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Original Research Article

Evaluation of the immunohistochemical expression of HER2/NEU in urinary bladder neoplasms in a tertiary care hospital

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ABSTRACT

Background: HER2/NEU expression in urothelial neoplasms has been reported to range from 9% to 80% on immunohistochemistry. We studied the expression of HER2/NEU in urothelial neoplasms and its variability according to histological grade along with the prognostic value.
Methods: The present study was conducted on 60 formalin-fixed, paraffin-embedded specimens of urinary bladder neoplasms, which comprised 45 cases of high-grade urothelial carcinoma followed by 13 cases of low-grade and 2 cases of PUNLMP. These were then subjected to IHC by HER2/NEU. Membrane positivity for HER2/NEU was noted.
Results: A total of 65% of cases showed positive membranous HER2/NEU expression. HER2/NEU expression was noted in 82.6% of cases of high grade while 8.4% of cases of low grade were positive for HER2/NEU expression.
Conclusions: The majority of high grade invasive urothelial carcinomas showed positive HER2/NEU expression with a significant p-value <0.001. Thus patients with high grade urothelial carcinoma may benefit from HER2/NEU targeted therapy.

Keywords: HER2/NEU, High-grade, IHC, Low-grade, PUNLMP, Urinary bladder neoplasms

INTRODUCTION

Urothelial carcinoma is the 10th most commonly occurring and 13th most common cause of cancer-related deaths worldwide. The estimated new number of cases and deaths are 5,73,278 and 2,12,536 respectively.[1]

In India, the incidence rate among men and women is 2.4 and 0.7 respectively, while the mortality rate is 1.3 in men and 0.3 in women.[2] The incidence and mortality of bladder cancer differ worldwide. It is six times more prevalent in countries with high HDI than those with low HDI.[3] The mean age according to National Comprehensive Cancer Network (NCCN) was 73 years stating that urothelial carcinoma is rare before the fifth decade.[4] Histologically, approximately 90% of bladder tumors are urothelial carcinomas and among these 90% originate from the urinary bladder itself. Other subtypes include squamous cell carcinoma and adenocarcinoma accounting for 5% and 2% of cases respectively.[5]

Smoking is the strongest risk factor associated with bladder cancer, representing approximately 50% of the cases followed by environmental exposure to aromatic amines, aromatic polycyclic hydrocarbons, and chlorate hydrocarbons are other important risk factors.[6] Water consumption with high levels of arsenic has also shown an increased risk of bladder cancer.[7] Schistosomiasis infection is another common cause of bladder cancer in regions of Africa and the Middle East.[8] The most common clinical presentation is painless hematuria which can be gross or microscopic. Other symptoms include

pain or burning during urination without evidence of urinary tract infection, and change in bladder habits, such as having to urinate more often or feeling the strong urge to urinate without producing much urine. Urinary Bladder cancer often causes no symptoms until it reaches an advanced stage that is difficult to cure. When the ureteral orifice is involved, pyelonephritis/ hydronephrosis may lead to flank/abdominal pain.[9]

Investigations required to diagnose bladder cancer involve a complete hemogram, biochemistry, urine cytology, and imaging. Imaging modalities include computerized tomography, magnetic resonance imaging (MRI), and ultrasonography. Cystoscopy is the gold standard method for the detection of bladder cancer. The transurethral resection of bladder tumor (TURBT) is a diagnostic, prognostic, and often therapeutic procedure followed. The treatment of bladder cancer depends on the grade and stage of the tumor.[10]

Human epidermal growth factor receptor 2 is a transmembrane tyrosine kinase receptor that is involved in cell growth, survival and migration. Increased activity of this molecule has been evaluated in breast cancer and was associated with poor prognosis and response to target therapy. A wide variability of HER2/NEU overexpression in bladder cancer, from 6 to 80%, has been reported generally related to high grade and stage and correlated with poor prognosis.[11] Immunohistostaining of HER2/NEU is shown in the cytoplasm and membrane of cells in urothelial carcinoma. The expression of HER2/NEU is increased with an increase in stage and recurrence. The HER2/NEU encoded protein molecule occupies a critical position in the biochemical pathways responsible for the transduction of mitogenic signals from a variety of growth factor receptors. In addition to its role in regulating normal cellular proliferation, overexpression of the HER2/NEU gene appears to play a role in neoplastic cell growth.[12]

