e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2024; 17(1); 22-27

Original Research Article

KI67 Immunoexpression and its Comparison with Histopathological Grading in Urothelial Neoplasms of Bladder - An Observational Study

Nisha Gangwal[1], Madhu Gupta[2], Yogi Raj Joshi[3], Shreya Agrawal[4]

[1]Resident Doctor, Department of Pathology, Dr. S.N. Medical College, Jodhpur, Rajasthan
[2]Senior Professor, Department of Pathology, Dr. S.N. Medical College, Jodhpur, Rajasthan
[3]Professor , Department of Pathology, Dr. S.N. Medical College, Jodhpur, Rajasthan
[4]Assistant Professor, Departmentof Pathology, Dr. S.N. Medical College, Jodhpur, Rajasthan

Received: 01-10-2024 Revised: 15-11-2024 / Accepted: 21-12-2024
Corresponding author: Dr. Shreya Agrawal
Conflict of interest: Nil

Abstract

Background: Bladder cancer is one of the common neoplasms of lower urinary tract with significant morbidity and mortality rates. Parameters like tumour grading, size, staging alone have minimal role to specify progression, disease recurrence and treatment response. Ki67 immunoexpression represents proliferative subsets of tumour cells indirectly denoting aggressiveness of the tumour. As molecular alteration precedes phenotypic change, immunohistochemical study may be a valuable tool for screening patients and early identification of aggressive cancers. This study aims to compare Ki67 immunoexpression and histopathological grading in urothelial neoplasms of bladder.

Aim: The primary objective is to correlate the grading of tumor with Ki 67 immunoexpression in various urothelial neoplasms of bladder.

Study Design: This is an Observational study which was done at department of Pathology, Dr. S. N. Medical College, Jodhpur, and Rajasthan, India. Total 40 paraffin embedded tissues of urothelial carcinoma cases were taken for the study. The intensity of Ki 67 nuclear staining was graded and compared with histopathological grading.

Results: In present study high grade carcinomas have high ki67 expression and graded as 3+ and 4+, whereas low grade carcinomas have low Ki67 expression and graded as 1+ and 2+. Out of 40 cases pTa, cases had 2+ Ki-67 positivity, with none showing higher positivity. In contrast, pT1 cases showed 4+ ki67 positivity. pT2a, and pT4a stages exclusively exhibited 4+ positivity, while pT2b had mix of 3+ and 4+ positivity. This indicated that higher stages generally correlate with increased Ki-67 positivity.

Conclusion: The observations of the study emphasize the use of Ki 67, in addition to tumour grade and stage to predict the clinical outcome of patients and thereby helps in identifying high risk patients. These patients are frequently followed up and may benefit by additional adjuvant therapies.

Keywords: UC (urothelial carcinoma), BC (Bladder cancer), TURBT (Transurethral resection of urinary bladder tumor), PUNLMP (papillary urothelial neoplasm of low malignant potential) HG (High grade).

Introduction

Bladder cancer is a common malignancy with higher incidence in men than in women, making it the 6th most common cancer in men. [1] Bladder cancer is a largely preventable disease due to its many modifiable risk factors. Tobacco smoking is the principal risk factor for Bladder cancer, accounting for about half of all Bladder cancer cases and 37% of its deaths. [2]Bladder cancer is divided clinically into three disease states: muscle-invasive, non-muscle-invasive, and metastatic cancer; each differs in tumor biology, phenotype, prognosis and management. [3]The most common type of bladder cancers is urothelial carcinoma (UC), which represents more than 90% of all bladder cancers. [1] This type of cancer is notably common among older adults, with risk factors including smoking, chemical exposure, and chronic bladder inflammation. Presenting symptoms such as gross or microscopic haematuria, urinary frequency, and pelvic pain lead to its detection. [4]Approximately 70-80% of patients with primarily diagnosed bladder cancer present with non-invasive (non-muscularis propria invasive) disease (NMIBC). With these tumors, recurrence is common (occurring in 50-70% of cases), but progression occurs in only 15-25% of cases. Muscle invasive bladder cancer (MIBC) is highly aggressive and can rapidly progress and

metastasize. [5] Despite improvement in therapeutic strategies, most patients face death. [6]Current clinical and pathologic parameters, such as tumor grade, stage, and vascular and lymphatic extension provide important prognostic information. [7] In addition, some molecular markers have been identified in recent studies. [8]Analysis of new molecular markers in predicting prognosis of bladder cancer is a recent topic of interest among the pathologists due to lack of specific serum prognostic markers. [9]

