EXPRESSION OF P53 IN UROTHELIAL CARCINOMA AND ITS CORRELATION WITH THE GRADE OF THE TUMOR – A TWO YEARS STUDY IN A TERTIARY HOSPITAL

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ABSTRACT

BACKGROUND

In India, Urothelial carcinoma of bladder constitutes about 7% of all male cancers. It is associated with high morbidity and mortality. Current grading system in application by WHO/ISUP divides Urothelial malignancies as low and high grade tumors by morphological criteria. Grading Urothelial carcinomas depending on morphological criteria alone is sometimes difficult in limited tissue. In such cases tumor grading by alternative methods like IHC expression is useful. In the present study, we have evaluated the diagnostic utility of p53 in grading Urothelial carcinomas**.**

MATERIALS AND METHODS:

The present study is an observational study conducted at department of Pathology, Gandhi Medical College/Hospital, Secunderabad for over a period of two years from June 2016 to May 2018. A total of 30 TURBT and Cystectomy specimens diagnosed as Urothelial carcinoma on routine H&E were included in the study. These tissues were later subjected to IHC markers for p53. The slides are reviewed and expression of these markers was evaluated and correlated with grade. The results were statistically analyzed**.**

RESULTS:

Out of 30 cases, Non invasive low grade cases were 12(40%) and Non invasive high grade cases were 06 (20%), and infiltrating urothelial carcinoma cases were 12(40%). In the present study we have made an effort to evaluate the expression of these p53 proteins in carcinoma bladder and correlate their expression with the grade of the tumour.

CONCLUSIONS
Grading of urothelial carcinoma of bladder is an important factor in management and prognosis of patients. We observed that as the tumour grade increases, expression of p53 is also increasing and it was statistically significant**.**
KEYWORDS: Urothelial carcinoma, WHO/ISUP grading, p53, Immunohistochemistry.

INTRODUCTION

Urothelial carcinoma of bladder is the 4th most common cancer in men and 8th most common malignancy in women in the western world. In India, it constitutes about 7% of all male cancers. It is associated with high morbidity and mortality[1] .

Various risk factors are associated with development of bladder carcinomas including cigarette smoking, arylamines, aniline dyes, auramines, phenacetin, cyclophosphomide, Schistosoma haematobium infestation, and radiation exposure.

Grading of bladder tumors is an important prognostic factor[2,3]. Hence in addition to early diagnosis, the prognostic indicators are important in these patients to plan the treatment modality.

Low grade carcinomas are associated with good prognosis as compared to high grade carcinomas. As further management and prognosis of patients is based on accurate grading of these tumors, therefore, the emphasis on categorization [4] .

Morphology alone is not of much use to distinguish these lesions. Immunohistochemical markers have been of some help in identifying lesions of various categories, as the pattern of expression of these markers vary in different lesions. Multiple immunohistochemical markers particularly p53 have been investigated in several studies for use as diagnostic and prognostic aids in urothelial tumors [5-7].

In the present study we have made an effort to evaluate the expression of P53 in carcinoma bladder, relate its expression to the grade of the tumour. Separation of low and high grade tumors can sometimes is difficult especially in small biopsies which may show crushing and cautery artifacts.

Therefore aim of this study is to determine the usefulness of p53 immunohistochemical stain as an adjunctive tool in grading urothelial carcinomas.

-P53:

It was identified in 1979 by Lionel Crawford, David P. Lane, Arnold Levine, and LIoyd Old. Its role as a tumour suppressor gene was revealed in 1989 by Bert Vogelstein and Arnold Levine[8,9].

TP53 is a tumour suppressor gene located on chromosome 17p. Phosphoprotein produced by this gene is p53. Mutations of the p53 are most common genetic defects identified in human tumours[10].

The p53 tumour suppressor gene has been shown to regulate cell growth by arresting cells in G1 phase[11]. The p53 protein increases in response to damaged DNA, causing a

cellular arrest to allow for possible repair or to start the sequence of programmed cellular death, apoptosis[12,13].

Point mutations that lead to the loss of tumour suppressor genes have been implicated in uncontrolled cellular growth leading to tumour formation.

Normal wild-type p53 protein has a half-life of 6-30minutes and generally will not accumulate to high levels for detection by IHC staining[14]. However protein encoded by mutated p53 remains active for a long period resulting in its accumulation in nuclei & is detectable with IHC and thus correlates with p53 gene mutation.

