A STUDY OF P53 EXPRESSION IN UROTHELIAL NEOPLASMS OF URINARY BLADDER

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ABSTRACT

BACKGROUND

Urothelial Cell Carcinoma (UCC) of urinary bladder is the seventh commonest cancer wordwide.[1] At initial diagnosis, 30% of UCC display solid and invasive growth patterns and are locally advanced or metastatic at the time of diagnosis. 70% of tumours are noninvasive papillary UCC confined to the epithelium and subepithelial connective tissue,[2] which can be managed by endoscopic resection. A significant number of post-resected cases, progress for recurrence of tumour and infiltration to muscle layers. Invasive bladder cancer has high morbidity and uniform mortality when it is metastatic. There are no effective tools to predict aggressiveness of tumour, so that these cases can be managed more successfully. Mutated Tp53/p53 is the genetic abnormality most frequently associated with UCC and related to cell transformation, malignancy and high recurrence rates.[2]

MATERIALS AND METHODS

This is a descriptive study conducted in the departments of urology and pathology and during the period of March 2014 to February 2015. All consecutive cystoscopic biopsies, Trans urethral resection of bladder tumour (TURBT) and radical cystectomy specimens histopathologically diagnosed as UCC were included in the study. p53 expression was assessed by immunohistochemistry. Positive and negative controls were used. Bivariate analysis was done using Chi-square test in all cases.

RESULTS

A total of 80 cases were analysed. Significant association of p53 expression was found in higher grades of tumour. Also, noted relation of p53 mutation with tumour size, multifocality, multiplicity, muscle invasion and tumour stage, which were statistically not significant.

CONCLUSION

Bladder tumour grade shows significant association to p53 expression. Papillary neoplasm of low malignant potential (PUNLMP) tumours are negative for p53, and in the present study, there was significant difference in p53 over expression low-grade papillary UCC compared with PUNLMP. 90% of low-grade papillary tumours were p53 positive. This indicates a crucial role of p53 mutation in further tumour progression from PUNLMP to low-grade UCC. p53 mutation may have a role in transformation of low to high-grade TCC, and in this study, we found increased p53 expression with increased grade. But, though tumour size, multifocality, recurrent tumours and advanced stage show positive relation to p53, the association couldn't be proved to be statistically significant.

KEYWORDS

p53 Mutation, Urothelial Cell Carcinoma, p53 Immunoreactivity.

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BACKGROUND

Urothelial Cell Carcinoma (UCC) of urinary bladder is the fourth commonest cancer in men in developed countries and seventeenth in women representing 3.2% of all adult cancers. The overall male:female ratio is 3:1. It is also the 9th most common cause of death in the world.[3,4] At initial diagnosis, 70% of tumours are Non-Muscle Invasive Bladder Cancer (NMIBC), which can be managed by endoscopic resection. Following resection, depending upon the risk characteristics of the tumour, further treatment with intravesical chemotherapy or immunotherapy is indicated to prevent recurrences. However, a significant number of post-resected cases recur (50% in 4 years) or show stage progression with muscle invasion (10-15%). The most important factors for early recurrence are prior recurrence rate, number of tumours and tumour grade. For progression, stage and grade are the most important prognostic factors.[3] T1G3 patients have the worst prognosis with 1 and 5 year progression rates of 11.4% and 19.8%.[3] It is now established that acquired genetic alterations play a major role in bladder cancer and that some germline genetic variants have been associated with the risk of developing distinct subphenotypes of bladder cancer according to tumour stage and grade.[5] Tp53/p53 is the most important human tumour suppressor gene and somatic alterations in Tp53/p53 is one of the most frequent alterations in bladder cancer, especially with the more aggressive tumours.[6] TP53

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codes for a protein that regulates cell cycle and hence function as tumour suppressor.[7] Human p53 gene is located on the chromosome 17 (17p13.1). The name is due to the molecular mass (53 kilodalton). Tp53 is described as the guardian of the genome as it conserves the genome stability. Tp53 up regulates the activity of many genes modulating apoptosis; therefore, mutation of Tp53 inhibits apoptosis. Wild type Tp53 protein has a short half-life; however, the protein encoded by mutated Tp53 remains active for long period. Therefore, mutation of Tp53 gene results in accumulation of p53 protein in cell nuclei and has been studied in detail by many research groups.

Aims and Objectives

Aim of this study is to study the expression of mutated p53 with regard to grade and stage of the tumour and to compare the intensity of p53 immunoreactivity with the different grades of the tumour.

