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A clinico-immunohistopathological study of urothelial neoplasms with an expression of HER2/Neu and Ki-67 in malignant lesions presenting to a tertiary centre

Rasheed Fatima [1], Shilpa Karamchedu [1*], Venkata Hari Charan Bagadi [1], Misbah Tabassum [1], Manisha Goud Punjala [1], Florence Nightingale [1], Suresh Karamchedu [1]

1. Department of Pathology, SVS Medical College and Hospital, Yenugonda, Mahabubnagar, Telangana-509001, India.
*Correspondence: Shilpa Karamchedu. Department of Pathology, SVS Medical College and Hospital, Yenugonda, Mahabubnagar, Telangana-509001, India.
Tel: +91 8074001312; Email: dr.kshilpa@rediffmail.com

Abstract

Background: HER2/neu is associated with increased tumor grade, and Ki-67 is related to tumor recurrence and stage progression. This study aims to evaluate the clinical profile in patients with urothelial neoplasms and correlate the expression of HER2/neu and Ki-67 in urothelial carcinomas.

Methods: This is a five-year retrospective study from June 2017 to May 2022 with forty cases from the Department of Pathology. A microscopic examination was done to assess the tumor stage and histological grade. Immunohistochemistry was performed with HER2/neu and Ki-67.

Results: 65% (26/40) of the cases were diagnosed as infiltrating urothelial carcinoma, 30% (12) of the cases as low-grade non-invasive urothelial carcinoma, one case as a papillary urothelial neoplasm of low malignant potential (PUNLMP), and one case as urothelial papilloma. 95.5% of infiltrating carcinomas and 84.5% of low-grade non-invasive carcinomas were Ki-67-positive. A significant correlation was observed between the expression of Her-2/neu and Ki-67 and the 2016 World Health Organization (WHO) grading system of urothelial carcinoma. Moderate-to-strong HER2/neu overexpression (2+ or 3+) was observed in only 18% of cases.

Conclusion: The expression of ki-67 increased with an increase in the grade of tumor, which shows its prognostic importance. The relation between Ki-67 expression and the histologic grade of the tumor and the presence of lymphovascular invasion was significant. A significant correlation was observed between the grade of the carcinoma and the immunohistochemical expression of HER2/neu, in which 19.3% of high-grade carcinoma cases showed immunohistochemical expression of the HER2/neu marker. Further studies with a larger population group are required to establish the role of HER2/neu as a prognostic marker.

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Highlights

What is current knowledge?

Immunohistochemical expression of HER2/neu and Ki-67 markers can be used to assess high grade urothelial tumours, but the choice between low- and high-grade urothelial tumors is crucial.

What is new here?

Ki-67 expression helps in discrimination between cases of bladder dysplasia and carcinoma and it helps to identify the high-grade carcinoma. A nonsignificant relation between HER2/neu positivity and Ki-67 expression resulted in this study. A significant relation seen between Ki-67 expression and presence of lymphovascular invasion and histologic grade of tumour.

Introduction

Urological cancer, also known as urinary bladder cancer, is the tenth most prevalent cancer worldwide, and its prevalence is increasing in developed countries (1). 90% of bladder cancer cases were caused by urothelial cells in the bladder, although on rare occasions in the urinary system. Bladder cancer affects men four times more than women. While bladder cancer is the 13th most lethal cancer, it is estimated that approximately 200,000 people died from it in 2018, accounting for 2.1% of all cancer deaths (2). Bladder cancer is most common in older people. Tobacco use is the leading risk factor, accounting for 50-65% of new cases yearly. Smoking has been shown to increase the risk of bladder cancer by three to four times (3). The second preventable risk factor for bladder cancer is occupational exposure to aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons. Tumor stage and grade are critical predictive indicators in clinical application. Urothelial (transitional) tumors are the most frequent bladder tumors, such as non-invasive urothelial (transitional cell) tumors and infiltrating urothelial carcinoma.