P53 mutations are most commonly seen in colorectal, lung, breast cancers and urological neoplasms.

This gene is typically affected by missense point mutations In exon 5-8 that paradoxically stabilize the protein product leading to massive accumulation in the tumour cell nuclei[15].

MATERIALS AND METHODS

This is an observational study done at Department of Pathology, Gandhi Medical College/Hospital, Secunderabad in the state of telangana, India for a period of 24 months, i.e. June 2016 to May 2018. Formaline fixed TURBT and Cystectomy specimens were included and patients who were already on chemotherapy were excluded from the study. This study was approved by the institutional Human Ethics Committee.

The specimens were fixed in 10% formalin for 24 hours and then meticulously grossed and submitted for processing. The tissue bits were routinely processed and sections of 3-4 micron thickness were cut and stained with Haematoxylin and Eosin stains. The sections were then studied under microscopy and the results were recorded. Immunohistochemistry (IHC) was done using Dako antibody to p53 (Monoclonal mouse antihuman p53 protein IS616, clone D0-7)

Immunoreactivity is indicated by nuclei of tumour cells staining brown with P53

MORPHOLOGICAL EVALUATION OF p53

The immuno-quantification was performed using percentage of tumour cells that react with the antibody. Each slide was evaluated at x40 magnification to find areas with maximum positive cells, followed by examination under x400 magnification to calculate percentage of positive cells to total cells were calculated. At least 500 cells were counted, and only the cells that were definitely positive for the desired marker were considered

A semi quantitative scoring system employed to assess the level of reactivity.

Table 1 - Scoring of p53[16]

Percentage of positive cells	Score
Negative stain (No staining)	0
0-25%	1
26-50%	2
51-75%	3
76-100%	4

The results were statistically analysed using Pearson Chi-squaretest and Fisher exact test, p-value was calculated wherever they wereappropriate. The correlation between Histological grading&IHCexpression was analysed.

OBSERVATIONS AND RESULTS

Out of 30 cases, Non invasive low grade cases were 12(40%) and Non invasive high grade cases were 06 (20%), and infiltrating urothelial carcinoma cases were 12(40%).

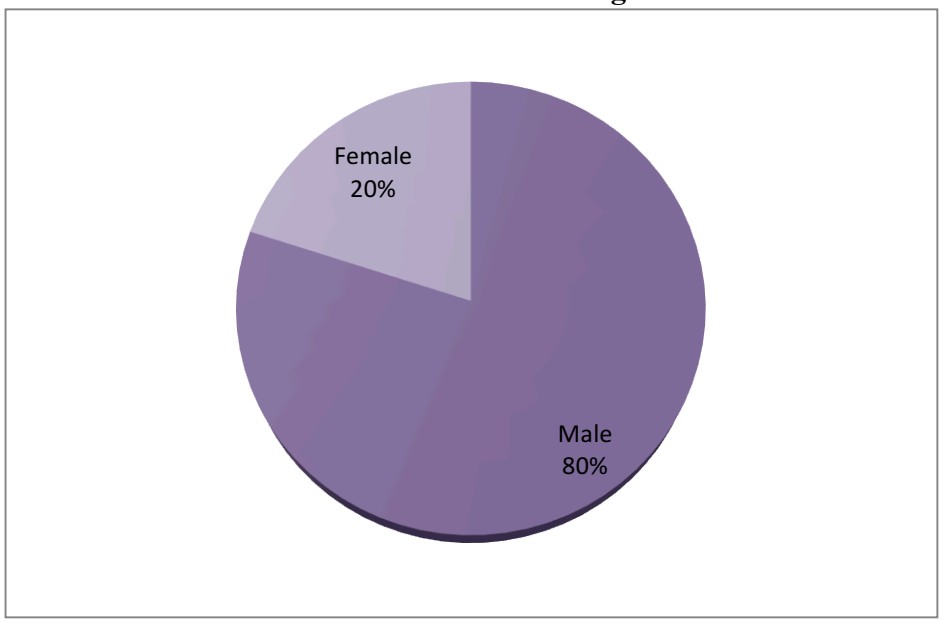
Table 2 - Distribution of cases according to histological grade of tumour

Tumour grade	Total cases	Percentage
Non invasive low grade	12	40%
Non invasive high grade	06	20%
Infiltrative	12	40%