MATERIALS AND METHODS

All consecutive cystoscopic biopsies, TURBT and radical cystectomy specimens histopathologically diagnosed as UCC bladder included in this study. Clinical details of all cases were collected using a structured proforma. Formalin fixed paraffin embedded sections were cut into 5 micrometre thick sections and stained with H and E. Each case was reviewed and graded according to WHO2004 Classification. p53 expression was assessed by immunohistochemistry using mouse monoclonal antibody (PathnSitu). Positive and negative control slides were included in each run of staining. Expression of p53 was calculated as a percentage of labelled nuclei per 500 cells counted in the most immunoreactive region of the tumour and categorised into negative, weak, positive and strong positive. Negative expression was reported when less than 10% are stained. 10-20% was taken as weak positive and more than 20% strong positive. All results were entered in excel sheet and analysed by statistical software SPSS. Categorical variables analysed by proportion and compared using Chi-square test.

Definition of Variables Used in Study Size of Tumour- Tumour with size more than 3 cm taken as large and size less than 3 cm as small tumours.

Multifocal Tumours- Presence of more than one tumour in cystoscopy or imaging or intraoperatively or gross pathology was taken as multifocal.

Histopathological Grade of the Tumour- PUNLMP (papillary urothelial neoplasm of low malignant potential), low-grade and high-grade according to WHO/ISUP classification.[3]

Low-grade papillary tumour with increased cell size, nuclear atypia and occasional mitotic figures.

High-grade papillary cell dyscohesion, cell pleomorphism, nuclear atypia, prominent nucleoli and frequent mitosis.

OBSERVATION AND RESULTS

A total of 80 cases, which included 58 TURBT specimens, 20 cystoscopic biopsies and 2 radical cystectomies.

Age at Presentation- Age ranged from 40 to 88 with mean age 63.2 years. Most patients were in seventh decade.

Age	Count	Percentage
40-49	5	6.3%
50-59	24	30.0%
60-69	29	36.3%
70-79	18	22.5%
80-89	4	5.0%
Total	**80**	**100%**

Table 1. Percentage Distribution of Sample According to Age

Gender- 59 cases were males and 21 were females (Figure 1).

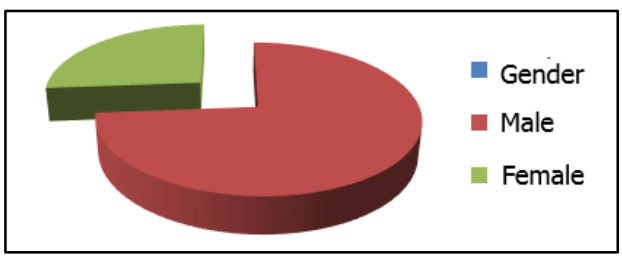

Figure 1. Percentage Distribution of the Sample According to Gender

Number of Tumours- 77.5% of cases showed single tumour while 22.5% cases, the tumour was multifocal (Table 2).

Number of Tumours	Count	Percentage
Single	62	77.5%
Multiple	18	22.5%
Total	**80**	**100%**

Table 2. Distribution According to Number of Tumours

Tumour Size- Out of 80 cases, 54 (67.5%) were small tumours and 26 (32.5%), large tumours (Table 3).

Tumour Size	Count	Percentage
Small tumours (<3 cm)	54	67.5%
Large tumours (≥3 cm)	26	32.5%
Total	**80**	**100%**

Table 3. Percentage Distribution of the Sample According to Tumour Size

Histopathological Features- Among the 80 cases, 39 cases were diagnosed as low-grade papillary urothelial carcinoma, 37 cases as high-grade papillary urothelial carcinoma and 4 cases as Papillary Urothelial Carcinoma of Low Malignant Potential (PUNLMP) (Table 4).

Grade	Count	Percentage
PUNLMP	4	5%
Low-grade papillary	39	48.%75
High-grade papillary	37	46.25%
Total	**80**	**100%**

Table 4. Distribution According to Histopathological Grade

Squamous differentiation was noted in 6 and sarcomatoid in one case of high-grade tumours.

Invasion of Tumour

A. Invasion to Lamina Propria- Of the total 80 cases, 28 (35%) tumours showed invasion into lamina propria.

B. Invasion to Muscle Layer- In 18 (23%) cases, the sample did not show any muscle tissue and invasion could not be assessed. In the rest 62 cases, which contained muscularis propria in the biopsy, 49 (79.3%) cases were non-muscle invasive and 13 cases (79.03%), muscle invasive (Table 5).