Several studies have found an association between the Ki-67 labeling index and well-known prognostic variables such as grade and stage (5). Overexpression of the HER2/neu protein and gene amplification have been observed in urothelial carcinomas. A study discovered a link between the immunohistochemical expression of HER2/neu and Ki-67 and the 2016 WHO grading system of urothelial cancer (6).

No previous studies have been conducted on this topic in our region. Hence, the current study aims to assess the immunohistochemistry expression of Ki-67 and HER2/neu in urinary cancer and its correlation with clinicopathological data.

Methods

A five-year retrospective and prospective study was conducted on urothelial samples collected in the Department of Pathology from June 2017 to May 2022. The Institutional Ethical Committee, SVS Medical College, Mahabubnagar, approved the study procedure.
Inclusion criteria: Radical cystectomy specimens and specimens diagnosed with urothelial carcinoma.
Exclusion criteria: Surgical cystectomy specimens with different causes of urine bladder.

Each block was stained with hematoxylin and eosin (H&E), and additional slices were mounted on charged slides for immunohistochemical staining with a monoclonal antibody against the Ki-67 antigen clone MIB-1 and a monoclonal rabbit anti-human c-erbB-2 oncoprotein (HER2/neu).

Ready-to-use antibodies were used, i.e., Anti-c-erbB2/Her2-neu (CloneCB11) Mouse Monoclonal Antibody (Biogenex company) and Ki-67 (CloneMiB-1) Mouse Monoclonal Antibody (Biogenex company).
Nature of Specimen: Transurethral resection of bladder tumor.

Microscopy was used to determine the tumor stage and histological grade. Following the WHO classification, the TNM classification was used for tumor staging (2016). The paraffin blocks represented urothelial carcinoma of the bladder surgically removed by transurethral resection of the bladder tumor. H&E stained sections of 5mm thickness; some sections were placed on positively charged slides and were immunohistochemically stained for her2/neu and Ki-67. The H&E stained sections were examined for histological type, grading, and staging of the tumors—Monoclonal Mouse Anti-Human antigen clone was used for immunohistochemistry. Stained sections were evaluated under the light microscope.

For Ki-67, a minimum of 1000 tumor cells were counted under 400 magnification from immunopositive regions. The proportion of positive cells was computed as follows: 0 = 0-10% of tumor cells stained, 1 = 11-25% of cells stained, 2 = 26-50% of cells stained, and 3 = >50% of cells stained.
Quantification: 0 = negative (1-2), 1+ = mild (3), 2+ = moderate (4), and 3+ = strong (5-6).

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Brown membranous staining intensity and pattern were used to score HER2/neu: 0 = no staining or less than 10% of tumor cells; 1+ = partial faint membrane staining in more than 10% of tumor cells; 2+ = circumferential weak to moderate staining in more than 10% of tumor cells; 3+ = circumferential strong membrane staining in more than 10% of tumor cells (Table 1).

Statistical analysis: The data was analyzed using the Statistical Package for Social Science Software (SPSS), version 16.0. Descriptive statistics were employed to characterize variables; the mean and standard deviation were utilized for quantitative variables. The chi-square test was used to compare groups for qualitative factors. A p-value of less than 0.05 was considered statistically significant.

Results

The patients' ages ranged from thirty to eighty years; the mean age was 59.2±8.9 years. Among forty cases, the majority (13 cases) were aged between 61-70 years (32.5%), followed by 11 (27.5%) cases between 51-60, six (15%) cases between 41-50, six (15%) cases between 71-80, and four (10%) cases between 30-40. The male-to-female ratio was 3:1.

