IJHS

Original Article

Association of smoking, p53 and Ki-67 immunomarkers with bladder neoplasms in tribal region of India

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WEBSITE: ijhs.org.sa
ISSN: 1658-3639
PUBLISHER: Qassim University

ABSTRACT

Objectives: Going back to the basics of urinary bladder neoplasms and correlation of histopathological diagnosis with age, sex, clinical features, smoking in tribal population of India in today's day and age. Along with that we are also determining the role of p53 and Ki67 immunomarkers in grading and staging of Urinary Bladder Neoplasms and their correlation with history of smoking.

Materials and Methods: This retrospective study was done in a tertiary care hospital affiliated with medical college in tribal region of India. Total 72 cases of transurethral resection of bladder tumors and bladder biopsies were studied over a period of 2 years. The histopathological grading and staging was done according to the WHO/ISUP 2016 classification. The histopathological diagnosis was further correlated with age, sex, clinical features, and history of active smoking. Along with that immunomarkers p53 and Ki-67 were done and correlated with grading and staging of tumors and with history of smoking. The cut-off used was more than 20% positivity for high expression and < 20% for low expression. For descriptive, data median (Interquartile Range) and ratio were used. For categorical variables percentage (%), Fisher exact test and Chi-square test were used. Results were evaluated with SPSS software program. $P < 0.05$ was considered statistically significant.

Results: Out of 72 cases studied, 66 cases were neoplastic. Out of them, bladder neoplasms were common in age group of 41–60 years and there was a male preponderance. Most common clinical feature was hematuria. Smoking was not a risk factor for the development of bladder neoplasms but bladder neoplasms in smokers were associated with higher grading and staging. The most common neoplasm was High Grade Papillary Carcinoma (43.05%). Stage T1 and Stage T2 were seen in 3.03% and 27.8% of cases. P53 and Ki-67 immunomarkers showed higher expression in tumors of higher grade and stage.

Conclusion: The take away from this study is bladder neoplasms that are quite versatile and histopathology is the gold standard to confirm these. However, p53and ki-67 gives important information regarding prognosis of tumors and helps in stratification of patients in high risk and low risk groups. Development of bladder carcinoma was independent of history of smoking; however, smoking is associated with higher grading and staging of bladder neoplasms and is also associated with higher expression of p53 and Ki-67 immunomarkers.

Keywords: Bladder cancer, Ki-67, p53, Transitional cell carcinoma

Introduction

Urinary bladder neoplasms represent a global health problem.[1] Bladder cancer is ranked 10th in incidence in the world. In India, it is ranked 17th in incidence and 21st in mortality. Although bladder cancer can occur at any age, and many demographic studies have shown that individuals aged ≥65 years have 11 times higher incidence than those younger than 65 years.[2] Bladder cancer is more common in men and the possible reason in gender-based disparity in incidence.[2]

Tobacco consumption is a significant risk factor for bladder cancer.[2] Not many studies are done to correlate smoking with staging and grading of bladder neoplasms hence this study aims to study effect of smoking on staging and grading along with its influence on p53 and Ki-67 immunomarker expression.

Gross and microscopic hematuria constitutes the main symptoms in many bladder neoplasms.[3]

Cystoscopy is used to screen for bladder neoplasms. Transurethral resection of bladder tumors (TURBT) is a therapeutic procedure which is used for evaluation of various clinical and prognostic factors like tumor differentiation, and depth of infiltration along with complete removal of non-invasive papillary tumors of bladder.

