and tumour grade in patients with urinary bladder carcinoma.*

Keywords: *Urinary bladder, Carcinoma, Tumour.*

Introduction: Bladder cancer is the 10th most commonly diagnosed cancer worldwide, with approximately 573,000 new cases and 213,0009 deaths.[1] It is more common in men than in women with a ratio of 3:1 globally.[2] The incidence and mortality rates are of 9.6 and 3.2 per 100,000 in men and women respectively.[3] Though it is more common in well-resourced countries the incidence is gradually increasing more in developing countries like Bangladesh; due to urbanization, increased use of chemicals, increased tobacco consumption and increased arsenic concentration in the drinking water.[4] Most of the cases (about 90%) of urinary bladder tumors are of epithelial origin, the remainders are of mesenchymal tumors.[5] Most epithelial tumors are urothelial or transitional cell type, but squamous and glandular carcinomas also occur.[6] The clinical challenges with these neoplasms are biological behaviour of the tumor, individualized risk for recurrence, progression and individualized therapeutic strategy. In recent years value of immuno-histochemistry (IHC) has raised as an important tool not only for diagnosis but also for predicting recurrence, progression, drug resistance and prognosis in urothelial carcinoma. So, an immuno-marker that can assess biological behavior of the tumor, predict individualized risk of tumor recurrence and progression as well as prognosis and selecting therapeutic strategy for better outcome of the patient is needed. Increased cellular proliferation correlates with biological aggressiveness of bladder tumours.[7] Ki-67 is a nuclear protein present during different stages

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of the cell cycle. It is widely used to assess the activities of cell proliferation in various cancers by immunohistochemistry.[8] A significant correlation of cell proliferation assessed by Ki-67 and histopathological assessment of malignancy have been reported.[9,10] Remarkable variation between Ki-67 value and histopathological grade of the tumour has been reported, so it might be helpful to outline the individual therapy and prognosis. p63 is the tumour marker that is a member of the p53 gene family, located in chromosome 3q27-28. It is primarily expressed in the basal compartment of epithelia, including epidermis, oral mucosa, cervix, vaginal epithelium, urothelium, prostate and breast. It has been postulated that epithelial stem cells reside in these basal layers. Thus, p63 is crucial for the development and maintenance of stem cells in stratified epithelia and its differentiation. Loss of p63 expression results in up-regulation of genes associated with invasion and metastasis.[11] It is involved in all aspects of tumorogenesis and cancer progression. Expression of p63 by tumour cells indicates limited potential to progress to invasive disease and provides excellent prognosis.[12] Considering the above mentioned facts the present study aimed to investigate the immunohistochemical expression of Ki-67 and p63 in urinary bladder (UB) urothelial carcinoma.

Methods:

A Cross-sectional Observational study was carried out at the Department of Pathology, Dhaka Medical College around a period of two years from September 2019 to August 2021. Prior to commencement of this study, the study protocol was approved by the ethical review committee. All the sample collected by transurethral resection of bladder tumors (TURBT) or cystectomy attending the pathology department of Dhaka Medical College with a histopathologic diagnosis of bladder urothelial carcinoma (invasive and noninvasive) were included in this study. A total of 36 cases were enrolled in this study with non-probability, consecutive sampling method. During the study period the patients who received neo-adjuvant or adjuvant chemotherapy due to carcinoma bladder, TURBT samples with absent muscularis propria, autolyzed tissue and inadequate tissue in the block were excluded. Three sections from representative paraffin block (with maximum tumor bulk) for each cases were taken. One section is stained with Hematoxylin & Eosin (H&E) and other two were selected for immunohistochemical stain with Ki-67 and p63. All the relevant information was collected from patient including clinical history, findings of cystoscopy and radiology & imaging. H&E stained sections of each case were re-evaluated to confirm the histopathological diagnosis of urothelial carcinoma, grading and to determine the presence of adequate tumor tissue to perform immunostain. Presence of muscularis propria in the sample and muscle invasion was also evaluated. After histological examination relevant points were taken from the report and included in the prescribed proforma. Immunostaining for Ki-67 and p63 was done on all the 36 cases. Nuclear positivity in any number of cells was required to assign Ki-67 or p63 expressions. Percentage of expression was expressed as mean, median and range. Further subdivision of cases was done according to the median percentage of expression of each marker into high (>median) and low (≤ median).13 In this study median value of Ki-67 was 15. The cases expressing more than 15 was regarded as high expression and the cases expressing less than or equal to 15 was regarded as low expression. The median value of p63 was 25. The cases expressing more than 25 was regarded as high expression and the cases expressing less than or equal to 25 was regarded as low expression. Data analysis was carried out by using SPSS version 23.0. Exploratory data analysis was carried out to describe the study population. Categorical variables were summarized using frequency tables and continuous variables were summarized using measures of central tendency and dispersion such as mean and standard deviation. The association between categorical variables was determined by Fisher's exact test. The difference between continuous variables were determined by independent student t test. A level of $p< 0.05$ was considered as statistically significant.