Twenty-five cases had a smoking history. Occupational industrial exposure was seen in five cases. Ninety percent of cases had painless hematuria, and only 10% had dysuria. Infiltrating urothelial carcinoma accounted for 65% of bladder lesions, 30% of low-grade non-invasive urothelial carcinoma, 2% of PUNLMP, and 3% of urothelial papilloma. HER-2/neu and Ki-67 immunohistochemical expression showed no significant connection with age or gender. A significant correlation was found between the expression of HER-2/neu and Ki-67 and the WHO grading system of urothelial carcinoma (2016). Figure 1 depicts the immunohistochemical results of urothelial carcinoma and its invasiveness. Immunohistochemical analysis of urothelial carcinoma with Ki-67 nuclear and HER2/neu staining and their findings is depicted in (Figure 2).

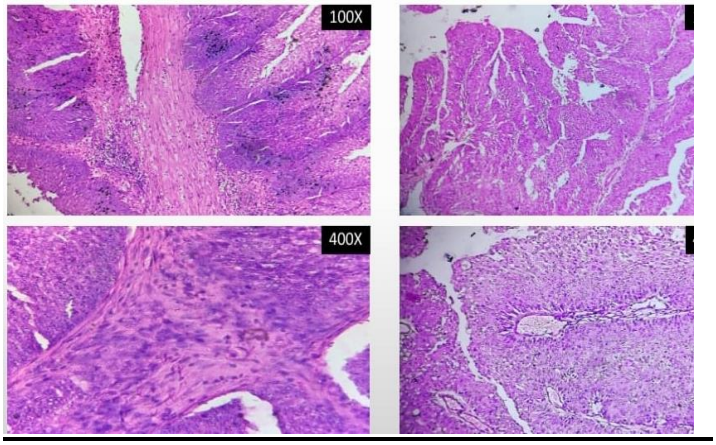

Figure 1. Immunohistochemical findings. a. high-grade muscle-invasive papillary urothelial carcinoma (PUC). b. low-grade PUC

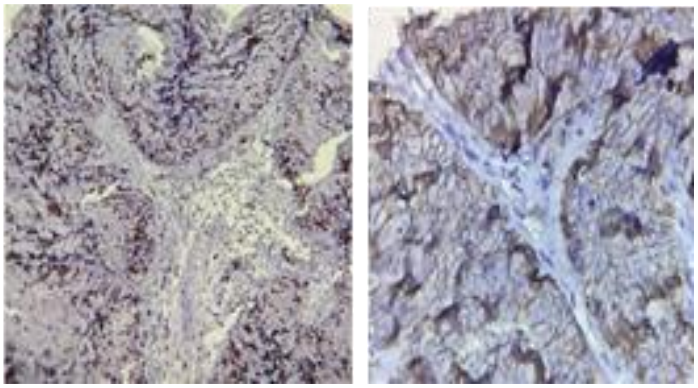

Figure 2. Immunohistochemical findings. a. Ki-67 nuclear staining. b. HER2/neu membranous staining

A significant correlation was observed between Ki-67 and Her2/neu and the 2016 WHO grading system of the tumor, especially for the Ki-67 marker (p< 0.001), with twenty-four out of twenty-six (92.3%) high-grade cases showing positive immunohistochemical Ki-67-expression and nine out of 12 (75%) low-grade cases showing positive immunohistochemical Ki-67-expression (Table 2). A significant association was observed between the immunohistochemical expression of HER2/neu and the 2016 WHO grading system of the tumor, with five out of twenty-six (19.3%) high-grade carcinoma cases showing positive immunohistochemical expression of the HER2/neu marker.

Table 2. Association of Ki-67 and Her2/neu expression and grade of urothelial carcinoma

Histochemical diagnosis with grade	Ki-67 expression		P value	HER2 expression		P value
	Positive	Negative		Positive	Negative	
Low grade-non -invasive urothelial carcinoma	9 (75%)	3 (35%)	< 0.001	0 (0%)	12 (100%)	< 0.001
Infiltrating urothelial carcinoma	24 (92.3%)	2 (7.7%)		5 (19.3%)	21 (80.7%)	
Total	33 (86.9%)	5 (13.1%)		5 (13.2%)	33 (86.8%)	

Discussion

The average age of the total patients in this study was 59.2, with a male-to-female ratio of 3:1. In contrast, in a study by Badawia B Ibrahim et al. (7), the mean age of the patients was 59.579.39 years, with a range of twenty-eight to seventy-seven years and a male to female ratio of 9:1.