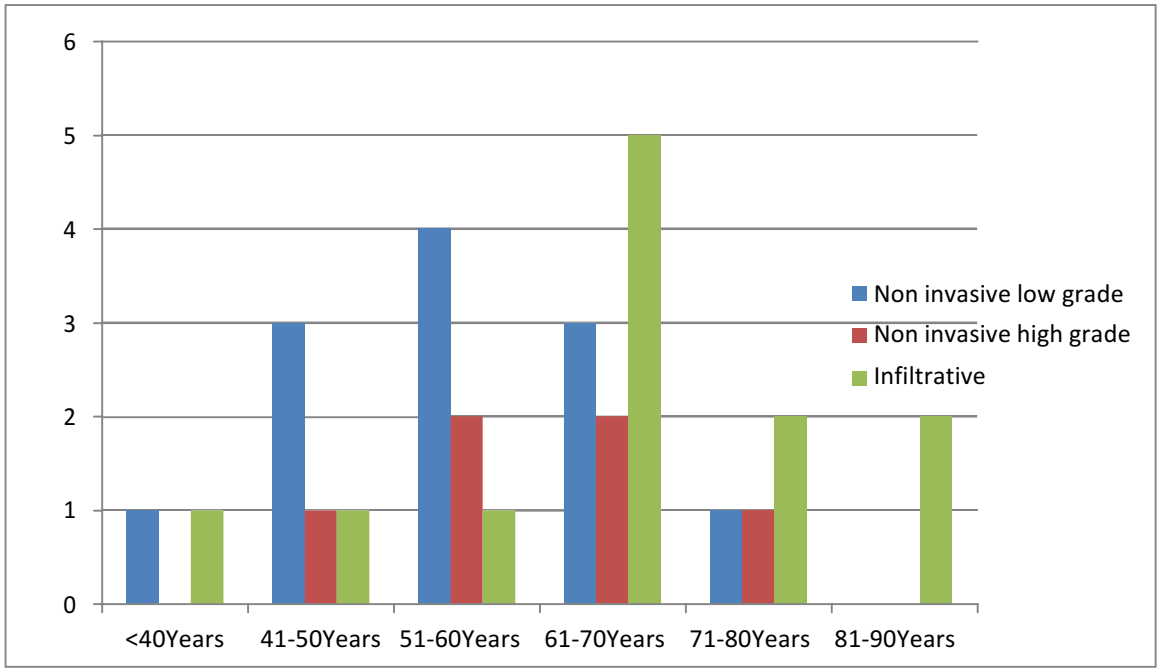
AGE AND SEX DISTRIBUTION

The age of the patients included in this study ranged from 23 to 87years witha mean of 57 years. Majority of patients (80%) belonged to 41 to 90 years age group. Non-invasive high grade and infiltrative urothelial tumors were predominantly seen at an older age. 24 cases were seen in males (80%) and 6 (20%) cases were seen in females with a male to female ratio of 4:1.

GRAPH NO.1 Distribution of cases according to sex



GRAPH NO.2 Distribution of cases according to age



EXPRESION OF p53

Out of 30 cases, p53 positive expression was noted in 18(60%) cases, negative expression was noted in remaining 12(40%) cases.

Table 3 - Expression of p53

p53 expression	Total cases	Percentage
Positive cases	18	60%
Negative cases	12	40%
Total cases	30	100%

Table 4 - Comparison of p53 expression in histological tumour grade

Tumour grade	Positive	Negative	Total
Non invasive low grade	5(42%)	7(58%)	12

Non invasive high grade	4(67%)	2(33%)	6
Infiltrating	9(75%)	3(25%)	12

p53 expression was noted in 5(42%) cases of low grade, 4(67%) cases of high grade and 9(75%) cases of infiltrating urothelial carcinoma.

We observed that as the tumour grade increases, expression of p53 is also increasing and it was statistically significant.

P value was calculated by using Fisher's exact test (p=<0.0001).