Histopathological examination is the gold standard to diagnose bladder neoplasms and to grade and stage the tumors. Use of immunohistochemistry is not necessary to diagnose bladder neoplasms but it helps in stratification of patients in low and high grade groups and to determine the prognosis. Most commonly used markers are p53, Uroplakin, Ki-67, etc.[3]

Few previous studies have shown the importance of p53 mutations and Ki-67 proliferations in pathogenesis and prognosis of urothelial carcinoma.[4-6] Ki-67 is high in tumors with rapid growth fraction or rapid proliferation and in general these tumors respond better to chemotherapy so it is essential to detect Ki-67 levels.[7] P53 is a marker of genomic instability and limitless replicative potential. Hence, it is an important for staging and prognosis of urothelial carcinoma.[8-10]

The main objective of this study is to determine incidence of bladder cancer in tribal population in India along with its association with various clinical features such as age, sex, and smoking. We carried out grading and staging of bladder neoplasms according to WHO/ISUP 2016 classification and TNM classification, respectively. We also tried to determine the role of smoking and the role of p53 and Ki-67 immunoexpression in grading and staging of bladder cancer and hence on prognosis of bladder carcinoma.

Materials and Methods

Study design

It is a retrospective study carried out in the Department of Pathology at a Tertiary Care Hospital in tribal region of India. The retrospective study was reviewed and approved by Ethical Board Committee of our institute.

Patient and tissue samples

TURBT and bladder biopsies received during 2-year period of December 2019 to November 2021 were included in the study. Total 72 cases of bladder TURBT and biopsy were included in the study.

Routine histopathology

Relevant history of the patients was taken and noted on a designated patient information form. The tissue specimens were received in 10% buffered formalin. After gross examination of the received biopsies, tissues were processed for paraffin embedding. After embedding, 3-5 micrometer thick sections were cut and slides were stained with hematoxylin and eosin. The prepared slides were seen under a microscope and histopathological examination was carried out and tumors were graded according to WHO/ISUP 2016 classification and staged according to the TNM Classification.

Immunohistochemistry

For immunohistochemistry study, 3–5 m sections were taken on poly-L-Lysine slides and underwent antigen retrieval in BioGenex microwave (EZ-R2). Peroxide block was applied for 5 min at room temperature. Immunohistochemical staining of P53 was done using antibodies EP9 (Rabbit monoclonal antibody) at 1:40 dilution in PBS. Immunohistochemical staining of Ki-67 was done using antibodies MIB-1 (Mouse monoclonal antibody). These antibodies were applied for 1 h at room temperature. Poly-HRP was applied for 30 min at room temperature. Chromogen DAB was applied for 20 min at room temperature and then counterstaining with hematoxylin was done. For control of p53 colonic carcinoma tissue was used as positive and negative control. There is staining by p53 in basal crypts but not in the luminal cells. For positive and negative control of Ki-67, chronic tonsillitis tissue was used. B cells in dark and light zones show nuclear reactivity but mantle zone B cells do not show positivity.

Evaluation of immunohistochemistry

Evaluation of IHC staining was done by taking a cutoff of 20% for both P53 and Ki-67. Both of them stain the nuclei. A total of 200 cells were counted in each patient. The percentage of cells positive was determined.

Criteria

Inclusion criteria

All the TURBT and bladder biopsies specimens of both sexes and all age groups were included in the study.

Exclusion criteria

The following criteria were excluded from the study:

- Poorly fixed/unfixed specimens.
- Inadequate biopsies.

Statistical Analysis For categorical variables Percentage (%), Fisher exact test, and Chi-square test was used. Results were evaluated with SPSS software program. $P < 0.05$ was considered statistically significant.

Results

Total 72 urinary bladder specimens were included in the study. Out of these 68 were TURBT specimens and 4 were bladder biopsies.

The bladder specimens accounted for 1.2% of all specimens received from December 2019 to November 2021. Total of 2112 neoplastic specimens were received and out of that 619 were malignant lesions. Out of all neoplastic specimens, 3.1% bladder specimens were received and out of all malignant neoplastic specimens 10.5% malignant bladder specimens were received.

Out of 72 bladder cases, six cases were non-neoplastic and 66 cases were neoplastic. The histopathological grading is as Graph 1. The most common histopathological grades were High Grade Papillary Urothelial Carcinoma (43.05%) followed by Low Grade Papillary Urothelial Carcinoma (19.44%).