Histopathological examination is the gold standard to diagnose bladder neoplasms and to grade and stage the tumor. Use of immunohistochemistry is not necessary to diagnose bladder neoplasms but it helps to determine the prognosis. [11]There have been a large number of IHC markers studied so far, but none of them has been used systematically in clinical practice. [12] Most commonly used markers are p53, Uroplakin, Ki-67, etc. [11]Antigen Ki-67 is a nuclear protein that is necessary for cellular proliferation. [13]Ki-67 is an excellent marker in determining the growth fraction of a given cell population. It represents proliferative subsets of tumour cells indirectly denoting aggressiveness of the tumour. As molecular alteration precedes phenotypic change

Material and Method:

This is an Observational study which was done at department of Pathology, Dr. S.N. Medical College, Jodhpur, and Rajasthan, India. Transurethral resection of bladder tissue andradical cystectomy specimen diagnosed as urothelial neoplasm are included in the study. 40 paraffin embedded tissues of urothelial neoplasm were taken. Two sections were taken for each case. One for immunohistochemistry and other for routine haematoxylin and eosin staining. Sections for IHC were cut at four micron thickness on to coated slides which were then incubated at 58 degree Celsius overnight. Initial sections were stained with haematoxylin and eosin stain.

Immunohistochemistry was done by two step indirect technique by using ki67 antibody. The entire sections in a slide were examined under high power objective for the presence of positive immunoreactivity. Tumor cells were read positive if there is golden brown nuclear staining of the neoplastic cells. The intensity of Ki 67 nuclear staining was assessed and graded according to Adams et al, 2000. Then ki 67 grading and histopathological grading were compared and statistically evaluated.

For Ki 67 labelling index each slide was examined under high power and hot spot were selected. Hot spot is an area with highest immunostained nuclei. 1000 tumor cell nuclei were counted and the labelling index is expressed as percentage of positive cells. Caution should be expressed while interpreting Ki67 positivity as any proliferating cell such as lymphocytes can be positive. Hence hot spot should always be selected after comparison with routine H&E sections.

Observations and results

Table 1: Distribution according to age

Age Groups (in Years)	N	%
≤50	4	10
51-60	11	27.5
61-70	16	40
≥71	9	22.5
Total	40	100
Mean ± SD	63.60±11.51	

Table 2: Sex distribution of all cases

Sex	N	%
Male	31	77.5
Female	9	22.5
Total	40	100

Table 3: Distribution according to WHO classification of urothelial neoplasms.

Histopathological Diagnosis	N	%
PUNLMP	2	5
Non-invasive Low Grade papillary UC	7	17.5
Non-invasive High Grade papillary UC	0	0
Invasive UC	31	77.5
Total	40	100

Table 4: Distribution of study patients according to Ki67 immunoexpression in urothelial neoplasms.

Ki 67	N	%
<10% positivity (1+)	2	5
10-25% positivity (2+)	9	22.5
25-50% positivity (3+)	11	27.5
>50% positivity (4+)	18	45

Table 5: Correlation between urothelial neoplasms and Ki67 expression

Urothelial neoplasms	Ki 67							
	<10% positivity (1+)		10-25% positivity (2+)		25-50% positivity (3+)		>50% positivity (4+)	
	N	%	N	%	N	%	N	%
PUNLMP	1	50	1	50	0	0	0	0
Non-Invasive Low Grade UC	1	14.29	6	85.71	0	0	0	
Non-Invasive High Grade UC	0	0	0	0	0	0	0	0
Invasive UC	0	0	2	6.45	11	35.48	18	58.06

Table 6: Correlation between PTNM staging and Ki67 expression in urothelial neoplasms

PTNM	Ki 67							
	<10% positivity (1+) (2)		10-25% positivity (2+) (9)		25-50% positivity (3+) (11)		>50% positivity (4+) (18)	
	N	%	N	%	N	%	N	%
PUNLMP	1	50.00	1	50.00	0	0.00	0	0.00
pTa (7)	0	0	7	100	0	0.00	0	0.00
pT1 (12)	0	0	2	16.67	8	66.67	2	16.67
pT2 (5)	0	0	0	0	0	0	5	100
pT2a (3)	0	0	0	0	0	0	3	100
pT2b (10)	0	0	0	0	3	30	7	70
pT3	0	0	0	0	0	0	0	0
pT4a (1)	0	0	0	0	0	0	1	100
pT4b	0	0	0	0	0	0	0	0

The table categorizes individuals into age groups. The majority are in the 61-70 years range (40%), followed by the 51-60 years range (27.5%), and the ≥71 years range (22.5%). The smallest group is ≤50 years, with 10%. The mean age is 63.60 years with a standard deviation of 11.51 years, reflecting a predominantly older population. The table shows the distribution of sex among 40 individuals, with 77.5% being male (M) and 22.5% being female (F) showing male predominance.

