Original Article

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Pathology Section

Immunoreactivity of p53 in Urothelial Carcinomas of the Urinary Bladder

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ABSTRACT

Introduction: Urothelial Carcinomas (UC) are heterogenous disease with unpredictable outcome. Risk stratification plays an important role in the management as 70% tumours are Non-Muscle Invasive (NMI) at the time of diagnosis. It is difficult to make prognosis for individual patients due to high rates of recurrence and progression. Over-expression of p53 cell cycle regulator protein is associated with high grade, high stage of bladder cancer and important in the multistep progression with an unfavourable prognosis. The p53 immunohistochemical staining intensity and score status identifies patients needing early cystectomy for treatment of NMI or aggressive adjuvant therapy after cystectomy.

Aim: To assess the immunohistochemical expression patterns of p53 in UC of the urinary bladder and to find out the relationship between p53 over expression with clinicopathological parameters like grading and pathological staging.

Materials and Methods: The study was conducted in JSS Medical College and Hospital, JSS Academy of Higher Education & Research, Mysuru. A total of 50 cases of UC of the bladder were included in the study (2 years each of retrospective-October 2012 to September 2014 and prospective study-October 2014 to September 2016) over a duration of two years.

The over expression of p53 antigen was evaluated by Immunohistochemistry (IHC) as intensity and a score based on the percentage of tumour cells staining positive after counting at least 500 cells in each case using high power objective of the microscope (x400).

RESULTS: Among 50 cases, 39 (78%) tumours were high grade and 11 were low grade. A 49 (98%) were positive for p53 over expression and 01 (2%) high grade non papillary tumour was negative. Higher intensity (3+) and score (4+) of p53 over expression was seen with higher grade than lower grade with statistically significant p-value (intensity - < 0.001, score - 0.006).

Also, when evaluated separately this correlation was found significant in high grade NMI (p-value: intensity- 0.021, score – 0.025) rather than muscle invasive tumours. No relationship was established between p53 over expression with pathological stage of tumour and other clinicopathological parameters.

CONCLUSION: Higher p53 over expression is associated with higher grade, particularly in NMI tumours which may go for progression and recurrence. Therefore, p53 over expression by IHC can provide important prognostic information in risk stratification of UC of the bladder. It aid in appropriate modification of treatment management and better understanding biological behaviour of UC.

Keywords: Cell cycle regulator protein, Non muscle invasive, Pathologic staging, Transitional epithelium, Tumour grade

INTRODUCTION

The Urinary bladder is a frequent site for development of cancer in the urinary tract system. In worldwide cancer incidence it ranks 9th and 7th most common malignant tumour in men and 17th in women [1,2].

India has an overall annual incidence rate of urinary bladder cancer of 2.25% (per 100,000 annually). This includes 3.67% between males and 0.83% between females [3,4]. About 95% of bladder tumours are of epithelial origin, the remainder being mesenchymal tumours. Most epithelial tumours are UC type which originate from transitional epithelium, but squamous and glandular carcinomas also occur [5].

The aetiology of bladder cancer is multifactorial, with tobacco smoking as the most common cause in most countries. Other aetiological factors include occupational exposure, analgesic abuse, genetic predisposition and chronic Schistosoma cystitis [2,6].

The UC show extremely unpredictable biological behaviour. Non-invasive bladder tumours (pTa) are confined to the mucosa and invasive tumours invade the lamina propria (pT1). pTa tumours are frequently low grade with recurrence rate of 34-72% and progression into lamina propria or muscle are low. pT1 tumours are low or high grade with more commonly papillary type having poor prognosis compared to pTa tumours. About 7-44% of pT1 tumours are anticipated to progress into muscle invasion in five years and recurrence of 15-70% within a year after transurethral resection. These tumours (pTa and pT1) are grouped as NMI for therapeutic purposes [2,7,8].

Cumulative effects of progressive nature in one or more initiating/promoting agents lead to neoplastic changes. As urothelial lesions are known for recurrences and progression to invasive tumours, analysis of bladder cancer by immunopathologic and molecular genetics is important. This has recognised a number of abnormalities in some of the genes and proteins that have been implicated in the development and progression of tumours principally in the p53 pathway mutation which play an important role in UC pathogenesis and also as prognostic factors [2,6,9-12].