Invasion to Muscle	Count	Percentage
Present	13	16.2%
Absent	49	61.2%
Inconclusive due to absence of muscle tissue	18	22.5%
Total	**80**	**100%**

Table 5. Frequency of Muscle Invasion

C. Invasion to Perivesical Fat- Two cases showed perivesical fat invasion, both were radical cystectomy specimens.

D. Invasion to Pelvic Organs and Pelvic Lymph Nodes- Two cases showed invasion to pelvic organs (prostate, cervix). Only one case showed involvement of lymph nodes.

Primary Tumour or Recurrent Tumour- Among 80 cases, 68 (85%) were primary tumours, while 12 (15%) were recurrent tumours (Table 6).

Primary or Recurrent	Count	Percentage
Primary	68	85.0%
Recurrence	12	15.0%
Total	**80**	**100%**

Table 6. Percentage Distribution of the Sample According to Primary or Recurrent

Out of the 12 recurrent cases, 8 were low-grade urothelial carcinoma and 4 were high-grade urothelial carcinoma.

Clinical Stage of Tumour- Out of 80 cases, majority of cases (52.5%) were in the stage $T_1N_0M_0$ (Table 7).

Stage	Count	Percentage
$T_aN_0M_0$	10	12.5%
$T_1N_0M_0$	42	52.5%
$T_2N_0M_0$	8	10.0%
$T_3N_0M_0$	17	21.3%
$T_4N_0M_0$	2	2.5%
T any N1M0	1	1.3%

Table 7. Percentage Distribution According to Clinical Stage

Immunohistochemical Staining for p53- In the present study, tumours expressing p53 in more than 10% of the nuclei were regarded as positive, which was found in 72 (90%) of the examined tumours. Whereas, in 8 (10%) cases, p53 was absent. Negative expression was observed in all cases of PUNLMP (Table 8).

p53 Expression	Count	Percentage
Yes	72	90.0%
No	8	10.0%

Table 8. Percentage Distribution of the Sample According to p53 Expression

Bivariate Analysis- Bivariate analysis was done using Chi-square test in all cases to assess the expression of p53 on the grade and behaviour of the tumour.

p53 Expression and Age of Patient- Among 80 cases, positive expression observed in 93.1% in age group below 60, 86.2% in age 60-69 and 90.9% above 70 years. P53 expression has no association to age (p value 0.672).

Age	p53 Expression				x^2	p value
	Yes		No			
	Count	Percentage	Count	Percentage		
<60	27	93.1	2	6.9	0.79	0.672
60-69	25	86.2	4	13.8		
≥70	20	90.9	2	9.1		

Table 9. Association of p53 Expression and Age of Patient

p53 and Gender- p53 overexpression seen in 91.5% males and 85.7% females. P53 expression has no correlation to age (p value 0.446).

Gender	p53 Expression				x^2	p value
	Yes		No			
	Count	Percentage	Count	Percentage		
Male	54	91.5	5	8.5	0.58	0.446
Female	18	85.7	3	14.3		

Table 10. Association of p53 Expression and Gender of Patient

p53 Expression and Tumour Size- Out of 80 cases, 88.9% of small tumours and 92.3% of large tumours shown p53 expression. But, this association was not statistically significant (p value 0.633).

Tumour Size	p53 Expression				x^2	p value
	Yes		No			
	Count	Percentage	Count	Percentage		
Small <3	48	88.9	6	11.1	0.23	0.633
Large ≥3	24	92.3	2	7.7		
Table. 11. Association of p53 Expression and Tumour Size						

p53 Expression and Tumour Number- 87.2% of single tumours and 100% of multifocal tumours showed positive p53 expression. But, the association was not statistically significant (p value 0.108).

Tumour Number	p53 Expression				x^2	p value
	Yes		No			
	Count	Percentage	Count	Percentage		
Single	54	87.1	8	12.9	2.58	0.108
Multiple	18	100	0	0.0		
Table 12. Association of p53 Expression and Tumour Number						

p53 and Grade of Tumour- 90% of low-grade tumours and 92.3% of high-grade tumours showed p53 overexpression. But, none of PUNLMP was positive for p53. This association was statistically significant (p value 0.010).

Grade of Tumour	p53 Expression				x^2	p value
	Yes		No			
	Count	Percentage	Count	Percentage		
PUNLMP	0	0.0	4	100	9.23	0.010
Low grade	35	89.74	4	10.26		
High grade	35	94.6	2	5.4		
Table 13. Association of p53 Expression and Tumour Grade						

p53 and Recurrence of Tumour- p53 overexpression observed in 88.2% of primary tumours and 100% of multiple tumours. But, this association was not statistically significant (p value 0.210).