According to WHO Classification, majority of patients fall into Invasive carcinoma (77.5%) followed by 17.5% have Non-invasive Low grade papillary urothelial carcinoma and 5% have PUNLMPthe table shows Ki-67 positivity levels among 40 individuals. The majority have high Ki-67 positivity (>50%, 4+) at 45%, while 27.5% have 25-50% positivity (3+). Lower positivity levels are less common, with 22.5% having 10-25% positivity (2+) and only 5% having less than 10% positivity (1+).

This indicates a predominance of high Ki-67 positivity in the cohort.The table correlates urothelial neoplasms with Ki-67 positivity levels among individuals. PUNLMP cases show 50% each for <10% (1+) and 10-25% (2+) positivity, with no higher positivity levels. Non-Invasive Low Grade UC cases have a significant percentage with 10-25% positivity (85.71%) and 25-50% positivity (14.29%) but none with >50% positivity. We have no Non-invasive High-grade UC cases in this study. Invasive UC cases shows maximum 58.06% (18) cases of >50% (4+) positivity and 3+ positivity in 11 cases. This highlight increasing Ki-67 positivity with higher WHO grades

The table shows the distribution of Ki-67 positivity across different PTNM stages. For PUNLMP, Ki-67 positivity is evenly distributed between <10% (1+) and 10-25% (2+), with no higher positivity levels. For pTa, all cases have Ki-67 positivity between 10-25% (100%), with none showing higher positivity.

In contrast, pT1 cases are distributed across several positivity levels, with a significant percentage showing 25-50% (66.67%) and 16.67% having >50% positivity. pT2, pT2a, and pT4a stages exclusively exhibit >50% positivity (100%), while pT2b has a mix of 25-50% (30%) and >50% (70%) positivity. This indicates that higher PTNM stages generally correlate with increased Ki-67 positivity.