The majority of cases (90%) had painless hematuria. Smoking accounted for 75% of cases among risk factors. According to Fortuny et al. (8), patients with a history of tobacco smoking were at a higher risk of urinary tract cancer, and 72% of patients presenting with urothelial- neoplasms had a smoking history.

Out of forty patients, twenty-six had infiltrating urothelial carcinoma, 12 had minimal grade non-invasive urothelial- carcinoma, one had PUNLMP, and one had urothelial papilloma.

Identifying prognostic indicators in urothelial cancer has received significant attention around the world. HER2/neu is among the most often increased oncogenes in bladder cancer. It appears to play a role in tumor etiology; nevertheless, its expression varies between studies (9).

HER2/neu expression was detected in five cases (13.2%) in our investigation.

According to Badawi B Ibrahim et al. (7), positive HER2/neu expression was detected in 63.3% of cases and negative in 36.7% of cases. Of sixty instances, 35% have high Ki-67 expression, 33.33% have moderate, 23.3% have mild, and 8.3% have negative Ki-67 expression.

A study of 111 bladder cancer samples found HER2/neu over-expression in 22% of the samples (10). Another study found HER2/neu over-expression in 74% of this number.

Except for perineural invasion, the relationship between HER2/neu expression and the clinicopathologic characteristics was negligible in this investigation (11). In the present study, a significant correlation was found between Ki-67 and Her2/neu and the 2016 WHO tumor grading system, especially for the Ki-67 marker (p 0.001), with twenty-four out of twenty-six (92.3%) high-grade cases showing positive immunohistochemical Ki-67 expression and nine out of 12 (75%) low-grade cases showing positive immunohistochemical Ki-67 expression. A significant link was found between the immunohistochemistry expression of HER2/neu and the 2016 WHO tumor grading system, with five out of twenty-six (19.3%) high-grade carcinoma cases showing positive immunohistochemical expression of HER2/neu marker.

In the study by Noora Ali Jawad et al., a significant correlation was observed between Ki-67 and HER2/neu and the 2004 WHO grading system of the tumor, with twenty-one out of twenty-five (84%) high-grade cases showing positive immunohistochemical Ki-67 expression and 10 out of 20 (50%) low-grade cases showing positive immunohistochemical Ki-67 expression. This correlation was also significant for the immunohistochemical expression of HER2/neu (p = 0.015), with twenty out of twenty-five (80%) high-grade carcinoma cases showing positive immunohistochemical expression of the HER2/neu marker and nine out of twenty (45%) low-grade cases showing positive expression (12).

According to Abid A et al.'s study, eight cases were identified as low-grade non-invasive urothelial carcinoma, 78% as infiltrating urothelial carcinoma, and one as a PUNLMP. Ki-67 positivity was seen in 96.8% of infiltrating carcinomas and 87.5% of low-grade non-invasive carcinomas (13).

Therefore, Ki-67 expression is useful for distinguishing between bladder dysplasia and carcinoma cases and detecting high-grade cancer cases (14). Ki-67 is now used by 90% of pathologists worldwide to identify bladder cancer (15). Several studies have found a link between Ki-67 expression and the tumor grade, as well as the occurrence of lymphovascular invasion. They also found a non-significant association between Ki-67 expression and HER2/neu positivity (16). A biopsy or transurethral resection reveals a different grade than what is discovered in the operative specimen (17). The grading system is designed for non-invasive urothelial neoplasms and lacks discriminatory power when applied to invasive components. Infiltrating carcinomas act like high-grade carcinomas. In the present study, 92.3% of infiltrating carcinomas and 75% of low-grade non-invasive carcinomas were positive for Ki-67. Jawad et al. found a similar result (12). Elkady et al. discovered a substantial correlation between Ki-67 and the tumor grade (18). In the present study, the Ki-67 score was high (3+) in all higher-stage tumors (pT2) and lower in pTa and pT1. The Ki-67 value and the tumor stage were shown to be significantly correlated.