Non invasive UC of the bladder is a highly unpredictable disease in spite of the emergence of new diagnostic tools and it is difficult in individual patients to make prognosis. Applying IHC to vigilantly selected proteins will recognise prognostic factors. The levels of expression of cell cycle regulators such as p53, p16, p21, pRb and cyclin D1 proteins, separately or in combinations by IHC can be exploited as prognostic factors which has been described by many researchers [13,14].

Numerous studies have evaluated relationship of p53 mutations, abnormalities and prognosis in UC. Several studies shows that the evidence of over-expression of p53 is a potential prognostic factor and correlated the association of p53 mutation with high grade, high stage and an unfavourable prognosis [2,6,9-12,15].

The present study is taken up to evaluate the relationship of p53 over expression by both intensity and score with clinicopathological parameters of UC of the bladder and to understand the prognostic implications in the Indian population.

MATERIALS AND METHODS

The study was undertaken at the Department of Pathology, JSS Medical College, JSS Academy of Higher Education & Research, Mysuru.

A total of 50 cases of UC of the bladder were included in the study (2 years each of retrospective -October 2012 to September 2014 and prospective study -October 2014 to September 2016) over a duration of two years. All cases diagnosed histopathologically as UC of the urinary bladder were included in the study. All non-malignant lesions and malignancies other than urothelial carcinomas of the urinary bladder were excluded from the study.

The materials for the study were Trans Urethral Resection of Bladder Tumour (TURBT) and cystectomy specimens received at the department of pathology, JSS Hospital after obtaining approval from the hospital ethics committee (IEC no.-ECR/387/Inst/KA/2013/RR-16) and consent of the patient. All specimens were received in 10% formalin.

Relevant clinical information including age at diagnosis, gender, presenting complaints and history of smoking were obtained according to the proforma. The specimens were then subjected to gross description and type of specimen (TURBT/Cystectomy), weight (TURBT) and tumour location (Cystectomy) were noted. TURBT chips were embedded entirely. Adequate sampling by appropriate tissue section was done for cystectomy specimens. Microscopic features were studied with routine Haematoxylin and Eosin (H&E) stained sections. Histological type, grade, depth of invasion and pathological stage of tumour were noted. For all retrospective cases (2012-2014), clinical and histopathological data were retrieved and 4µm thick sections were obtained from representative paraffin blocks and studied. The WHO–2016 and American Joint Committee On Cancer (AJCC) and TNM Pathologic Staging of Urinary Bladder Carcinomas-eight edition guidelines were followed for classification, grading and pathological staging of tumours [6,12,16,17].

Paraffin blocks best representing the tumour in each case were selected after reviewing the H&E slides for IHC staining. A 3-4 µm thick sections were taken on Poly-L-Lysine coated slides.

The slides were baked at 60°C for one hour in hot air oven. Slides were deparaffinised and rehydrated. Retrieval solution (Tris buffer for antigen retrieval) was brought to boil in the pressure cooker. Slides were placed in metal staining racks and lowered into pressure cooker ensuring that the slides were completely immersed in the retrieval solution. When the pressure cooker reached operating temperature and pressure, it was timed for upto two to three whistles. The pressure cooker was removed from the heat source and the slides were allowed to cool for 30 minutes in the same solution. The slides were washed with wash buffer for one minute. Peroxide block was applied for 10 minutes and washed with wash buffer for one minute. The sections were incubated with primary antibody (DAKO, FLEX Monoclonal Mouse Anti-Human p53 Protein Clone DO -7 Ready to Use, Code IS616) for 20 minutes and washed twice with wash buffer. The sections were then incubated with LABELLED POLYMER – HRP (DakoEn Vision + Dual Link System – HRP, DAB+, Code K4065) for 20 minutes and washed thrice with wash buffer. The bound antibody was visualized using a DAB-chromogen substrate which was prepared by adding 50µL of DAB Chromogen to 1 ml of DAB. The sections were washed with wash buffer and counterstained with haematoxylin and again rinsed in water for 5 minutes. Sections from colorectal carcinoma were taken as positive control and sections from the adjacent normal colonic mucosa were taken as negative control.

Brown granular nuclear reactivity was taken as positive. Staining characteristics of the tumours were agreed upon by two people involved in the study. Results of the histological and IHC analysis were recorded.