Primary or Recurrence	p53 Expression				x^2	p value
	Yes		No			
	Count	Percentage	Count	Percentage		
Primary	60	88.2	8	11.8	1.57	0.210
Recurrence	12	100	0	0.0		
Table 14. Association of p53 Expression and Tumour Recurrence/Primary						

p53 and Clinical Stage of Tumour- TaN0M0 80%, T1N0M0 90.5%, T2N0M0 87.5%, T3 T4 tumours 100% were p53 positive. But, the relation was not statistically significant (p value 0.597).

Stage	p53 Expression				x^2	p value
	Yes		No			
	Count	Percentage	Count	Percentage		
TaN0M0	8	80.0	2	20.0	3.67	0.597
T1N0M0	38	90.5	4	9.5		
T2N0M0	7	87.5	1	12.5		
T3N00	17	100	0	0.0		
T4N0M0	2	100	0	0.0		
TaN1M0	1	100	0	0.0		
Table 15. Association of p53 Expression and Tumour Stage						

p53 and Muscle Invasion- 89.8% of muscle invasive tumours were positive for p53, but non-muscle invasive showed a rate of 76.9% only. But, the relation was statistically significant (p value 0.107).

Invasion to Muscle	p53 Expression				x^2	p Value
	Yes		No			
	Count	Percentage	Count	Percentage		
Present	10	76.9	3	23.1	4.47	0.107
Absent	44	89.8	5	10.2		
Inconclusive	18	100	0	0.0		
Table 16. Association of p53 Expression and Tumour Invasion to Muscle						

DISCUSSION

The age at presentation ranged in the present study from 40 to 88 years with a mean age of 63.2 years. Most patients belonged to the age group of 50-70 years. This finding is similar to the study by Karan Thurath Taufiq and Shuaib. H. S. Al-Thalib where mean age was 62.64 years and another study by Mojgan Karbakhsh et al[8] in 960 cases where the peak incidence was 62.14 years.

The gender distribution in this study, male: female ratio 2.8:1 shows a definite male preponderance. This is comparable to studies conducted by Johansson (3:1)[9] and Rajesh Singh (1.5:1).[10] In the present study, p53 expression was observed in 90% of patients. P53 expression was mainly found in seventh decade even if the relation was not statistically significant. 92.3% of large tumours showed p53 over expression, but only 88.9% positivity in small tumours. Also, multifocal tumours showed 100% positivity for p53 opposite to 88.2% in unifocal tumours. All the recurrent tumours were p53 positive compared to 88.2% in primary tumours. These observations were statistically significant as sample size was inadequate. This has to be confirmed in recruiting adequate sample.

Analysis of 80 cases of UCC showed that the difference in nuclear p53 accumulation between PUNLMP and low and high-grade UCC was statistically significant. Expression of p53 was absent in all cases of PUNLMP. This result is comparable with other similar studies done by M. Kalantari, Hassan Ahmadnia et al,[11] Turk N.S, Zafer Aybek et al[12] and Roy Chowdhury et al.[13]

Regarding intensity of staining, which was assessed by the p53 index, 10% of the low-grade carcinoma and 7.7% of the high-grade carcinoma showed negative staining (negative p53 index). 37.5% of the low-grade carcinoma and 33% of high-grade carcinoma showed low-intensity staining (low p53 index). 52% of low-grade carcinoma and 59.5% of high-grade carcinoma showed strong intensity staining (high p53 index).

In this study, though the frequency of p53 expression was higher for higher stage tumours, statistical significance was nil. But, this relation was proved by studies conducted by Ibrahim et al[14] and Galmozzi et al.[15]

CONCLUSION

Tumour grade shows significant association to p53 expression. PUNLMP tumours are negative for p53. In the present study, a significant difference in p53 over expression was noted between PUNLMP and low-grade papillary UCC. 90% of low-grade papillary tumours were p53 positive. This indicates a crucial role of p53 mutation in further tumour progression from PUNLMP to low-grade UCC. The finding that increased p53 expression with increased grade supports the role of p53 mutation in disease progression. Since, there was no significant statistical difference between low-grade and high-grade UCC in p53 nuclear accumulation, it is probable that multiple genomic alterations may play a role along with p53 mutation for transformation of low to high-grade UCC. Tumour size, multifocality, recurrent tumours and advanced stage show positive relation to p53. But, the association couldn't be proved statistically significant in this study probably because of low sample size. Further studies with adequate sample size may define it better.

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