The incidence of overexpression of HER2/NEU in bladder cancer ranges from 9% to 34% of most cancers tested. In transitional bladder cell carcinoma, it was found that HER2/NEU is over-expressed with a greater frequency in higher grades and stages than in lower grades and stages. Several studies found that patients harboring tumors with HER2/NEU overexpression were twice likely to experience recurrence and die from their cancer, compared to patients with HER2/NEU negative tumors. A subset of high-grade NMIBCs contains HER2/NEU amplification and is associated with more aggressive behavior. The results obtained by quantitative methods in other studies showed HER2/NEU oncoprotein to be more significantly expressed in the malignant group compared to the benign and normal groups, and they concluded that the quantitative assessment of HER2/NEU expression in malignant tumors aided by other proliferation markers such as synthetic phase fraction (SPF), DNA index (DI) and ploidy is useful in selecting patients for more aggressive treatment or for predicting outcome.[12]

Early detection and treatment of bladder tumors improve the five-year survival rates by up to 80%. So, this IHC-based study was conducted to evaluate the correlation of HER2/NEU expression in urinary bladder neoplasms, as it can provide more aggressive targeted therapy for patients thereby increasing the survival rate.

METHODS

A descriptive study was conducted in The Department of Pathology, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India for one year from November 2021 to November 2022. 60 cases were studied and sampling is done by keeping in view the availability and feasibility of the participants, a non-random convenient sampling technique was adopted.

Inclusion criteria include all the cystectomy specimens and urinary bladder biopsies received from urology and surgery department (diagnosed as urinary bladder neoplasms), all urinary bladder slides and blocks (diagnosed as urinary bladder neoplasms) received for review in The Department of Pathology, Guru Gobind Singh Medical College, Faridkot.

Exclusion criteria include all inadequate biopsies, autolyzed specimens and non-neoplastic conditions.

Data collection tools

This present study comprised of urinary bladder specimens that were received in the Department of Pathology over a period of one year. In each case, introductory data and clinical information regarding age, sex, size of tumor and clinical symptoms were noted in a prestructured proforma. The tissue was formalin fixed, paraffin embedded and then stained with haematoxylin and eosin for histopathological typing and grading. Grading of urothelial tumors was done according to WHO classification. All the cases were then subjected to immunohistochemistry for HER2/NEU expression.

IHC of HER2/NEU was done by taking sections of 3-5 µm which were cut and mounted on poly-L-lysine coated slides. These slides were dried overnight at 37ºC and dewaxed in xylene and hydrated. Antigen retrieval was done by using 1500 mL of citrate buffer solution at pH 6.0 which was heated, till it boiled in a stainless steel pressure cooker. The slides were positioned into metal staining racks and lowered into pressure cooker ensuring slides were completely immersed in unmasking solution and lid was locked. When the pressure cooker reached operating temperature and pressure (after about 5 minutes) a timer was started for 1 minute. When the timer rang, pressure cooker was removed from heat source and run under cold water with lid on. Lid was not removed until the indicators show that pressure had been released.

Later lid was opened, slides were removed and placed immediately into a bath of tap water. Neutralization of endogenous peroxidase was done using Peroxidase Block for 5 minutes, followed by two washings in Phosphate Buffer Saline/Tris buffer saline each for 5 minutes. Incubation with Protein Block for 5 minutes was done and two washes in tris buffer for 5 minutes each were given. The primary antibody was put on the sections and sections were kept for 1 hour in the moist chamber, followed by 2 washes in tris buffer for 5 minutes each. The post primary block was then applied for 30 minutes at room temperature. Again 2 washings of tris buffer were given for 5 minutes each. Incubation of slides was done with Polymer for 30 minutes. Again 2 washings were given with Phosphate Buffer for 5 minutes each with gentle rocking. Slides were then covered with DAB for 2-3 minutes. All these steps were performed in a moist chamber. Sections were later washed in deionised water for 5 minutes. Haematoxylin counterstaining was done for 2-5 minutes and sections washed under running tap water. Dehydration and clearing of the sections was done in propanol and xylene, respectively followed by mounting with DPX and sections were viewed under the microscope.

Results and interpretation of IHC scoring

Positive and negative controls were run with every batch of the IHC. Positive control tissue had coloured end product at the site of target antigen. Negative control tissue section did not have the above colour, which meant that the tissue was having antibody specific antigen.

For HER2/NEU Receptor: Brown membrane staining was taken as positive for HER2/NEU. Depending upon intensity and percentage of cells, a score of 0-3 was given.

Table 1: Scoring of HER2/NEU13

Staining pattern	Score	Expression
No staining/membrane staining <10% of the tumor cells	0	Negative
Faint/barely perceptible membrane staining in >10%of tumor cells. The cells are only stained in part of their membrane.	1+	Negative
Weak to moderate complete membrane staining observed in >10% of tumor cells.	2+	Weakly positive
Strong complete membrane staining is observed in >10% of tumor cells	3+	Strongly positive

All +2 and +3 cases were considered as positive.[13-15]

Data analysis plan

The data related to clinical details was entered in the form of data matrix in Microsoft excel and analysis was done by using SPSS 23.0 ver. The descriptive statistics for categorical variables were represented in form of frequencies and percentages. The association between categorical variables was assessed by Pearson chi-square test. A p-value of <0.05 was considered as significant.