Tumours with nuclear immunoreactivity of more than 10% were considered positive, in accordance with previous studies [8,15,18]. The entire section was screened to determine the region with the maximum proportion of stained nuclei. The percentage of nuclei stained was assessed by counting 500 cells using high power (x400) objective in areas of maximum expression of the marker. p53 over expression was recorded

as intensity of staining and score based on the percentage of cells staining positively. Staining intensity was scored on a 4 point scale [19]; Negative (No staining) 0, Weak 1+, Moderate 2+ and Strong3+. The staining score (extent) was based upon the proportion of tumour cells positively stained; Zero (< 10%), 1+ (11 – 25%), 2+ (26 - 50%), 3+ (51 – 75%) and 4+ (>76%). The relationship of clinicopathological parameters with p53 over expression was analysed separately for intensity and score.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM Statistical Package for the Social sciences (SPSS) Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY). The rates and proportions of discrete variables were determined using the chi-square test. The Fischer's-Exact test was also used to examine significance of association between clinicopathological parameters and p53 over expression. Correlations between tumour grade, stage and p53 over expression was evaluated using Spearman's rank correlation coefficient (r). The Mann-Whitney U Test was also used to assess the significance of difference in p53 over expression between categories of clinicopathological parameters. The two-sided p-value of <0.05 was considered to indicate statistical significance.

RESULTS

Among the cases received, only six (12%) were Cystectomies and rest 44 (88%) cases were TURBT. Recurrence of tumour was noted in two cases. The youngest patient in the present study was 28-years-old and oldest was 85-years-old. The maximum number (17) of cases was in the age group of 61 to 70 years and only one case was seen below 30 years of age. The other clinicopathological variables noted were history of smoking, common clinical presentation, tumour focality, tumour configuration, tumour grade and pathological stage of the tumour [Table/Fig-1].

Histopathologically, out of 36 papillary tumour [Table/Fig-2a,b,c,d and Table/Fig-3a,b], squamous differentiation [Table/Fig-3c,d] was seen in three. In the 14 non papillary tumours[Table/Fig-4a,b], one each of nested [Table/Fig-4c,d], sarcomatoid [Table/Fig-5a,b] variant and poorly differentiated UC [Table/Fig-5c,d], along with two squamous differentiations were noted.

A total of 25 of the 36 papillary tumours were high grade [Table/Fig-2c,d] and 11 were low grade [Table/Fig-2a,b]. All 14 Non papillary tumours were high grade. On histopathological tumour grade, high grade were more common than low grade. The pathological staging showed highest cases in pTa than pT1 and above. Out of 50 cases, 39 cases showed muscle tissue. These 39 cases were classified as NMI (pTa and pT1) and muscle invasive (pT2 and above) based on absence or presence of muscle infiltration by tumour cells respectively. The maximum number of cases were seen in NMI category [Table/Fig-1].

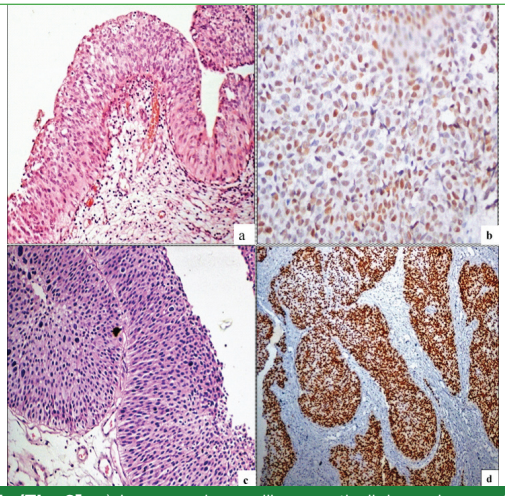
	Characteristic	Total No of cases 50	p53 intensity p-value	p53 score p-value
1	p53 over expression	49 (98%) positive	-	-
		1 (2%) negative		
2	Age (years)	mean age of 63.38	0.834	0,605
		70 years and below-36		
		Above 70 years -14		
3	M:F Ratio	6.14	-	-
4	History of smoking	36 (72%) *HG -27, † LG-09	0.409	0.932
5	Common clinical presentation	Gross haematuria 48 (96%)	-	-
6	Tumour focality	Multifocal -29 cases (58%) *HG -25, † LG-04	0.209	0.932
		Unifocal-21 cases (42%) *HG -14, † LG-07		
7	Tumour configuration	Papillary-36 (72%) *HG -25, † LG-11	0.124	0.429
		Non papillary-14 (28%) *HG -14, † LG-00		
8	Tumour grade	High grade- 39 (78%) Papillary-25, Non papillary-14	< 0.001	0.006
		Low grade -11 (22%) Papillary-11, Non papillary-00		
9	Pathological stage of the tumour	Invasive (pT1 & above)-23	0.239	0.492
		Non invasive (pTa)-27		
10	Total No of cases with muscle tissue	39		
		Non muscle invasive (pTa,pT1)- 23 *HG-18, † LG-5	0.021	0.025
		Muscle invasive (pT2& above)-16 *HG-14, †LG-02	0.053	0.075