The most common age group affected for neoplastic lesions was 41–60 years as shown in Graph 2.

There was a male preponderance, and male to female ratio was 3.1:1 as shown in Graph 3.

The most common clinical feature was hematuria (37.87%) followed by dysuria (16.66%).

Out of 66 neoplastic cases of bladder, 42.43% of patients had history of smoking. Out of 50 neoplastic cases in male, 27 (54%) had history of smoking. Out of 16 neoplastic cases in female 01 (6.25%) had history of smoking as shown in Graph 4. Furthermore, smoking was associated with p53 and Ki-67 immunoexpression as shown in Graph 5. The association between smoking and development of neoplastic lesions was not significant (Statistical Value = 0.307, *P* value was not < 0.05). However, smoking was associated with higher immunoexpression of p53 and Ki-67 in neoplastic lesions and it was statistically significant (Statistical Value= < 0.00001, $P < 0.05$).

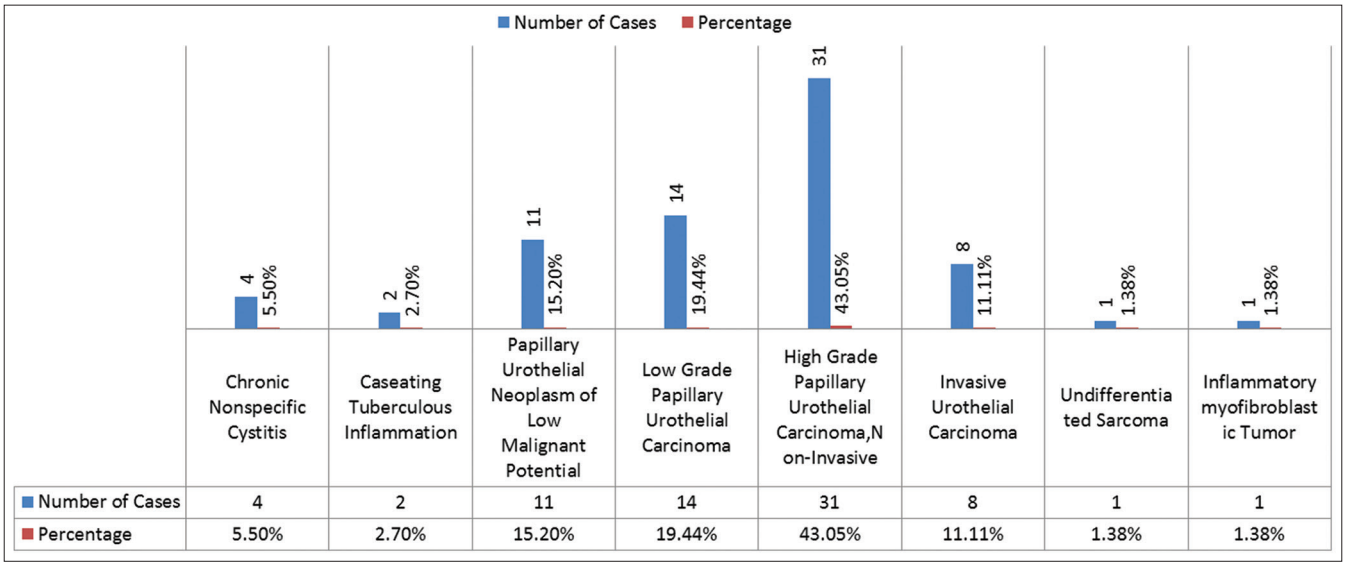
Out of 66 neoplastic cases, 69.69% were classified as stage Ta as they did not show lamina propria and muscularis propria invasion. 3.03% were classified as Stage T1 as they showed invasion only into the lamina propria. About 27.8% cases showed muscularis propria invasion and hence were classified as T2. The data are as shown in Graph 6. The association between p53 and Ki-67 was statistically significant as per Fisher Exact Test and Chi-square Test (Statistical Value= <0.00001, $P < 0.05$). Hence, it was observed that higher stage of the disease correlated with higher immune-expression of P53 and KI-67 immunomarkers.

