Section: Pathology

Original Research Article

ROLE OF P53 AND KI67 IN URINARY BLADDER CARCINOMAS

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ABSTRACT

Background: Urothelial carcinoma comprises 90% of bladder tumor. The p53 gene is a tumor suppressor gene located on chromosome 17p; important for genome stability, response to genotoxic stimuli, and activation of apoptosis. Nuclear antigen Ki-67 coded by gene on chromosome 10 is absent in resting cells (G0 phase) and hence exclusively positive in the nuclei of proliferating cells.

Materials and Methods: This was a prospective study. All cases presenting with hematuria were subjected to ultrasonography and cystoscopy then TURBT chips or cystectomy was performed in patients showing growth by cystoscopy. In the IHC the homogenous nuclear positivity was seen as dark brown colour. The percentage of immunopositivity was calculated by counting atleast 1000 tumor cells in areas of maximum positivity. The cells having nuclear positivity are calculated in the ratio of total number of cells. The results were interpreted taking the cutoff value as 20% and divided into three categories as immune negative, 20% as high expression for both immunomarkers positivity. Data was analysed using chi square statistical methods. P value less than 0.05 was considered as significant.

Results: P53 positivity with more than 20% expression was found in high grade urinary bladder carcinomas and cases with pT2 stage. Some Low grade urothelial carcinomas with lamina propria invasion (pT1) also showed high p53 expression. There were high grade tumors also showing low expression of p53. So, prognosis was good in those cases. Ki67 expression was increased with increased grading and staging of bladder carcinomas. In Squamous cell carcinoma p53 showed low expression and ki67 showed high expression. Adenocarcinoma of the bladder showed high p53 and ki67 expression. Other high grade non papillary urothelial carcinomas also showed high p53 and ki67 expression.

Conclusion: Urothelial carcinoma is the seventh most common cancer in the world. Many factors have been known as risk factors of this condition. Spectrum of p53 and Ki67 are useful as potential prognostic markers in bladder cancers.

Key words: p53, Ki67, Urinary bladder carcinoma.

INTRODUCTION

Urothelial carcinoma comprises 90% of bladder tumor. Most common presentation is gross or microscopic painless haematuria. Cystoscopy and biopsy of suspicious area are gold standard for diagnosis. Incidence increases with age, smoking, tobacco use, industrialization, petrochemicals arylamines exposure and urbanization. Most cases are seen in patients of more than 50 years of age.[1,2]

As there is more risk of tumor recurrence, stage progression, and tumor-related mortality with increasing grade and infiltrative tumor pattern, histopathological grading and staging of urothelial carcinomas can be an important tool in determining the prognosis of the disease. However, these pathologic variables exhibit limited ability to predict the response toward treatment more so where are different treatment approaches and follow-up schedules. Hence, there is a clear need for molecular

studies which may be helpful for accurate prediction of the behaviour of these cases.[3,4]

The p53 gene is a tumor suppressor gene located on chromosome 17p; important for genome stability, response to genotoxic stimuli, and activation of apoptosis. In normal tissues, p53 gene product is 393-amino acid nuclear protein with shorter half-life while mutated p53 gene product having longer half-life is accumulated which allows its detection by immunohistochemistry easily.[5]

Proliferation abnormalities resulting from disruption of cell cycle regulators and also abnormal cellular proliferation can be easily detected by immunohistochemistry using Ki-67 labelling index which is a measure of cell growth fraction and therefore also of biological aggressiveness of malignancy. Nuclear antigen Ki-67 coded by gene on chromosome 10 is absent in resting cells (G0 phase) and hence exclusively positive in the nuclei of proliferating cells. P16 protein, epidermal growth factor receptor (EGFR), cytokeratin 7 and high molecular weight 34 beta E12 cytokeratin (HMW-cytokeratin characteristic of basal cells), bcl2, bax are the other immunohistochemical markers used for urinary bladder carcinoma.[6-8]

MATERIALS AND METHODS

This was a prospective study. All cases presenting with hematuria were subjected to ultrasonography and cystoscopy then TURBT chips or cystectomy was performed in patients showing growth by cystoscopy. Clinical data including registration number, age, sex, family history, addiction history, occupational history, clinical presentation, cystoscopy finding and other relevant investigation reports were collected. Tissue samples in 10% buffered formalin were received as TURBT chips or bladder biopsy in histopathological section and processed. 3-5 micron thick H&E stained sections were analyzed for histopathological study. For immunohistochemical study 2-4 micron thick sections were taken on poly L lysine coated slides and subjected to antigen retrieval by microwave heat method. Then IHC staining of P53 & Ki67 was performed using primary antibodies DO7 and sp6.