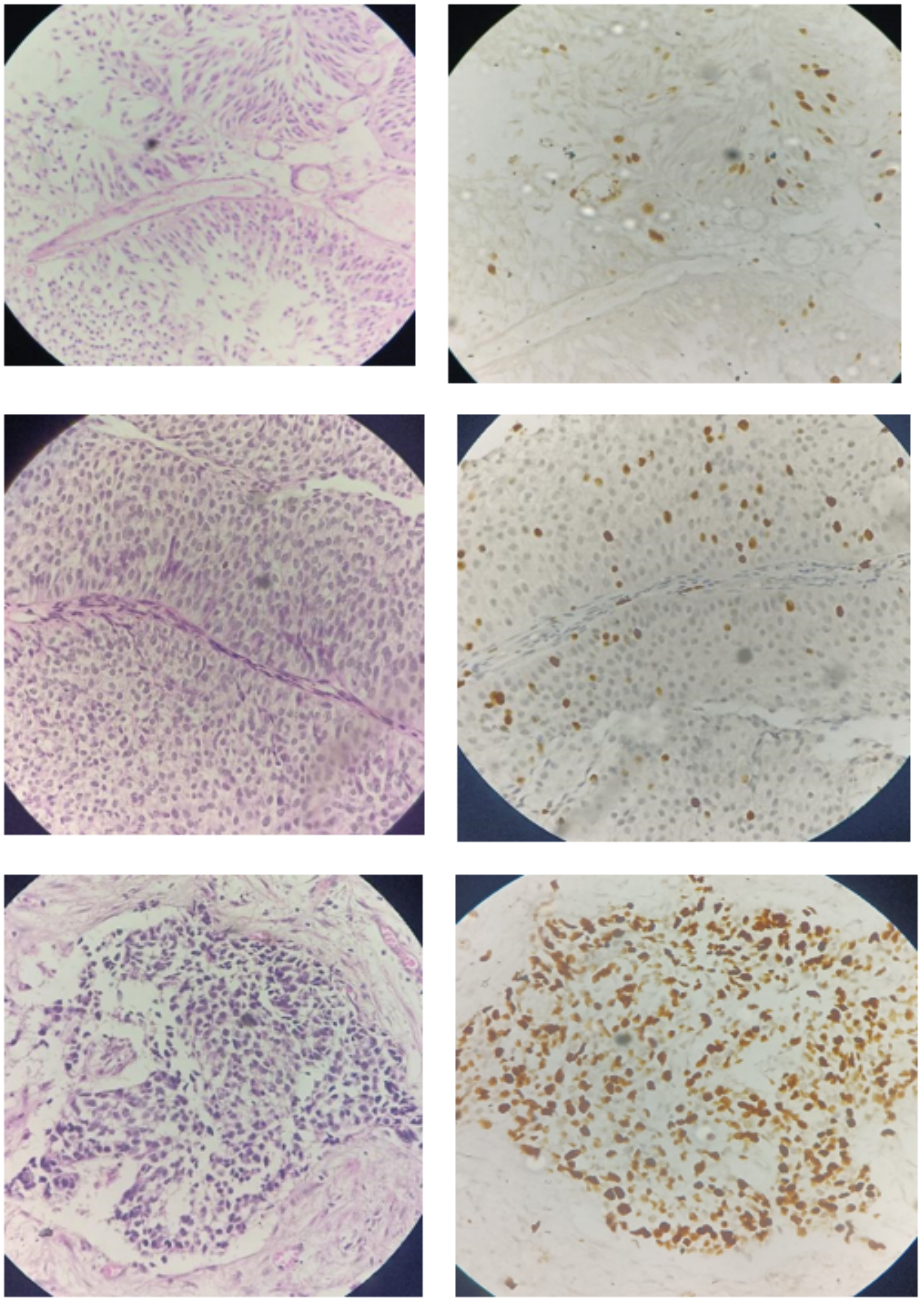

Fig 1: Comparison of KI 67 Scoring with Histological Grade of Urothelial neoplasm PUNLMP – KI 67 Score 1+ Non-invasive Low Grade Papillary UC – KI 67 Score 2+ Invasive Urothelial Carcinoma – KI 67 Score 4+

Discussion

Urothelial carcinoma of the urinary bladder is one of the most common malignancies in the world, and the prevalence varies across different countries and may differ between the regions of the same country. [14,15]

Uncontrolled cell proliferation is considered to be an important step in tumorigenesis. Ki-67 is a well-known nuclear cell proliferation marker that can be visualized and examined through immunohistochemical staining. Ki-67 expression is frequently found in several malignant cancers, including breast, colon, and ovarian cancers. The positive Ki-67 expression, or high index proliferation, has been associated with adverse histological and pathological features with poor recurrence and cancer-free survival, particularly in patients with upper tract urothelial cancer receiving nephroureterectomy. [16,17]So, predicting the grade of carcinoma and condition of muscle invasion might aid in choosing the most appropriate treatment plan to prevent spread, to increase the chance of survival and to improve the quality of life. Molecular biomarkers might be proved very beneficial in this case but serum

biomarkers to predict the disease progress of bladder carcinoma are yet to established. The present study aimed the role of ki-67 in distinguishing the histopathological grade and muscle invasion of urothelial carcinoma.

A total of 40 patients were taken in this study according to selection criteria. Bladder carcinoma is a disease of mostly the middle aged or the elderly people, but it can occur at any age, commonly between 50 and 80 years. The mean age in our study is 63.60 years with majority are in the 61-70 years range (40%). In this study clear male predominance was observed with 77.5% male patients and 22.5% females. In our study mostly patients presented with hematuria followed by frequency and dysuria,and around 72.5% smokers.

According to WHO classification , majority of patients fall into invasive urothelial carcinoma (77.5%) followed by 17.5% have non -invasive low grade urothelial carcinoma, Among high grade 45% have muscle invasion out of which 25% have deep muscle invasion and 7.5% have superficial muscle invasion. According to pTNM staging most common stage is pT3, affecting 32.5% of cases. Muscle invasion was significantly associated with grade of tumor. Ki-67 were higher in patients with high grade tumor which was statistically significant, with according to Ki67 grading, majority (45%) Ki positivity >50% (4+). On comparing pTNM with Ki67 we found that pT2, pT2a and pT4a stages exclusively exhibit >50% positivity (100%), Thus, we found that higher pTNM stages generally correlated with increased Ki-67 positivity.

Conclusion

These observations emphasize the use of Ki 67, in addition to tumour grade and stage to predict the clinical outcome of patients and thereby helps in identifying high risk patients. These patients are frequently followed up and may benefit by additional adjuvant therapies. Clinical importance of this marker prompts multi-institutional prospective studies with large sample size. Awareness among public is needed as they ignore haematuria symptoms and presents to the clinicians at advanced stage of the disease. Reason behind aggressive tumors in elderly is not known and hence research should be carried out further.