[Table/Fig-1]: Clinicopathological characteristics of 50 urothelial carcinoma of the bladder cases with p-value.
* High grade, †Low grade

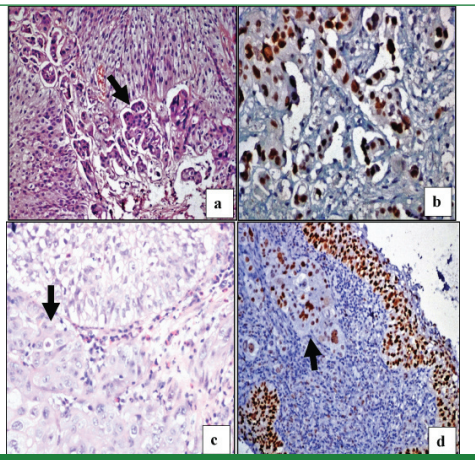
Out of 50 cases, the p53 over expression 49 (98%) were positive (> 10% cells positively stained), and 1 (2%) case was negative (<10% cells positively stained). Majority of the cases showed strong intensity of p53 over expression 3+ (46%) and staining in >75% cells, i.e., score 4+ (48%) [Table/Fig-2d]. No significant correlation was found between p53 over expression and the two age groups (70 years and below and Above 70 years), M:F ratio, history of smoking, tumour focality

and tumour configuration [Table/Fig-1]. Positive correlation was found between the grade and p53 over expression intensity and score [Table/Fig-6]. The distribution of intensity and score was seen different between the two grades. Higher grade showed higher expression of intensity and score. (Spearman's Rank correlation test, Intensity: r – 0.475, P < 0.001, Score: r – 0.381, P – 0.006) (Mann-Whitney U test, p-value: Intensity – 0.001, Score – 0.008).

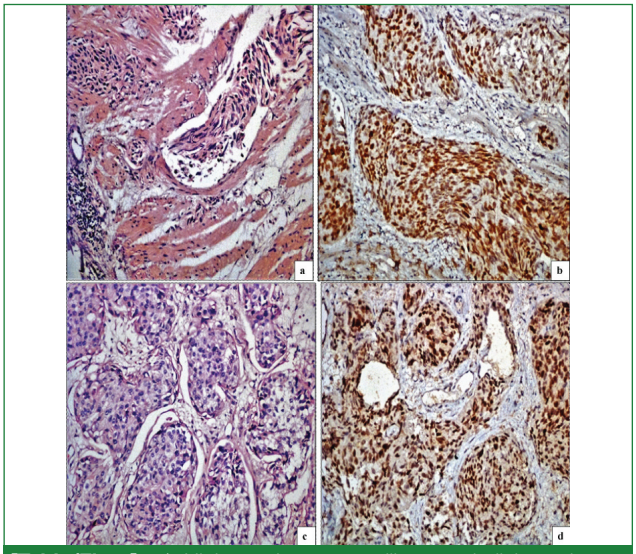
Around 11 (40.7%) of non invasive (pTa) tumours showed an intensity of 3+ and 12 (44.4%) showed a score of 4+. Whereas 12 (52.2%) invasive (pT1 & above) tumours showed both an intensity of 3+ and score of 4+. No significant correlation was noted between pathological stage and p53 over expression.



