INTRODUCTION
Urinary bladder cancers accounts for approximately 7% of all cancers in western population. Male to female ratio is 4:1. Many social, occupational and environmental factors are responsible for these tumors.1 Around 90% of these tumors are papillary urothelial neoplasms. Many known risk factors, like cigarette smoking, arylamine exposure, schistosoma infection, analgesics, immunosuppressant drugs and radiation are responsible for bladder cancers.

Urothelial lesions range from benign lesions to aggressive cancers so that a complete range of papillary hyper plastic lesions, preneoplastic, non-invasive papillary lesions and invasive papillary cancers exist.2 Their differentiation depends upon cytological features of malignancy into high and low grade. They are staged according to their biological behavior of invasion beyond basement membrane. Approximately 10% of low grade and 80% of high grade cancers are invasive.3 The grey area between benign and malignant is occupied by papillary urothelial neoplasms of low malignant potential (PUNLUMP). PUNLUMP exhibit many features of benign papillomas of bladder and show only subtle features of thickened urothelium and nuclear enlargement. They cannot be distinguished from papillary cancers on cystoscopy. PUNLUMP recur frequently and may progress to high grade malignancy.4 So, it is of utmost importance to confidently differentiate between these tumors on microscopic examination.

A molecular model for bladder carcinogenesis has been investigated. It is initiated by deletion of tumor suppressor genes on chromosome 9 (seen in superficial tumors) and progression to invasive cancer by p53 mutations.1 Expression of p53 has been studied in upper urinary tract urothelial cancers and is seen to be associated with higher grade, stage and female gender.5 However, no study has been conducted previously in Pakistan regarding expression of p53 alone in malignant urothelial neoplasms as an indicator of prognosis. Accuracy of overexpression of p53 as supportive ancillary test in differentiating low grade, high grade and
predicting stage of urinary bladder cancer will help in proper and definite diagnosis of urothelial cancers.\textsuperscript{6,7} The objective of this study was to determine association of immunohistochemical expression intensity of p53 with grade and stage of urothelial cancers.

**METHODOLOGY**

Seventy biopsies of transurethral resection/radical cystectomy specimens of papillary urothelial neoplasms of bladder, submitted at Pathology Department, were included in the study from January to December 2016. All low grade and high-grade papillary urothelial cancers of patients presenting for first time were included through non-probability consecutive sampling technique. All inadequate/insufficient tissue biopsies, recurrent tumors and tumors other than papillary urothelial cancers and patients undergoing chemo/radiotherapy for previous malignancy were excluded. Clinical, radiological and cystoscopic findings of patients were noted from charts of patients in urology ward by concerned urologist after taking informed consent. Hematoxylin-eosin (HE) stained slides were prepared after processing, and cases were graded according to WHO 2004 grading system. TNM system was used for pathological staging as pTis, non-invasive papillary urothelial carcinoma; pT1, tumor invades lamina propria; pT2, tumor invades muscularis propria; and pT3, tumor invades perivesical tissue (proven on histology after cystoscopic biopsy/radical cystectomy).\textsuperscript{8} On selected slides, IHC p53 was applied and its expression intensity was noted according to percentage of positive cell present. Immunohistochemistry was performed using a streptavidin-biotin-peroxidase technique with a monoclonal antibody to p53 protein. Five µm sections were cut from paraffin embedded samples on poly-L-lysine coated slides, deparaffinized in xylene and then dehydrated. The slides were then treated by microwave heating in citrate buffer (pH 0.6) for 10 minutes for antigen retrieval. Endogenous peroxidase activity was blocked by using three percent hydrogen peroxide solution. The sections were incubated with primary antibody p53 at 1:50 dilution for one hour at room temperature. Samples were washed with phosphate buffered saline and incubated with biotin conjugated secondary antibody. Incubation with streptavidin-biotin system was repeated at room temperature for 30 minutes. Specimens were then immersed in diaminobenzidin tetrahydrochloride. Hematoxylin was used as counterstain. Slides were rinsed and mounted. Non-neoplastic bladder mucosa served as internal control. Slides were examined by two pathologists under microscope. Nuclear immunoreactivity was considered positive if present in >10% of tumor cells, and negative if <10% of tumor cells.\textsuperscript{6} Intensity was calculated as weak (less than 15% cells) and strong (more than 15% cells).\textsuperscript{9,10}

Data was analyzed by SPSS version 21. Linear-by-linear association was calculated between p53 expression and stage of urothelial tumors, Chi-Square test was used to see association between grade and intensity of p53. Qualitative variables, like grade and stage of carcinoma along with p53 expression, were calculated in terms of frequencies and percentages. P≤0.05 was taken as significant.