For p53, more that 20% immunoexpression was seen in 12.5% cases of PUNLMP, 14.28% cases of Low Grade Papillary Urothelial Carcinoma, 45.16% cases of High Grade Papillary Urothelial Carcinoma, and 87.5% of cases of Invasive Urothelial Carcinoma. The details are as shown in Graph 6. Hence, it is evident that p53 immunoexpression increased as grade of tumor increased refer Figures 1 and 2.

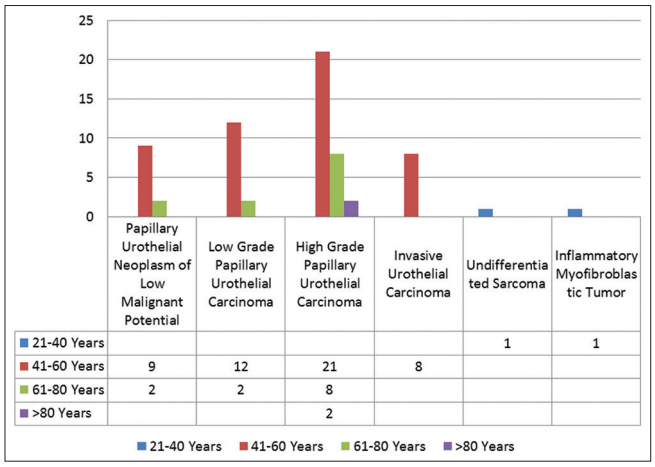
For Ki-67, more that 20% immunoexpression was seen in 9.09% cases of PUNLMP, 7.14% cases of Low Grade Papillary Urothelial Carcinoma, 54.83% cases of High Grade Papillary Urothelial Carcinoma, and 100% of cases of Invasive Urothelial Carcinoma. The details are as shown in Graph 6. Hence, it is evident that Ki67 immunoexpression increased as grade of tumor increased refer Figures 1 and 2.

Discussion

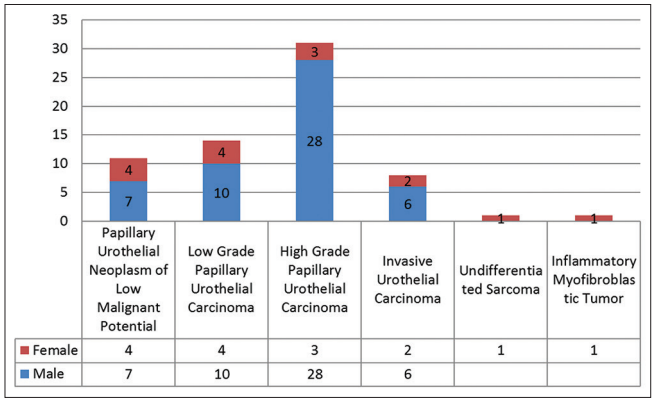
Epidemiological aspect of this study helps us in determining incidence and clinic-pathological status of bladder neoplasms in a secluded tribal region of India. Hence, correlations of basic epidemiological statistics are done with the world statistics as well as statistics in India.