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According to Zambelli et al. (19), the Ki-67 score substantially correlates with the tumor's histological grade and clinical stage. Suwa et al. (20) discovered a link between Ki-67 expression and the grade and stage of urothelial cancer. In the study by Sentürk et al., Ki-67 expression in bladder cancer increased with the pathological stage and histological grade (21).

Conclusion

Ki-67 expression was elevated with tumor grade, indicating its prognostic significance. Ki-67 expression was found to be significantly associated with the tumor histologic grade and the existence of lymphovascular invasion. A significant association was observed between the immunohistochemistry expression of HER2/neu and the tumor grade, with 19.3% of high-grade carcinoma patients showing positive immunohistochemical expression of the HER2/neu marker. More research with a broader demographic group is needed to establish the role of HER2/neu as a prognostic indicator.

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Ethical statement

The study was performed per the Declaration of Helsinki, and written informed consent was obtained from all participants. The study received approval from the Ethics Committee of SVS Medical College, Mahabubunagar (No: IEC/SVS/Path/2019/02).

Conflicts of interest

The authors declare no conflict of interest regarding the publication of this article.

Author contributions

BV, TM, and PM analyzed and interpreted the patient data regarding the urothelial neoplasms and the transplant. FR and KS performed the histological examination, and was a major contributor in writing the manuscript. NF and SK were supervised the total work and editing of manuscript. All authors read and approved the final manuscript.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424. [View at Publisher] [Google Scholar]
2. Mushtaq J, Thurairaja R, Nair R. Bladder Cancer. Surgery. 2019;37(9):529-37. [View at Publisher] [Google Scholar] [DOI]
3. Al-Zalabani A, Stewart K, Wesselius A, Schols AMWJ, Zeegers MP. Modifiable risk factors for the prevention of bladder cancer: A systematic review of meta-analyses. Eur J Epidemiol. 2016;31(9):811-51. [View at Publisher] [Google Scholar] [DOI] [PMID]
4. Zeegers M, Swaen GMH, Kant I, Goldbohm R, Brandt PAVD. Occupational risk factors for male bladder cancer: results from a population-based case cohort study in the Netherlands. Occup Environ Med. 2001;58(9):590-6. [View at Publisher] [Google Scholar] [DOI] [PMID]
5. Gönül II, Akyürek N, Dursun A, Küpeli B. Relationship of Ki67, TP53, MDM-2 and BCL-2 expressions with WHO 1973 and WHO/ISUP grades, tumour category and overall patient survival in urothelial tumours of the bladder. Pathol Res Pract. 2008;204(10):707-17. [View at Publisher] [Google Scholar] [DOI] [PMID]
6. Madhu K, Ponwang K, Suresh B, Atin S, Rahul Janak S, Sankhwar, et al. Immunohistochemical expession of markars of ki67 and her2 neu and its corelation with clinicopathological parameters of urothelial tumours. International Journal of Current Research. 2016;8(12):43704-08. [View at Publisher] [Google Scholar]
7. Ibrahim BB, Mahmoud SA, Mohamed AA, El Hanbuli HM. Expression of HER2/neu and Ki-67 in Urothelial Carcinoma and their Relation to Clinicopathological Data: An Egyptian Study. Journal of Clinical & Diagnostic Research. 2021;15(3):6-9. [View at Publisher] [Google Scholar]