GROSS PHOTOGRAPHS

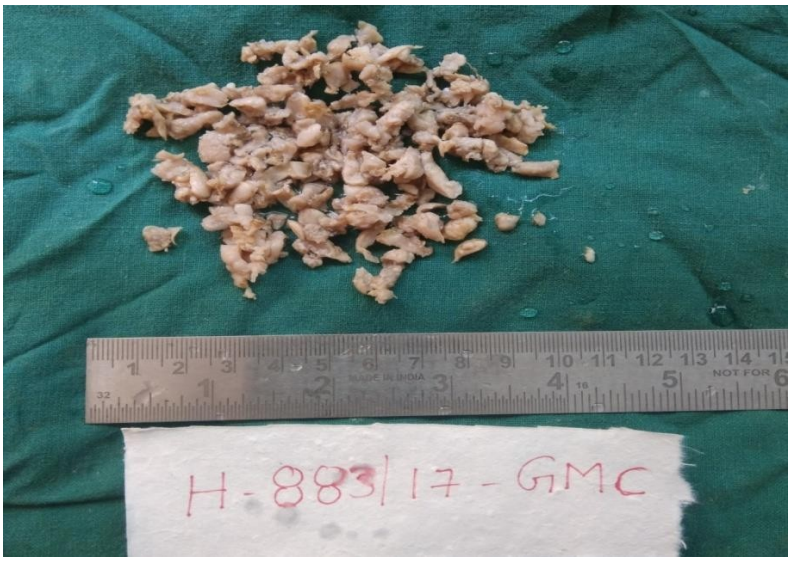


Fig 1A - Biopsy no:883/17 – Transurethral resected specimen of bladder

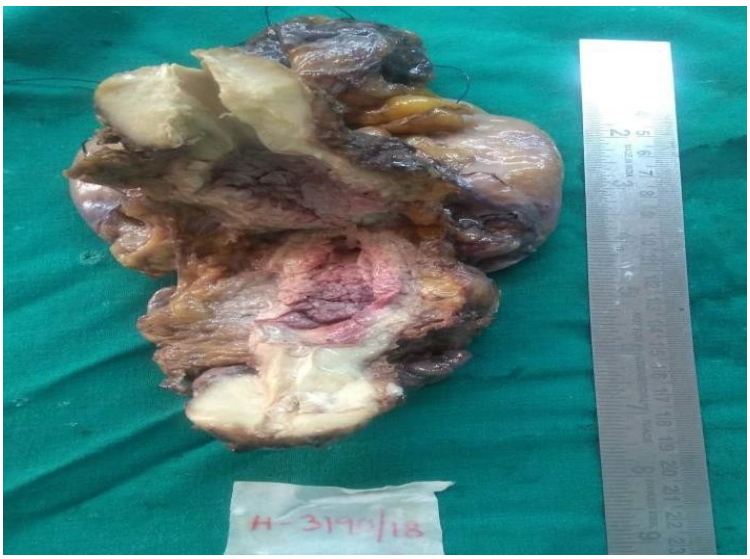


Fig1B - Biopsy no:3190/18 – Cystectomy specimen

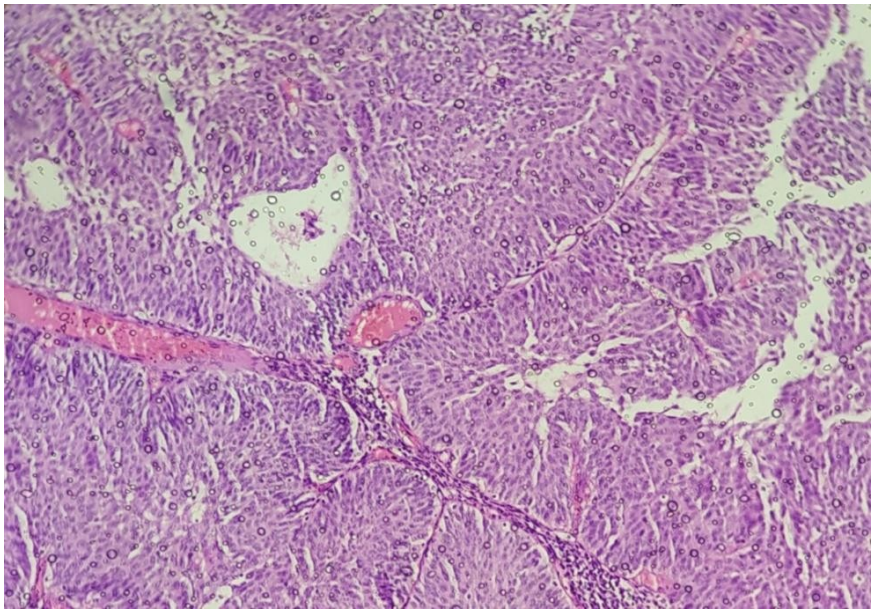


Fig 2a – Showing low power (10x) view of Non invasive low grade carcinoma (H & E)