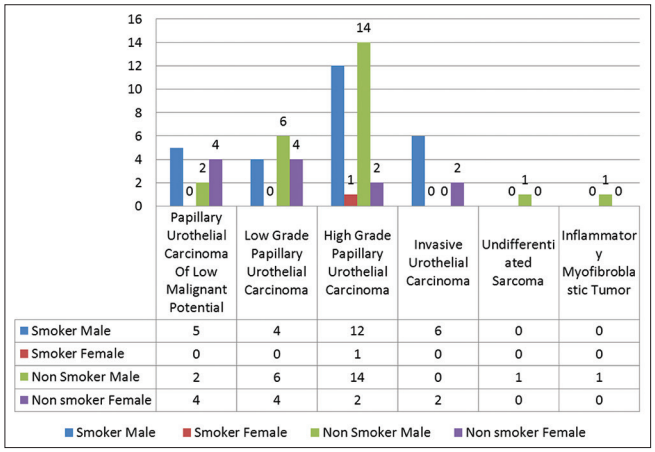
Graph 1: Histopathological subtypes



Graph 2: Neoplastic lesion in various age groups



Graph 3: Neoplastic lesion in male and female



Graph 4: Histopathological correlation with history of smoking

Bladder cancer was most commonly seen in 41–60 years age group in our institute. According to Siegel *et al*.,[11] bladder cancer diagnosis is in people above 55 years of age and average age of diagnosis is 73. This study included statistics from all countries worldwide. In the study of Gupta *et al*.,[12] which was carried out in Indian Subcontinent, the mean age was 60.2 ± 4.4 years. Hence, bladder cancers occurred in younger age group in India especially tribal areas as compared to the other countries.

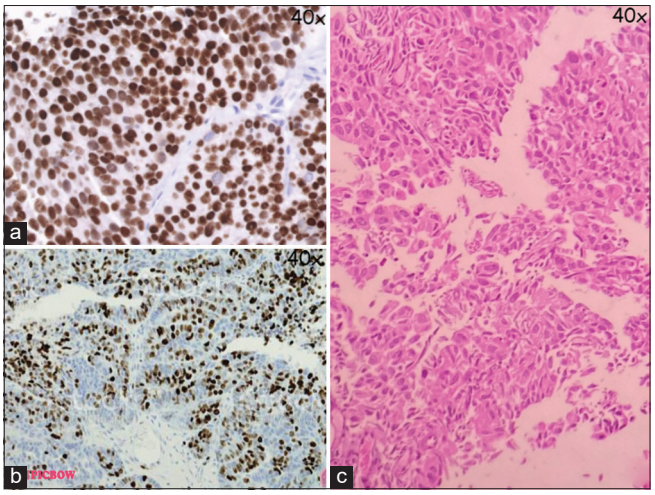


Figure 1: (a) P53 immunoexpression in high grade papillary urothelial carcinoma, (b) Ki-67 immunoexpression in high grade papillary urothelial carcinoma, (c) H&E stained section of high grade papillary urothelial carcinoma