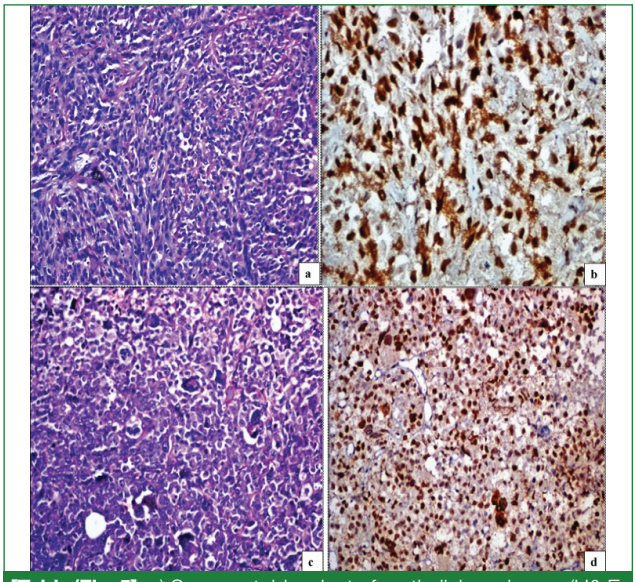
[Table/Fig-2]: a) Low grade papillary urothelial carcinoma (H&E; x 400); b) p53 IHC staining with brown nuclear positivity with Intensity 1+ and Score 3+ (p53 IHC, x200); c) High grade papillary urothelial carcinoma (H & E, x 400); d) p53 IHC staining with brown nuclear positivity with Intensity 3+ and Score 4+ (p53 IHC, x400).



[Table/Fig-3]: a) High grade papillary urothelial carcinoma showing invasion of the lamina propria by tumour cells (arrow) (H & E, x100); b) Tumour cells invading the lamina propria showing positive p53 IHC staining (p53 IHC, x200); C) High grade papillary urothelial carcinoma with Squamous differentiation (arrow) (H&E, x200); d) High grade papillary urothelial carcinoma with Squamous differentiation (arrow) showing positive p53 IHC staining (p53 IHC, x100).



[Table/Fig-4]: a) High grade non papillary urothelial carcinoma showing invasion of the muscularispropria by tumour cells (H& E, x100); b) Tumour cells invading the muscularispropria showing positive p53 IHC staining (p53 IHC, x100);
c) Nested variant of urothelial carcinoma (H& E, x100); d) Tumour cells staining positive for p53 IHC in nested variant of urothelial carcinoma (p53 IHC, x100).



[Table/Fig-5]: a) Sarcomatoid variant of urothelial carcinoma (H& E, x100); b) Tumour cells staining positive for p53 IHC in sarcomatoid variant of urothelial carcinoma (p53 IHC, x200); c) Poorly differentiated urothelial carcinoma (H& E, x40); d) Tumour cells staining positive for p53 IHC in Poorly differentiated urothelial carcinoma (p53 IHC, x100).

(Spearman's Rank correlation test, Intensity: r – 0.17, P – 0.239, Score: r –1.0, P – 0.492).

In cases where muscle tissue was present (39 out of 50), statistically significant correlation was found between high grade and p53 over expression in non muscle invasive tumours [Table/Fig-7]. A 66.7% of high grade superficial

Grade	i) p53 intensity					Total	p-value
	0	1+	2+	3+			
High	1(2.6%)	9(23.1%)	6(15.4%)	23(59.0%)		39(100%)	< 0.001
Low	0(0.0)	8(72.7%)	3(27.3%)	0(0.0%)		11(100%)	
Total	1(02%)	17(34%)	9(18%)	23(46%)		50(100%)	
Grade	**ii) p 53 score**					**Total**	**p-value**
	0	**1+**	**2+**	**3+**			
High	1(2.6%)	6(15.4%)	3(7.7%)	6(15.4%)	23(59.0%)	39(100%)	0.006
Low	0(0.0%)	3(27.3%)	5(45.5%)	2(18.2%)	1(9.1%)	11(100%)	
Total	1(02%)	9(18%)	8(16%)	8(16%)	24(48%)	50(100%)	

[Table/Fig-6]: Relationship between tumour grade and p53 over expression in non muscle invasive disease and muscle invasive disease { i) intensity; and ii) Score} by Spearman's Rank correlation test.