Results:

A total of 36 patients were enrolled in this study after meeting the inclusion criteria. The mean age of the patients was 56.94 years, ranging from 30 to 84 years old. The majority of the cases were in age group of 51-60 years (38.9%). The age distributions of the patients is shown in table 1.

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Table 1: Distribution of the cases by age (n=36)

Age (years)	n	%
≤30	2	5.6
31-40	3	8.3
41-50	6	16.7
51-60	14	38.9
61-70	8	22.2
71-80	1	2.8
81-90	2	5.6
Mean±SD	56.94±13.46	

Maximum patients was in the age group 51-60 years (38.9%)

Clear cut male predominance was found in this study. About 88.90% patients were male and 11.10% were female. The male female ratio was 8:1. The distribution of occupation of the cases is shown in table 2. Majority of the cases were farmer (50%).

Occupation	n	%
Tailor	3	8.3
Farmer	18	50.0
Barber	1	2.8
Shopkeeper	3	8.3
Housewife	4	11.1
Rickshaw puller	2	5.6
Businessman	4	11.1
Day labourer	1	2.8

According to the radiologic and cystoscopic findings majority of the tumors 21 (58.3%) involved the lateral wall of urinary bladder. least site of involvement was found in the anterior wall of the UB (5.9%). Tumor location of the cases is described in figure 2. Among the cases of urothelial carcinoma, 32 specimen (88.9%) were collected by TURBT and only 4 specimen (11.1%) were by cystectomy.

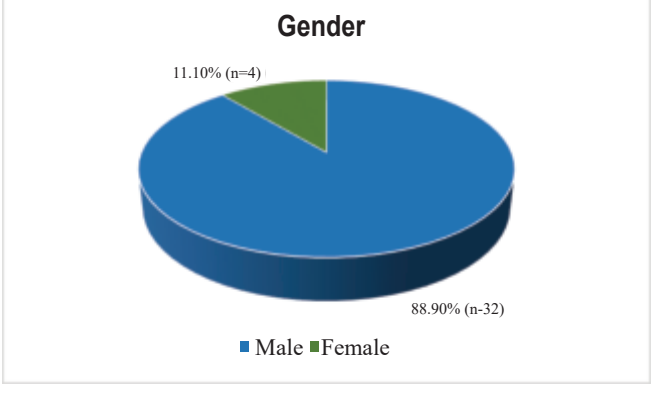

Figure 1: Distribution of the cases according to gender (n=36)

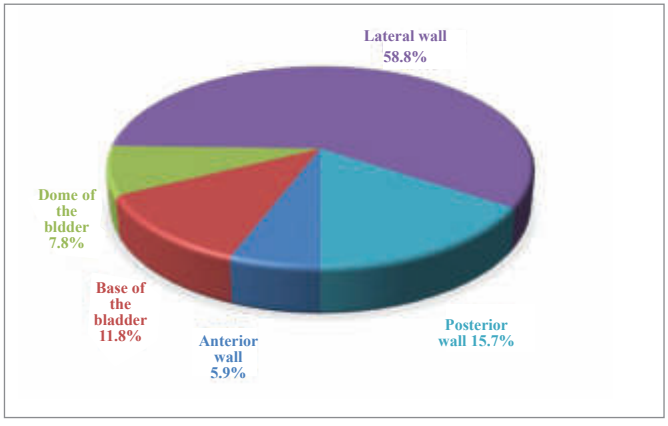

Figure 2: Distribution of the cases according to location (n=36)

Histopathological characteristics of tumors, grade and stage of muscle invassion among the cases is described in table 3. Majority of patients was found papillary urothelial carcinoma (88.9%). About 80.6% of patients had high grade tumor. Muscle invasive nature of the tumours was found in 19 cases (52.77%).

Table 3: Characteristics of tumors among the cases (n=36)

Histological type of tumor	n	%
Papillary urothelial carcinoma	32	88.9
Urothelial carcinoma	3	8.3
Urothelial carcinoma with glandular differentiation	1	2.8
Grade of tumor		
High grade	29	80.6
Low grade	7	19.4
Muscle invasion stage		
Non invasive	17	47.23
Invasive	19	52.77

Among the patients with high grade urothelial carcinoma, 65.5% patients had muscle invasion. All the low grade tumours were non-invasive. A significant statistical association was observed between muscle invasion and high grade tumour ($p<.05$).

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Table 4: Association between grading of tumor and muscle invasion (n=36)

	Grade of tumor		p value*
Muscle invasion	**High grade n=29**	**Low grade n=7**	
Invasive	19 (65.5)	0	<.001
Non invasive	10 (34.5)	7 (100)	

*p value was determined by chi square test

In the relationship between Ki-67 and grading of tumor; mean Ki-67 was statistically higher in patients with high grade tumor (p<.05).