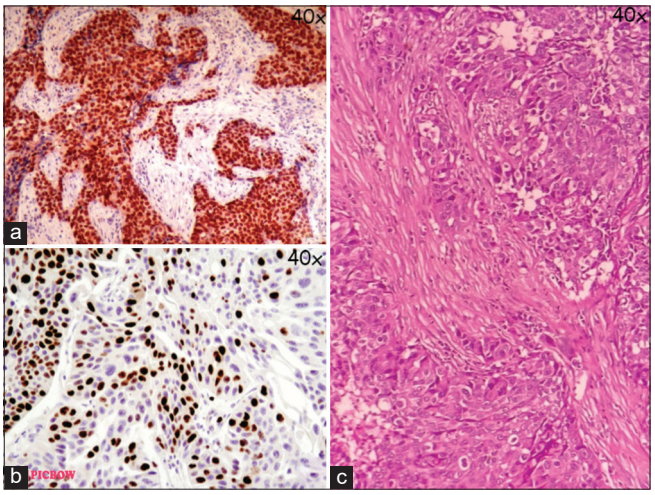
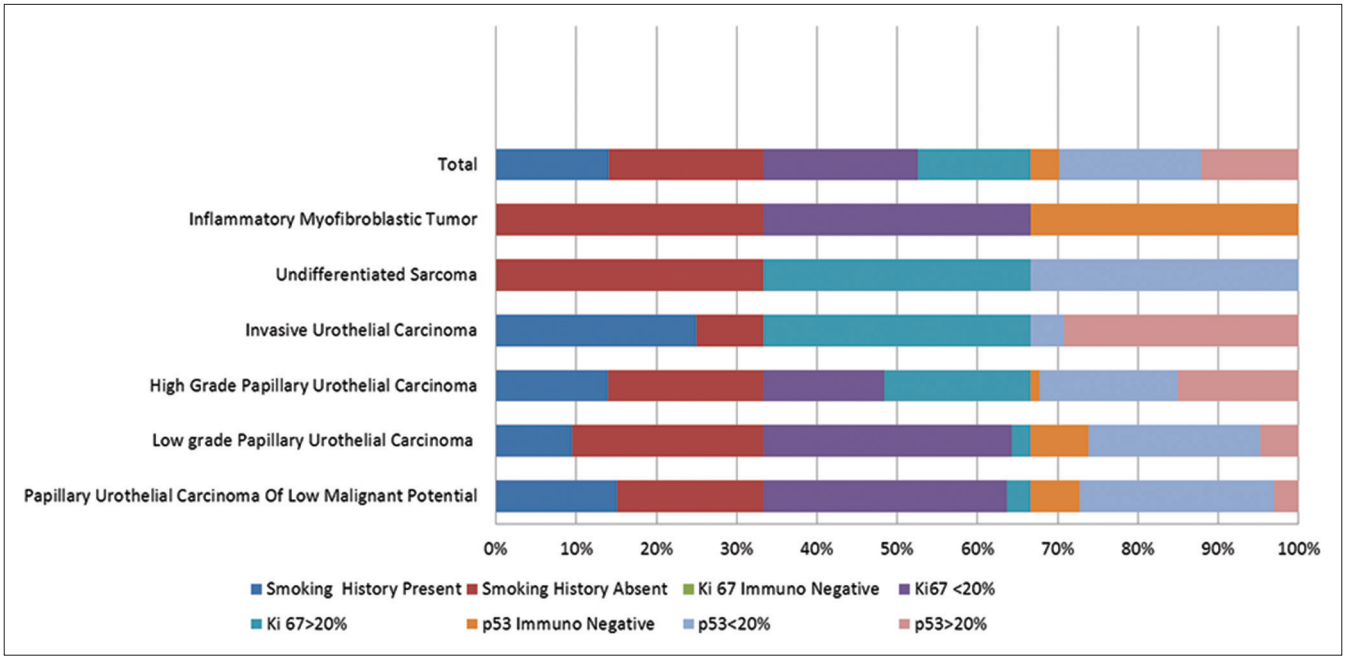


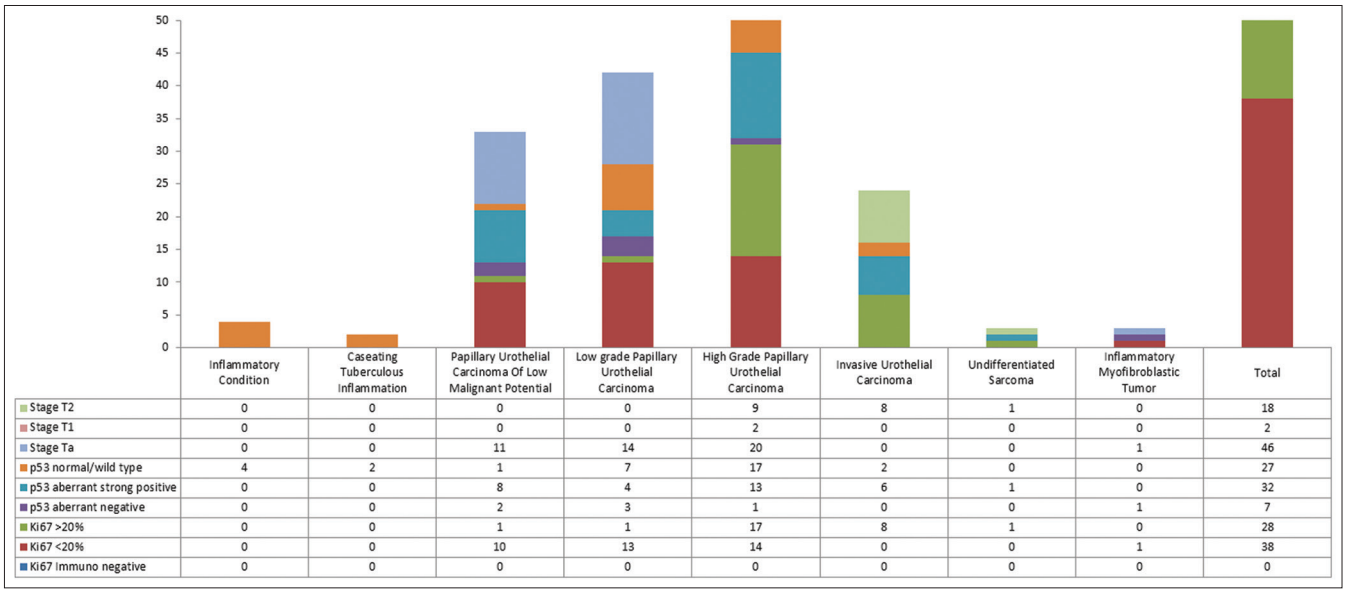
Figure 2: (a) P53 immunoexpression in invasive urothelial carcinoma, (b) Ki-67 immunoexpression in invasive urothelial carcinoma, (c) H&E stained section of invasive urothelial carcinoma