The basic principle, as with any other special staining method is a sharp localization of target components in the cell and tissue, based on a satisfactory signal to noise ratio. Amplifying the signal, while reducing non –specific background staining (noise) is the major strategy to achieve a satisfactory and practically useful result.

In the IHC the homogenous nuclear positivity was seen as dark brown colour. The percentage of immunopositivity was calculated by counting atleast 1000 tumor cells in areas of maximum positivity. The cells having nuclear positivity are calculated in the ratio of total number of cells. The results were interpreted taking the cutoff value as 20% and divided into three categories as immune negative, 20% as high expression for both immunomarkers positivity. Data was analysed using chi square statistical methods. P value less than 0.05 was considered as significant.

RESULTS

Most common age of presentation of carcinoma of bladder is between 41-60 years with a mean age of 53 years. Maximum number of patients were Males (89.2%). Male: Female ratio is 8.2:1. Most common presenting symptom was haematuria. Addiction history (cigarette smoking) was present in 74 % of patients followed by occupational exposure, tobacco and others. On histopathological examination majority cases were papillary urothelial carcinomas. Squamous adenocarcinomas are less common. P53 positivity with more than 20% expression was found in high grade urinary bladder carcinomas and cases with pT2 stage. Some Low grade urothelial carcinomas with lamina propria invasion (pT1) also showed high p53 expression. There were high grade tumors also showing low expression of p53. So, prognosis was good in those cases. Ki67 expression was increased with increased grading and staging of bladder carcinomas. In Squamous cell carcinoma p53 showed low expression and ki67 showed high expression. Adenocarcinoma of the bladder showed high p53 and ki67 expression. Other high grade non papillary urothelial carcinomas also showed high p53 and ki67 expression.

Table 1: Number of cases studied

No of cases	Urothelial Carcinoma	Squamous cell carcinoma	Total
n=84	82	01	84
Male	74	01	76
Female	8	00	08

Table 2: Age and sex distribution of Urothelial carcinoma

Age Group	Male	Female	Total
20-40 years	02	00	02
41-60 years	49	07	56
>60 years	23	01	24
Total	74	08	82

Table 3: Histological grading and staging of urothelial carcinoma (n=82) with age and sex distribution

Variables	Uroepithelial Low Malignant Potential		Low Grade Urothelial Carcinoma		High Grade Urothelial Carcinoma	
No of cases	01		30		51	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
	01	00	22	08	51	00
Age						
20-40 years	00	00	1	00	01	00
41-60 years	01	00	15	07	33	00
>60 years	00	00	06	01	17	00
Stage						
pTa (no invasion)	01	00	03	00	07	00
pT1 (lamina Propria Invasion)	00	00	20	05	17	02
pT2 (Muscle Invasion Present)	00	00	02	00	25	01

Table 4: Distribution pattern of p53 IHC staining according to histologic type and grade of Urinary bladder carcinoma

Type and grade of Urinary Bladder Carcinoma	P53 Immuno Expression		
	Immunonegativity	**<20% Expression**	**>20% Expression**
LGUC	**02**	**18**	**10**
HGUC	**01**	**05**	**45**
SCC	**00**	**01**	**00**
Adenocarcinoma	**00**	**00**	**01**

Table 5: Distribution pattern of p53 IHC staining according to histologic stage of Urinary bladder carcinoma

Type and grade of Urinary Bladder Carcinoma	P53 Immuno Expression		
	Immunonegativity	**<20% Expression**	**>20% Expression**
pTa	**00**	**09**	**02**
pT1	**01**	**16**	**26**
pT2	**00**	**02**	**26**

Table 6: Distribution pattern of ki67 IHC staining according to histologic type and grade of Urinary bladder carcinoma

Type and grade of Urinary Bladder Carcinoma	Ki67 Immuno Expression		
	Immunonegativity	**<20% Expression**	**>20% Expression**
LGUC	**00**	**28**	**02**
HGUC	**00**	**15**	**36**
SCC	**00**	**00**	**01**
Adenocarcinoma	**00**	**00**	**01**

Table 7: Distribution pattern of ki67 IHC staining according to histologic stage of Urinary bladder carcinoma

Type and grade of Urinary Bladder Carcinoma	Ki67 Immuno Expression		
	Immunonegativity	**<20% Expression**	**>20% Expression**
pTa	**00**	**09**	**02**
pT1	**00**	**24**	**17**
pT2	**00**	**01**	**27**