Muscle Invasion	Grade	i) p53 intensity					Total	p-value
		0	1+	2+	3+			
Non Muscle Invasive	High	1(5.6%)	3(16.7%)	2(11.1%)	12(66.7%)		18(100%)	0.021
	Low	0(0.0%)	3(60.0%)	2(40.0%)	0(0.0%)		5(100%)	
	Total	1(4.3%)	6(26.1%)	4(17.4%)	12(52.2%)		23(100%)	
Muscle Invasive	High	0(0.0%)	3(21.4%)	3(21.4%)	8(57.1%)		14(100%)	0.053
	Low	0(0.0%)	2(100%)	0(0.0%)	0(0.0%)		2(100%)	
	Total	0(0.0%)	5(31.3%)	3(18.8%)	8(50.0%)		16(100%)	
Total		1(2.6%)	11(28.2%)	7(17.9%)	20(51.3%)		39(100%)	
Muscle Invasion	**Grade**	**ii) p53 Score**					**Total**	**p-value**
		0	**1+**	**2+**	**3+**	**4+**		
Non Muscle Invasive	High	1(5.6%)	3(16.7%)	0(0.0%)	1(5.6%)	13(72.2%)	18(100%)	0.025
	Low	0(0.0%)	1(20.0%)	2(40.0%)	2(40.0%)	0(0.0%)	5(100%)	
	Total	1(4.3%)	4(17.4%)	2(8.7%)	3(13.0%)	13(56.5%)	23(100%)	
Muscle Invasive	High	0(0.0%)	1(7.1%)	3(21.4%)	3(21.4%)	7(50.0%)	14(100%)	0.075
	Low	0(0.0%)	1(50.0%)	1(50.0%)	0(0.0%)	0(0.0%)	2(100%)	
	Total	0(0.0%)	2(12.5%)	4(25.0%)	3(18.8%)	7(43.8%)	16(100%)	
Total		1(2.6%)	6(15.4%)	6(15.4%)	6(15.4%)	20(51.3%)	39(100%)	

[Table/Fig-7]: Relationship between tumour grade and p53 over expression in non muscle invasive disease and muscle invasive disease { i) intensity; and ii) Score} by Spearman's Rank correlation test.

tumours expressed intensity 3+ and 72.2% of high grade tumours expressed score 4+.(Spearman's Rank correlation test, Intensity: r –0.478 , P –0.021, Score: r – 0.449, P – 0.025). Whereas, 57.1% high grade muscle invasive tumours expressed intensity 3+ and 50% expressed score 4+. However, this difference of expression was not found to be statistically significant. (Spearman's Rank correlation test, Intensity: r –0.492, P–0.053, Score: r – 0.477, P – 0.075).

DISCUSSION

UC are heterogenous disease with variable outcome and commonly present with pTa or pT1 stage in 75% of patients. It is typically divided into two types, the papillary and nonpapillary types. The primary therapy is TURBT for NMI and intravesical Bacilli Calmette-Guérin (BCG) is the treatment option for high-grade tumours. Cystectomy becomes the treatment of choice for muscule invasion [2,16,20]. However, a scoring system with risk tables to predict recurrence and progression of disease was developed by The European Organisation for the Research and Treatment of Cancer-Genito-Urinary Cancer Group (EORTC-GUCG).The scoring system is based on the six most significant clinical and pathologic factors: Multifocality of tumour, tumour size, previous recurrence rate, presence of concurrent Carcinoma-in-situ, tumour grade and pT category [2,20].

Despite the emergence of new diagnostic tools, NMI UC is still an extremely unpredictable tumour and it is difficult to make

prognosis for individual patients. It is possible that applying IHC to cautiously selected proteins will identify prognostic factors. Many researchers considered to find patients at risk for disease progression by performing IHC to analyse cell cycle regulators such as p53, p16, p21, pRb, and cyclin D1 by levels of expression of these proteins, separately or in combinations as prognostic factors which contribute to the pathogenesis of UC. Numerous studies have evaluated association of abnormalities of p53 which frequently undergoes mutation in bladder carcinoma and have suggested that p53 alteration was associated with poor prognosis in UC [8,10,12-14].