In our institute male to female ratio for bladder cancer is 3.1:1. According to Bray *et al*.[13] bladder cancer is around 4 times more likely to be diagnosed in men than women. In the study of Gupta *et al*.,[12] the male to female ratio was 8.6:1. The study of Gupta *et al*.,[5] was carried out in 2009 and hence there is a significant change in ratio of male: female affected by bladder cancer in today's day and age. Also in Indian sub-continent at that time smoking and occupational risk of bladder cancer was more in male hence the higher incidence in males. According to study of Kanade *et al*.,[14] male to female ratio in bladder carcinoma was 4.3:1.

In our study, the most common clinical features were hematuria followed by dysuria. Similar findings were found in study by Shephard *et al*.[15] where hematuria and dysuria were most common clinical findings.



Graph 5: Distribution of p53 and K-67 immunohistochemical staining with history of smoking and grading



	Inflammatory Condition	Caseating Tuberculous Inflammation	Papillary Urothelial Carcinoma Of Low Malignant Potential	Low grade Papillary Urothelial Carcinoma	High Grade Papillary Urothelial Carcinoma	Invasive Urothelial Carcinoma	Undifferentiated Sarcoma	Inflammatory Myofibroblastic Tumor	Total
Stage T2	0	0	0	0	9	8	1	0	18
Stage T1	0	0	0	0	2	0	0	0	2
Stage Ta	0	0	11	14	20	0	0	1	46
p53 normal/wild type	4	2	1	7	17	2	0	0	27
p53 aberrant strong positive	0	0	8	4	13	6	1	0	32
p53 aberrant negative	0	0	2	3	1	0	0	1	7
Ki67 >20%	0	0	1	1	17	8	1	0	28
KI67 <20%	0	0	10	13	14	0	0	1	38
Ki67 Immuno negative	0	0	0	0	0	0	0	0	0

Graph 6: Distribution pattern of P53 and KI-67 immunohistochemical staining with grading and staging of tumors

In our study, we tried to associate smoking as a risk factor for development of bladder cancer. In the current study 42.43% of patients had history of smoking. However, in our study association between smoking and development of bladder neoplasms was not significant. These findings do not correlate with studies of Van Osch *et al*.[16] and Hemelt *et al*.[17] who discovered that active smokers were at increased risk of bladder cancer. This discrepancy might be seen due to variation in sample load and different sets of inclusion and exclusion criteria. Furthermore, environmental carcinogens which might be prevalent in tribal region may cause bladder cancer rather than smoking.