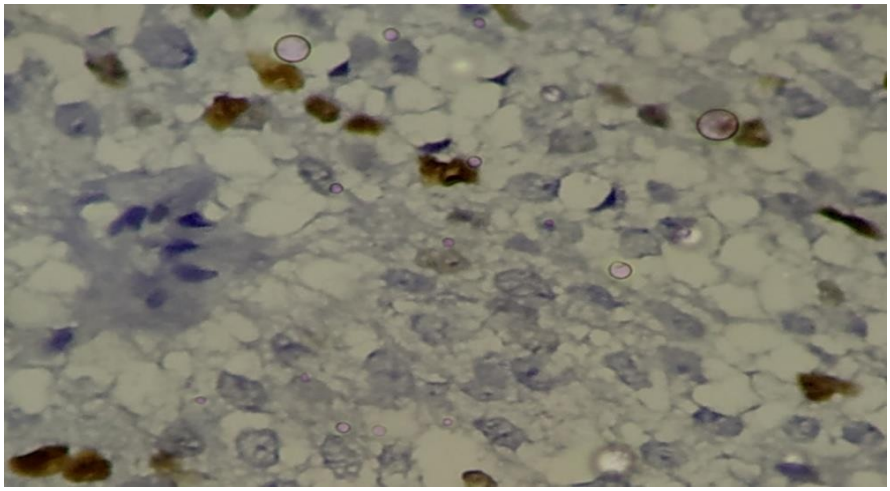


Fig 2b showing high power (40x) view of IHC stain p53 nuclear expression.

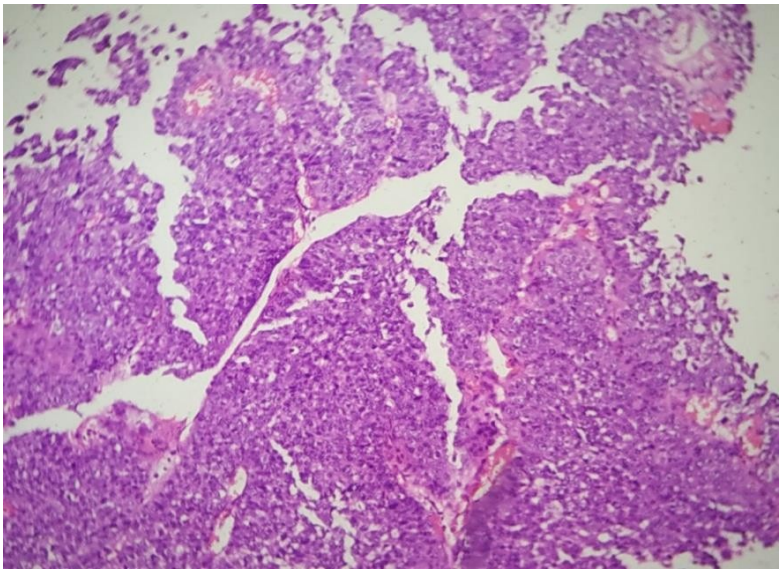


Fig 4a Showing low power (10x) view of Non invasive high grade carcinoma (H &E)