**RESULTS**

A total of 70 patients were included in the study. Out of them, 61 (87.1%) were males and 9 (12.9%) females. Their ages ranged from 25-90 years. Mean age was 57 ±13.9 years. Most common age group was found to be 56-65 years (n=22, 31.4%). Size of bladder lesions ranged from 1-8 cm. Mean size was 3.23 ±1.78 cm; 23% (n=16) of biopsies were 3 cm. Regarding the site of the lesion, lateral walls of bladder 42 (60%) were most frequently involved followed by posterior wall (n=19, 27%) and dome (n=1, 1%) of bladder. Fifty-five (78.6%) of patients were smokers. Most common symptom was hematuria (n=63, 90%) followed by retention of urine (n=6, 8.6%) and dysuria (n=1, 1.4%).

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<th>Table I: Association of tumor grade with p53.</th>
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<td>p53 Low grade</td>
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<td>n=25 (35.7%)</td>
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<tr>
<td>Negative</td>
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<td>Positive</td>
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Figure 1: Negative staining of p53x400 (low grade carcinoma).

Figure 2: Weak staining of p53x400 (low grade).

Figure 3: Strong staining of p53x400 (high grade carcinoma).

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Bladder cancer is one of the common tumors in third world population and poses a constant threat of recurrence and progression of cancer. High morbidity and mortality rate can be due to lack of education of population regarding risk factors and late presentation in tertiary care hospitals. Most of the patients presented with invasive bladder carcinoma such as stage T1 and beyond. A multicenter study conducted in Poland on 1,360 patients in year 2012-2013 showed that 39.2% of patients had stage Tis, 37.9% had T1, and 21.8% had reached stage T2. In this study, 6 (8.6%) patients had non-invasive tumors (Tis), 29 (41.4%) had lamina propria invasion (pT1), 29 (41.4%) had muscle invasion (pT2), 5 (7.1%) showed pT3a, macroscopic perivesical tissue invasion, and 1 (1.4%) showed pT3b macroscopic perivesical tissue invasion. This high frequency of high grade tumors may point toward late presentation of patients to hospitals, inaccessibility of frequent medical facilities to community or inefficient screening methods available in Pakistan.

The most common complaint of patients was hematuria. Any PUNLUMP patient was not found in this series of patient. This could be due to either ignorance of early symptoms on patient's side or interobserver variability as was mentioned by Virchows in 2011. They might be right in suggesting that exclusion of PUNLUMP from histological grading might improve interobserver variability. Likewise, Sharma also found difference of opinion between PUNLUMP and low grade carcinoma.

High grade carcinoma was seen in 64% (n=45) of smokers. Multifocality was noted in 8 (11.4%) patients in contrast to the study by Comperat. The most common site of involvement was lateral wall (60%) in accordance with Naeem et al., who found lateral wall involvement in 44% of patients. Average size of tumor diameter was 3.23 cm (±1.78) and there was no correlation between size and histological grade of tumor. This did not match with the findings of Xin-Ke Zhang in China. Male to female ratio was 6:1, age range was 25-90 years with median of 60 years and peak incidence of tumor in seventh decade. This was in accordance with the findings of Laishram, India.

Many different molecular, serological, and immuno-histochemical markers are under study to predict prognosis in urothelial malignancy. Traditional features like staging and grading are not sufficient to identify high risk patients. Recent advances in molecular diagnostics can improve risk stratification of patients and improve clinical outcomes. Stadler et al. indicated TP53 protein accumulation in nuclei of tumor cells and showed positive correlation between gene mutation, stage and grade. In this study, the authors found that 64.3% of the bladder-cancer patients were positive for nuclear protein TP53.
p53 staining. This is in accordance with findings of Bazrafshan and Sidransky, who found 60% and 61% mutations of p53 protein, respectively.\textsuperscript{21} Intensity of p53 when compared with grade of tumors showed weak staining in 69.7% of low grade lesions and 30.3% in high grade tumors. It may point towards progression of disease, indicating that as grade advances, nuclear accumulation of p53 increases and gives stronger intensity of stain. A strong intensity of p53 staining was noted in 5.4% of low grade and 94.6% of high grade tumors. Roy Chowdhury demonstrated nuclear p53 positivity in 90% of low grade and 100% of high grade tumors, but they inferred that p53 gene might be unrelated to development of urothelial neoplasm, since PUNLUMP showed negative staining.\textsuperscript{22} The present findings were dissimilar to that. Here, positive intense staining was found in high grade, weak staining in low grade and negative staining in tumor that showed rhabdoid differentiation. This may suggest that when urothelial papillary tumor dedifferentiates, it accumulates mutations other than p53. He et al. also showed role of RAS pathway activation in p53 deficient tumor cells and suggested prognostic role or RAS in urothelial cancers.\textsuperscript{23} Similarly, Zhou et al. showed role of fibroblast growth factor 3 (FGR3b) in cell cycle proliferation and progression.\textsuperscript{24}

CONCLUSION

The immunohistochemical expression of p53 increases and intensifies with advancing grade and stage of papillary urothelial carcinoma. If these tumors dedifferentiate towards sarcomatous morphology, they lose p53 staining and may accumulate mutations other than p53. It is concluded that p53 can be used as a prognostic indicator of higher stage and grade in bladder carcinoma.

REFERENCES


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