DISCUSSION

Urinary bladder carcinoma is a common multistage progressive malignancy ranking 9th in worldwide cancer incidence. From developed to developing countries its burden is increasing in past few days. It is also responsible for significant morbidity and mortality. In our study, 84 cases of urinary bladder carcinoma biopsies (TURBT and cystectomy specimens) were studied. Out of which 82 (97.6%) were urothelial carcinoma, 1 case of squamous cell carcinoma of bladder (1.2%) and 1 (1.2%) case of adeno carcinoma of bladder. From the cases of urothelial carcinoma 74 are male persons (89.2%) and 8 (10.8%) were female patients. Out of 74 male patients 23 patients (31.0%) were of age group more than 60 years & 49 cases (66.2%) were studied between 41 to 60 years of age. Out of 82 cases of male urothelial carcinoma 51 (62.2%) were of high grade, 30 (36.5%) were of low grade, 1 case (1.3%) was of low malignant potential. In females total 8 cases of urothelial carcinomas were studied. 1 female patient (12.5%) was of age group more than 60 years. 7 female patients (87.5%) with urothelial carcinoma were studied between 41 to 60 years of age.

So, from this study, majority of patients are male and are of more than 40 years of age group. This finding coincides with study done by Joshi Haematuria,[4] was the most common presenting symptoms as out of 84 cases 77 cases (91.6%) were presented with Haematuria. 6 cases (7.1%) were presented with burning micturition (Dysuria) and urinary tract infection. 1 case (1.3%) had presented pain in lower abdomen. Out of 82 cases of urothelial carcinoma 61(74.2%) male patients are with

addiction history of tobacco smoking, chewing of tobacco, and occupational history of exposure to arylamine and petrochemicals (chemical substances). The predominant histological variant was urothelial carcinoma. Squamous cell carcinoma was noted in 1 out of 84 (1.2%) cases. Beltran et al (2010),[5] described incidence of squamous cell carcinoma of bladder as approximately 1 -3%. 54 cases out of 82 cases (65.7%) were having no muscle invasion. Urothelial carcinomas were divided as low and high grades depending upon cellular atypia, polarity, epithelial thickness and mitotic activity (WHO-2004 grading system). High grade tumors were found to have more common in patients above 40 years of age. Staging was done according to American Joint Committee on Cancer TNM classification. In this study, out of 82 cases 11 (13.4%) cases were of stage pTa. 53 cases (64.6%) were of stage pT1 (with lamina propria invasion) and 28 cases (34.0%) were of stage pT2 (detrusor muscle invasion).

In this study it was observed that out of 84 cases of urinary bladder carcinoma 56 cases (66.6%) showed high p53 expression. 25 (29.5%) cases showed low p53 expression. 3 Cases (3.9%) were Immuno-negative. High expressions of p53 were seen mainly in high grade tumors i.e 45 cases as compared to 10 cases of low-grade tumors whereas Low expression was noticed in 18 patients of low-grade tumors and in only 5 cases of high-grade tumors. 10 cases of low-grade carcinoma showed high p53 immuno expression. So, prognosis was poor in these cases.15 cases of high grade carcinomas shows lows p53 expression. So, prognosis was good in these cases. This difference of p53 expression was statistically significant. Out of 2 cases of immune-negativity, 1 case was of pT1 stage and one case was of pT2 stage. Squamous cell carcinoma showed low expression of p53. Adenocarcinoma of bladder showed high expression of p53. It can be inferred that from the above study, results with higher p53 expression may be an indicator for tumor progression and failure of local therapy. That also requires early surgical intervention specially for localized (noninvasive) carcinomas. Similar result was given by study done by Serth et al (1995).[10]

To study ki67 immuno expression 43 cases (51.1%) showed less than 20% immune expression and 40 cases (47.1%) showing more than 20% immune expression. All muscle invasive tumors(pT2) along with squamous cell carcinoma and adenocarcinoma showed high ki67 expression. Out of 11 patients with pTa stage 9 cases showed low ki67 expression. In pT1 stage of carcinomas, low and high expression of ki67 were noticed in 24 and 17 cases respectively. Difference was found to be statistically significant in terms of grading ($P < 0.05$) and staging. Expression of Ki-67 was consistently more increasingly changed with staging as compared to p53 expression. This study coincides with study done by Margulis et al (2009).[11]

CONCLUSION

Urothelial carcinoma is the seventh most common cancer in the world. Many factors have been known as risk factors of this condition. Spectrum of p53 and Ki67 is useful as potential prognostic markers in bladder cancers.

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