Moreover, our study showed statistical association between smoking and higher p53 and Ki-67 immunoexpression. This is the novel point of the study. All studies done beforehand showed association between smoking and incidence of bladder cancer. However, this study shows that smoking causes bladder neoplasms with the higher grading and staging as they show higher grading and higher p53 and Ki-67 immunomarker levels. Pramod *et al*.[18] also found higher incidence of High Grade Papillary Urothelial Carcinoma and Invasive Urothelial carcinoma in smokers as compared to non-smokers.

In our study as stated before, we found that High Grade Papillary Urothelial Carcinoma was the most common followed by Low Grade Papillary Carcinoma of Bladder. Furthermore, Stage T2 was most commonly seen in High Grade Papillary Urothelial Carcinoma and Invasive Urothelial Carcinoma. Stage T1 was commonly seen in High Grade Papillary Urothelial Carcinoma. Similar findings were seen in study of Kanade *et al*.,[14] Sushmita *et al*.[3] and Dhatwalia *et al*.[19] done in India. In study of Goyal *et al*.,[20] low grade papillary carcinoma was common; the discrepancy was because this study was carried out in Nepal and hence environmental and genetic factors could have played a role.

P53 also known as guardian of genome and mutation in its pathway seems to be an important step for the development of urothelial carcinomas with higher grade and stage. In our study, we found significant statistical association between higher (more than 20%) p53 immunoexpression and higher grade of urothelial carcinoma and higher stage of urothelial carcinoma. Similar findings with this marker were noted in other studies.[4-7,21-24] Thus it can be inferred that higher p53 expression is associated with tumor progression and bad prognosis for patients suffering from urothelial carcinoma.

Our study found significant statistical association between higher Ki-67 immunoexpression, and higher grading and higher staging of tumors. Ki-67 being a proliferative marker gives information about proliferative activity of tumor and higher proliferative activity is associated with bad prognosis and higher staging of tumor. Similar association was found in other studies.[7,8,21-25]

Limitations of the study

1. Sample load is less in number. A study with more sample load should be carried out to get better statistics, result, and correlation
2. As all TURBT and bladder biopsies were inclusion criteria for sample even benign conditions were included in the study
3. Proper occupational history of patients should have been taken so that incidence of bladder carcinoma could have been correlated with occupational carcinogens. As this is a tribal area with majority of population working in industrial zones and factories, the possibility of an occupational carcinogen cannot be ruled out
4. The patients should have been followed up regularly to determine their long-term prognosis.

Conclusion

According to findings in our study, we conclude that the tribal area shows higher incidence of bladder cancer as compared to other regions of the country. Bladder carcinoma is common in middle to elderly age group and is seen frequently in age group of 40–60 years. It is more commonly seen in males and smoking is associated with higher grade and stage of urothelial carcinoma. Hematuria and dysuria were the most common symptoms among the patients of bladder cancer. High Grade Papillary Urothelial Carcinoma was the most common type. P53 and Ki-67 immunomarkers gives significant information staging and grading of tumor and hence is associated with prognosis. They help in stratification of patients in high risk and low risk groups. We would like the clinicians to keep this in mind and not rely solely on histopathological diagnosis for treatment of patients. As smoking was correlated with incidence of bladder cancer, other occupational carcinogens should also be investigated in respect to development of bladder neoplasms.

Authors' Declaration Statement

Ethics approval

The study was approved by ethical committee of the institute.

Consent for publications

All the authors of the study provide their consent for publication.

Data availability statement

The authors confirm that the data supporting the findings of this study are available at request from the corresponding author.

Competing interests

Nil.

Conflict of interest

The authors have no conflict of interest to declare.

Funding statement

The authors did not receive any financial support for research, authorship, and/or publication of this article.

Acknowledgment

None.

Author' contributions

Dr Brinda Adhaduk was associated with data acquisition and literature search. Dr Mitsu Vaishnav and Dr Sameep Garg were associated with analysis of data, manuscript preparation, manuscript editing, and review.

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