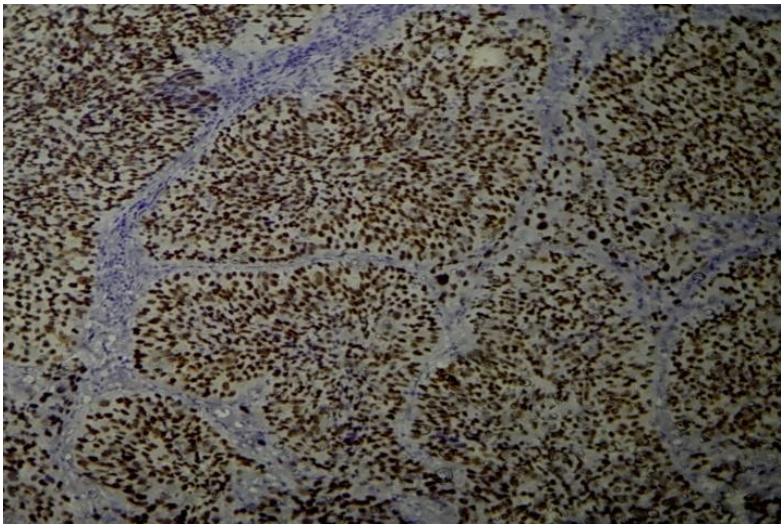


Fig 4b showing low power (10x) view of IHC stain p53 nuclear diffuse expression

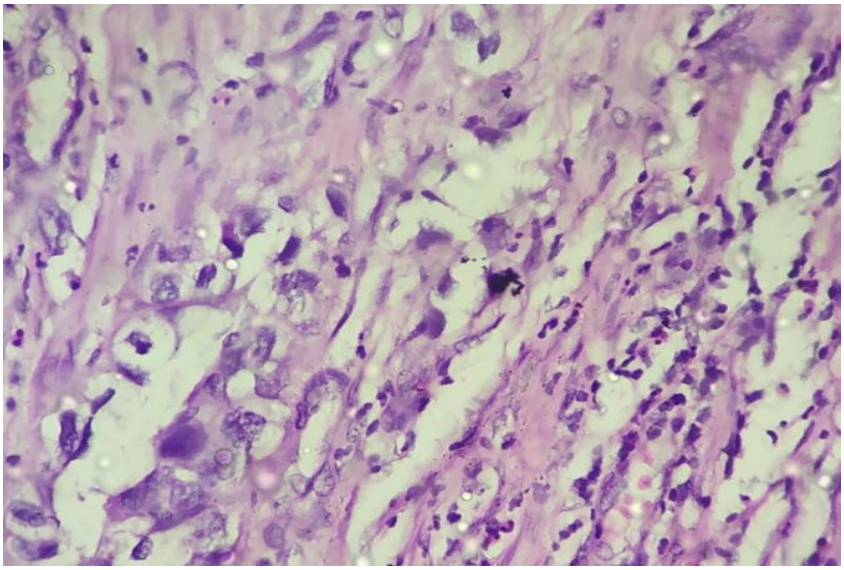


Fig 5a – Showing high power (40x) view of Infiltrative urothelial carcinoma (H & E)

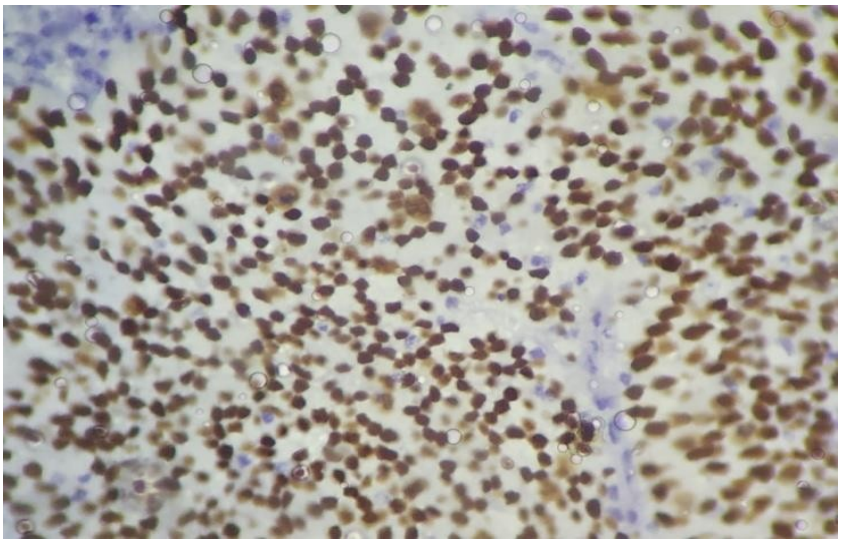


Fig 5b showing high power (40x) view of IHC stain p53 nuclear diffuse expression.

DISCUSSION

Urinary bladder carcinoma is a common multistage progressive malignancy and it is 4th most common cancer in men and 8th most common malignancy in women in the western world. The most common type of bladder cancer is urothelial carcinoma, followedby squamous cell carcinoma and adenocarcinoma are much less common [17]

Cystoscopy and biopsy/transurethral resection are the best techniques to confirm the histological diagnosis and determine the extent of disease within the bladder. Histological grade has great significance in noninvasive lesions, especially the papillary types. Recurrence and progression are influenced by the pattern of growth, grade, dimension,

multifocality, time of recurrence, and any prior bladder therapy. Grading can be subjective and so immunohistochemistry or molecular tests play a great role.