References

1. Guo, Charles C., Shen, Steven S., and Czerniak, Bogdan. ‘Recent Advances in the Classification of Bladder Cancer – Updates from the 5th Edition of the World Health Organization Classification of the Urinary and Male Genital Tumors’. 1 Jan. 2023: 1 – 14.
2. Zhang Y, Rumgay H, Li M, Yu H, Pan H, Ni J. The global landscape of bladder cancer incidence and mortality in 2020 and projections to 2040. J Glob Health. 2023 Sep 15; 13:04109.
3. Hasan A, Mohammed Y, Basiony M, Hanbazazh M, Samman A, Abdelaleem MF et al. Clinico-Pathological Features and Immunohistochemical Comparison of p16, p53, and Ki-67 Expression in Muscle-Invasive and Non-Muscle-Invasive Conventional Urothelial Bladder Carcinoma. Clin Pract. 2023 Jul 9;13(4):806-819.
4. Kaseb H, Leslie SW, Soon-Sutton TL, et al. Bladder Cancer. [Updated 2024 Jun 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from:https://www.ncbi.nlm.nih.gov/books/NBK536923/.
5. Moch H, Humphrey PA, Albright TM, Reuter VA, editors. WHO Classification of Tumours of Urinary System and Male Genital Organs. 4th ed. Lyon: International Agency for Research on Cancer; 2016.
6. Haque S, Dewan RK, Saleh S, Jennah SA, Jahan F, Akter F, Sultana T, Ferdaus NJ. Histomorphological study of urinary bladder tumor and statusofHER2/NeuandKi67expressioninurothelialcarcinoma.JHistopatholCytopathol.2018;2(2):99-108.
7. Mckenney, J.K. 'Bladder', in Lamps, L.W., McKenney, J.K. and Myers, J.L.(ed.)RosaiandAckerman'sSurgicalPathology,11thedition,Philadelphia:Elsevier.
8. Habuchi T, Marberger M, Droller MJ, Hemstreet III GP, Grossman HB, Schalken JA, Schmitz-Dräger BJ, Murphy WM, Bono AV, Goebell P, Getzenberg RH. Prognostic markers for bladder cancer: International Consensus Panel on bladder tumor markers. Urology 2005.
9. Wang L, Feng C, Ding G, Ding Q, Zhou Z, Jiang H, Wu Z. Ki67 and TP53 expressions predict recurrence of non-muscle-invasive bladder cancer. Tumor Biology 2014. DOI: https:// doi.org/10.1007/s13277-013-1384-9
10. Serdar A, Turhan C, Soner G, Cem SN, Bayram K, Damla BE, Erbil E. The prognostic importance of ecadherin and p53 gene expression in transitional bladder carcinoma patients. International Urology and Nephrology 2005.Vaishnav M, Garg S, Adhaduk B. Association of smoking, p53
11. Vaishnav M, Garg S, Adhaduk B. Association of smoking, p53 and Ki-67 immunomarkers with bladder neoplasms in tribal region of India. Int J Health Sci (Qassim). 2022 Nov-Dec;16(6):11-17.
12. March-Villalba, J. A., Ramos-Soler, D., Soriano-Sarrió, P., Hervás-Marín, D., Martínez-García, L., &Martínez-Jabaloyas, J.

M. (2018). Immunohistochemical expression of Ki-67, Cyclin D1, p16INK4a, and Survivin as a predictive tool for recurrence and progression-free survival in papillary urothelial bladder cancer pTa / pT1 G2 (WHO 1973). Urologic Oncology: Seminars and Original Investigations. doi:10.1016/j.urolonc.2018.10.

13. Abufaraj M, Shariat SF, Foerster B, Pozo C, Moschini M, D'Andrea D, Mathieu R, Susani M, Czech AK, Karakiewicz PI, Seebacher V. Accuracy and prognostic value of variant histology and lymphovascular invasion at transurethral resection of bladder. World Journal of Urology 2018.
14. Chaudry M.U., Akhtar F., Jamal N., Mansur H., Sarwar N., Saleem Y. Immunohistochemical expression of P16 in low grade urothelial carcinoma. Pak. Armed Forces Med. J. 2021;71:885–888.
15. Manini C., Angulo J.C., López J.I. Mimickers of Urothelial Carcinoma and the Approach to Differential Diagnosis. Clin. Pract. 2021;11:110– 123.
16. Quintero A, Alvarez-Kindelan J, Luque RJ, Gonzalez-Campora R, Requena MJ, Montironi R, Lopez-Beltran A. Ki-67 MIB1 labelling indexand the prognosis of primary TaT1 urothelial cell carcinoma of the bladder. J Clin Pathol. 2006 Jan;59(1):83-8.
17. Enache M, Simionescu C, Lascu LC. Ki67 and Bcl-2 immunoexpression in primitive urothelial bladder carcinoma. Rom J MorpholEmbryol. 2012;53(3):521-5.