To evaluate the p53 status, IHC is still the most commonly used method, even though it can be detected by molecular analysis. The mutated p53 gene protein product commonly accumulates in the tumour nuclei and is confirmed by IHC methods. In the present study 49 tumours were positive for p53 over expression and one high grade non papillary tumour was negative. This negative expression can be explained by the fact that p53 gene protein product does not accumulate in the nucleus in 15% to 20% of tumours, in spite of p53 gene mutation [8,21]. This is for the reason that some p53 gene mutations especially point mutations may result in lack of or severe decrease in p53 protein synthesis. On the other hand, in a proportion of tumours, in spite of the nuclear accumulation of p53 protein, there is no mutation in p53 gene. In this condition, it has been shown that some cellular oncogenic products, such as mouse double minute 2 (MDM2), which bind to and inactivate wild-type p53 protein, result in a long half-life of P53 protein. In fact, recent studies have revealed that over expression of MDM2 leads to over expression of P53, without any detectable P53 mutation [8,21]. However, p53 expression was statistically significant in high grade urothelial carcinomas in current study as indicated by such preceding studies [8,21].

In the present study, no statistically significant relationship was observed between age, gender, history of smoking and multifocality of tumour. Wide range of presenting age groups in the present study and different method of assessment of p53 over expression in the previous studies [9,15] (positive and negative based on percentage of cells positive) may be the reason for conflicting result. The low number of female patients was not statistically sufficient for a correlation with p53 over expression [8]. Smoking is associated with lower age, higher grade, higher stage and larger size of malignant bladder tumours at diagnosis. Also, established as a definite risk factor for recurrence and progression [8,22]. In the present study also, positive history of smoking was present in 72% of cases and 75% were found to be high grade. No studies evaluating the relationship of p53 over expression with history of smoking, tumour configuration and tumour focality were found in the literature.

The higher grade tumours were more commonly associated with p53 positivity in comparison with lower grade tumours [8,9,18,19]. This was found to be statistically significant in the present study also. However, in studies by Koyuncuer A and Yalcin O et al., no statistically significant relationship was observed in p53 expression and grade of tumour [15,23].

The p53 expression was also correlated with tumour stage. pTa tumours are less reactive than pT1-pT2 tumours. A higher percentage of muscle-invasive tumours were positively stained for p53 in comparison with NMI (pTa and pT1) tumours [8,15,19,24]. No significant relationship of p53 over expression was established with pathological stage in the present study and this disagreement with other studies can be due to the limited sample size, absence of muscle tissue in 11 cases, difference in stratification of the disease (based on depth of invasion) in different studies to assess the association with p53 over expression and no universal standardised protocol for interpretation of p53 over expression on IHC.

In NMI UC of the bladder more high grade tumours showed positivity for p53 over expression than low grade tumours with statistically significant difference between two grades [8,18]. This difference of expression was statistically significant in NMI UC rather than muscle invasive tumours in the present study also.

Prediction of recurrence and progression is very important in terms of selecting patients for early aggressive management in bladder cancer. NMI UC patients treated with maintenance BCG for 1–3 years have a diverse prognosis in respect to recurrence for first time, progression time and death. Patients at high risk of recurrence and/or progression still do poorly on current maintenance schedules. p53 is still a reliable marker in prognosis, especially in recurrences of pTa and pT1 urothelial cancers. So alternative treatments like adjuvant chemotherapy or early cystectomy can be considered [2,8,12,25].

The results of the present study suggest aggressive therapeutic approach for NMI UC of the bladder expressing high score and intensity of p53 protein. The IHC expression of p53 is a cost effective and commonly available investigation especially in a developing country where tobacco smoking and UC are on the rise. Hence p53 is useful as histopathological prognostic marker especially in NMI due to high rates of recurrence and progression.

Currently 2017 American Joint Committee On Cancer (AJCC) TNM Pathologic Staging of Urinary Bladder Carcinomas - eight edition guidelines recommends p53 as one of the novel prognostic factor for NMI UC for invasive disease and for metastatic risk and survival in muscle invasive UC [12].

LIMITATION

Limitations of the present study include small number of cases, presence of muscle tissue only in 39 cases, no universal standardised protocol for reporting of p53 over expression by IHC and no follow up of the patients as they are referred elsewhere. A prospective study with larger number of patients with adequate resection of tumour including muscle tissue and appropriate follow up may provide better insight and validate the findings.

CONCLUSION

p53 over expression by IHC can provide important prognostic information in risk stratification of UC of the bladder. The IHC detection of the p53 protein, which exploits the difference in life span between mutated and wild-type p53 has shown

to correlate strongly with mutations of the p53 gene in bladder cancer. Incorporating IHC p53 over expression in histopathology report can aid in appropriate modification of treatment management and better understanding biological behaviour particularly in NMI tumours of UC.

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