Table 5: Relationship between Ki-67 and grading of tumor (n=36)

	High grade n=29	**Low grade n=7**	**p value***
Ki-67	35.21±33.09	9.19±8.95	0.001

*p value was determined by independent student t test

No significant relation was observed between Ki-67 and muscle invasion of urothelial tumor.

Table 6: Relationship between Ki-67 and muscle invasion (n=36)

	Muscle invasion stage		**p value***
	Invasive	**Non-invasive**	
Ki-67	36.26±34.86	23.29±26.90	0.218

*p value was determined by independent student t test**.**

Mean p63 was higher in patients with low grade tumor but no statistical difference was observed (p>.05).

Table 7: Relationship between p63 and grading of tumor (n=36)

	High grade n=29	**Low grade n=7**	**p value***
p63	36.55±33.14	43.14±34.58	0.659

*p value was determined by independent student t tes**t**

No significant relation was observed between p63 and muscle invasion of urothelial tumor.

Table 8: Relationship between p63 and muscle invasion (n=36)

	Muscle invasion stage		**p value***
	Invasive	**Non-invasive**	
p63	34.58±33.04	41.47±33.62	0.540

*p value was determined by independent student t test.

Discussion:

Bladder carcinoma is the most common malignancy of the urinary tract.[14] Immunohistochemistry following surgical intervention (Cystectomy or TURBT) is the gold standard diagnostic modality for urothelial carcinoma. In developing countries like Bangladesh, patients of urothelial carcinoma usually present with late features when the disease stage has been progressed. So, predicting the grade of carcinoma and condition of muscle invasion might aid in choosing the most appropriate treatment plan to prevent further spread, to increase the chance of survival and to improve the quality of life. Molecular markers might be proved very beneficial in this case but serum biomarkers to predict the disease progress of bladder carcinoma are yet to established. The present study aimed the role of Ki-67 and p63 in distinguishing the histopathological grade and muscle invasion of urothelial carcinoma. A total of 36 patients were taken in this study according to selection criteria. All patients underwent either TURBT or cystectomy. The mean age of the patients was 56 years with a majority in age group 51-70 years. Bladder carcinoma is a disease of mostly the middle-aged or the elderly people, but it can occur at any age, commonly between 50 and 80 years.[15] The median age of bladder urothelial carcinoma is 69 years in males and 71 years in females, but the disease can occur at any age, even in children.[16] In this study clear male dominance was observed with 88.90% male and 11.10% female. Shen et al. did a study on patients with urothelial carcinoma and observed that male are 3 times more likely develop bladder cancer than women.[17] In this study 88.9% of patients had papillary urothelial carcinoma which was the commonest. Of all, 80.6% of patients had high grade tumor and 19.4% of patients had low grade tumor. Among the high grade tumor 65.5% was found muscle invasion but muscle invasion was not found in low grade tumour. Usually more than 70% of urothelial cases present as superficial urothelial carcinomas that are confined to the mucosal layer or

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invade the lamina propria without muscular is propria invasion.[18] But in present study we observed most of the patient's carcinoma already invaded muscle and majority are high grade tumor. Consulting with late features may be the one of the fact behind this. Muscle invasion was significantly associated with grade of tumor. Ki-67 were higher in patients with high grade tumor which was statistically significant. No significant relation was observed of both p63 and Ki-67 among patients with muscle invasion of urothelial tumor. p63 is deregulated in bladder carcinogenesis. The expression of the cell proliferation marker, Ki-67 revealed variability in the mitotic activity between NMIBC and the more aggressive muscle-invasive tumors, thereby reflecting its prognostic significance.[19] Hegazy et al. and Raheem et al. observed a significant correlation between p63 overexpression and tumor stage which doesn't corroborate with the present study.[20,21] Warli et al. observed significant association between Ki-67 and tumor grade which corroborate with present study.[22] Chandrasekar et al. and Ibrahim et al. also reported similar findings.[23,24]

Limitations:

- All samples were collected from a single site.
- Sample size was not representative to generalize the findings.
- Arrangement of fund by the patients for the immunocyto chemistry was a great challenge.

Conclusion:

Muscle involvement was significantly associated with high grade tumor. Ki-67 expression was statistically higher in high grade tumour. But no association was observed of p63 with tumour grade. None of the markers showed association with muscle invasiveness.

Recommendation:

1. Long term follow up of the patients to correlate expression of p63 and Ki-67 with progression and recurrence of the disease and survival would be beneficial to establish prognostic role of these biomarkers.

2. Multi-centered, population based study with reliable, reproducible and equivalent standardized techniques, use of other biomarkers would bring out more and precise representative data.

Author's Contributions:

All the authors were contributed in various parts of the publication from concept and design, acquisition of data, analysis & interpretation of data and drafting of the manuscript.

Declaration of Conflicts:

The authors declare that, there is no conflict of interest regarding the publication of this article.

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