Cell proliferation and mutations in cell cycle regulatory genes are hallmarks in various tumors including urothelial carcinomas**.** Being a cell proliferation regulating and pro-apoptotic gene, mutation in p53 increases expression of the mutant protein and regarded as a predictor of poor prognosis of urothelial tumors.

In our study expression of P53 is not done for papillomas and papillary urothelial neoplasms of low malignant potential as morphologically they lack fused and branching papillary architecture. Moreover they don't show cytologic atypia and mitotic activity, therefore their categorization is not difficult. On the other hand segregation of non invasive low and high grade and infiltrative urothelial carcinomas can be very difficult at times specially in limited tissue specimens. More-over risk factor assessment like exposure to carcinogens was not done as detailed history was not available.

We found that evaluation of p53 expression is relatively easy to interpret in urothelial malignancies, as p53 is a nuclear stain useful in assigning grade to urothelial malignancies in difficult situations as histologic reproducibility of tumor grade is poor.

In the present study we have made an effort to evaluate the expression of these p53 proteins in carcinoma bladder and correlate their expression with the grade of the tumour.

Comparison of p53 expression with histological grade of tumour in present study with other studies

Out of 30 cases, on histology (H&E sections) non invasive low grade tumours are 12 (40%), high grade cases were 6(20%) and infiltrating urothelial carcinoma cases were 12(40%).

In our study p53 nuclear expression was noted in 5(42%) cases of low grade, 4(67%) cases of high grade non invasive tumours and 9(75%) cases of infiltrating urothelial carcinoma.

We observed that as the tumour grade increases, expression of p53 is also increasing and it was statistically significant. P value was calculated by using Fisher's exact test ($p=<0.0001$).

Anadi RC et al[18] studied that Expression of p53 Protein by Immunohistochemistry in Urothelial Neoplasm. 3(25%) out of 12 cases of non-invasive low-grade papillary urothelial carcinoma, and16 (88%) out of 18 patients with non-invasive high-grade papillary urothelial carcinoma and all 10(100%) cases of invasive urothelial carcinoma showing p53 positivity.

Also revealed high p53 positivity in high-grade papillary urothelial carcinomas and invasive carcinoma.

Shazia Mumtaz et al[19] studied that positive expression of p53 was seen in 35 cases of high grade (72.9%), while only 17 cases (36.2%) of low grade tumors.

Qamar S et al[20] studied Out of 25 low grade lesions, 4 (16%) cases were p53 positive and out of 45 high grade lesions, 41 (91%) cases were p53 positive.

Kalantari MRet al[5] studied Nuclear P53 protein in invasive high-grade TCC was slightly more frequent than that in noninvasive low-grade papillary TCC (P = .35)
TengA.Onget al[21] studied number of p53 positive cases was significantly higher in high-grade (p = 0.006) and muscle-invasive tumours (p = 0.035).
Yin H et al[22]studied p53, ck20 and Ki-67 on 84 noninvasive papillary urothelial tumors. Using recent WHO/ISUP classification p53 was not expressed in low malignant potential cases. The p53 index of greater than 10% was observed in only 5 cases of low grade carcinomas, and in 42 of the 53 cases i.e. 79% the p53 expression was less than 5% and was considered negative

CONCLUSION

Urinary bladder carcinoma is a common multistage progressive malignancy with urothelial carcinomas comprising 90% of all primary bladder carcinomas.
Grading of urothelial carcinoma of bladder is an important factor in management and prognosis of patients. Separation of low and high grade tumors can sometimes is difficult especially in small biopsies on H&E. So, various ancillary techniques like Immunohistochemical markers have been of some help in identifying lesions of various categories, as the pattern of expression of these markers vary in different lesions.
In our study, significant difference in expression of p53 was found between non invasive low and high grade and infiltrative urothelial carcinoma. As the tumour grade increases, expression of p53 is also increases.
Many studies have shown variable associations between the expression of p53 and tumour grade. Hence, we conclude that similar studies have to be conducted in large population groups to obtain more reliable results.

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