8. Fortuny J, Kogevinas M, Chang-Claude J, ´lez CG, Hours M, KJ; Zeegers MPA, Tan FES, Dorant E, Brandt PA. The impact of characteristics of cigarette smoking on urinary tract cancer risk. Cancer. 2000;89(3):630-9. [View at Publisher] [Google Scholar] [DOI] [PMID]
9. Wester K, Sjostrom A, Torre M, Carlsson J, Malmstrom PU. HER-2 a possible target for therapy of metastatic urinary bladder carcinoma. Acta Oncol. 2002;41(3):282-8. [View at Publisher] [Google Scholar] [DOI] [PMID]
10. Bellmunt J, Werner L, Bamias A, Fay AP, Park RS, Riester M, Selvarajah S, Barletta JA, Berman DM, Muga SD, Salido M. HER2 as a target in invasive urothelial carcinoma. Cancer Med. 2015;4(6):844-52. [View at Publisher] [Google Scholar] [DOI] [PMID]
11. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part B: Prostate and Bladder Tumours. Eur Urol. 2016;70(1):106-19. [View at Publisher] [Google Scholar] [DOI] [PMID]
12. Noora Ali J, Hussam Hasson A, Mohammed Subhi K. Her2/ Neu and Ki -67 Immunohistochemical Expression in Transitional Cell Carcinoma of the Urinary Bladder (A Clinicopathological Study). IOSR Journal of Dental and Medical Sciences. 2016;15(2):6-12. [View at Publisher] [Google Scholar] [DOI]
13. Abid A, Sen S, Bandyopadhyay R. Clinicopathological study of urothelial neoplasms in urinary bladder with special reference to expression of Her2/neu and Ki-67 in malignant lesions. Indian J Pathol Oncol. 2021;8(3):369-76. [View at Publisher] [DOI]
14. Badawi MA, El-Sharkawy SL, Abbas NF, Abdel-Aal WE. Image analysis and Ki-67 expression in urothelial dysplasia and carcinoma. J Arab Soc Med Res. 2018;13(2):144-50. [View at Publisher] [Google Scholar] [DOI]
15. Lopez-Beltran A, Algaba F, Berney DM, Boccon-Gibod L, Camparo P, Griffiths D, et al. Handling and reporting of transurethral resection specimens of the bladder in Europe: A web-based survey by the European Network of Uropathology (ENUP). Histopathology. 2011;58(4):579-85. [View at Publisher] [Google Scholar] [DOI] [PMID]
16. Thakur B, Kishore S, Dutta K, Kaushik S, Bhardwaj A. Role of p53 and Ki-67 immunomarkers in carcinoma of urinary bladder. Indian J Pathol Microbiol. 2017;60(4):505-9. [View at Publisher] [Google Scholar] [DOI] [PMID]
17. Jewett HJ, Blackman SS. Infiltrating Carcinoma of the Bladder: Histologic Pattern and Degree of Cellular Differentiation in 97 Autopsy Cases. J Urol. 1946;56(2):200-10. [View at Publisher] [Google Scholar] [DOI] [PMID]
18. Elkady N, Sultan M, Elkhouly E. Evaluation of topoisomerase II, ki-67, and P53 expression in non-muscle-invasive urothelial carcinoma and their clinical significance. Indian J Pathol Microbiol. 2018;61(4):526-31. [View at Publisher] [Google Scholar] [DOI] [PMID]
19. Zambelli S, Zanin A, Gaglio A, Zai G, Bosco E, Andrion A, et al. Ki-67 scores and AgNor counts in transitional cell carcinoma of the bladder: apparent lack of prognostic value. Arch Ital Urol Androl. 1993;65(6):665-70. [View at Publisher] [Google Scholar] [PMID]
20. Suwa Y, Takano Y, Iki M, Asakura T, Noguchi S, Masuda M. Prognostic significance of ki-67 expression in transitional cell bladder carcinoma after radical cystectomy. Pathology. 1997;193(8):551-6. [View at Publisher] [Google Scholar] [DOI] [PMID]
21. Senturk N, Aybek Z, Duzcan E. Ki-67, p53, bcl-2 and bax expression in urothelial carcinomas of urinary bladder. Turk J Pathol. 2010;26(1):25-30. [View at Publisher] [Google Scholar] [DOI]

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