Ethical considerations

The study was carried out after seeking permission from Institutional Ethics Committee of Guru Gobind Singh Medical College and Hospital, Faridkot. Written informed consents were taken from all the participating patients.

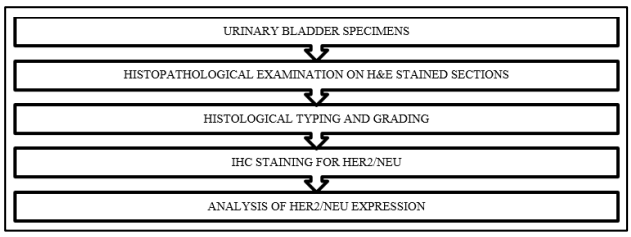

Figure 1: Flowchart.

RESULTS

In our study, demographic profile along with histological types and grades of urinary bladder neoplasms were observed (Table 2).

Out of the total of 60 cases of urothelial neoplasms, 65% (39 cases) showed positive HER2/NEU expression. All the cases (2/2) of PUNLMP exhibited negative expression for HER2/NEU. Among the non-invasive papillary urothelial carcinoma 15.3% (2/13) cases were positive for HER2/NEU expression while in invasive urothelial carcinoma 82.2% (37/45 cases) had positive membranous HER2/NEU expression respectively (Table 3).

Out of the total 60 cases, 82.6% (38/46) of high-grade urothelial carcinomas were positive for HER2/NEU expression and 8.4% (1/14) of low-grade urothelial carcinoma were positive for HER2/NEU expression (Table 4).

Hence, it was noted that as the grade of the tumor increased the expression of HER2/NEU also increased. A highly significant p-value of <0.0001 was seen with histological type and grade of tumor and HER2/NEU expression.

Table 2: Demographic observations with histological grade and types.

Variable	Frequencies
Age (Mean ± SD in years)	59.55±11.73
<40 years	8.3% (5/60)
41-70 years	75% (45/60)
>70 years	16.7% (10/60)
Gender (M:F ratio)	3.28:1
Males	76.7% (46/60)
Females	23.3% (14/60)
History of smoking	
Present	66.7% (40/60)
Absent	33.3% (20/60)
Presenting complaints	
Painless hematuria	86.5% (52/60)
Burning micturition	5% (3/60)
Urinary outflow obstruction	1.7% (1/60)
Combination of symptoms	6.8% (4/60)
Size of tumor on radiology	
0.1-3.0 cm	85% (51/60)
3.1-6.0 cm	11.7% (7/60)
>6.0 cm	3.3% (2/60)
Histological types	
PUNLMP	3.33% (2/60)
Non-invasive urothelial carcinoma	21.67% (13/60)
Invasive urothelial carcinoma	75% (45/60)
Histological grades	
High grade	79.3% (46/60)
Low grade	20.7% (12/60)
Muscularis propria invasion	
Present	30% (18/60)
Absent	70% (42/60)
Squamous differentiation and necrosis	
Present	4.44% (2/60)
Absent	95.56% (58/60)

Table 3: Correlation of histological type with HER2/NEU expression.

Histological type (n=60)	No. of HER2/NEU			
	Positive		Negative	
Papillary urothelial neoplasm of low malignant potential (n=2)	0	-	2	100%
Non-invasive urothelial carcinoma (n=13)	2	15.3%	11	84.7%
Invasive urothelial carcinoma (n=45)	37	82.2%	08	17.8%

P value = <0.0001

Table 4: Correlation of histological grade with HER2/NEU expression.

Histological grade	HER2/NEU expression	
	Positive	Negative
Low grade (n=12)	1 (8.4%)	11 (91.6%)
High grade (n=46)	38 (82.6%)	8 (17.4%)

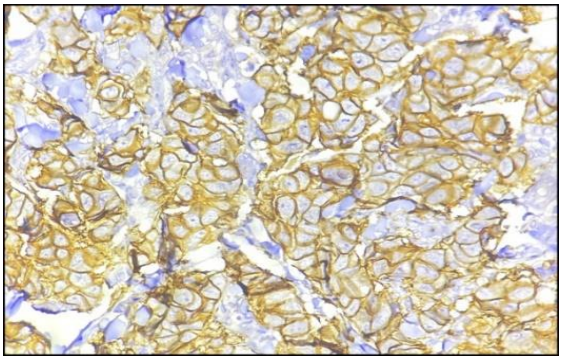

Figure 2: Control.

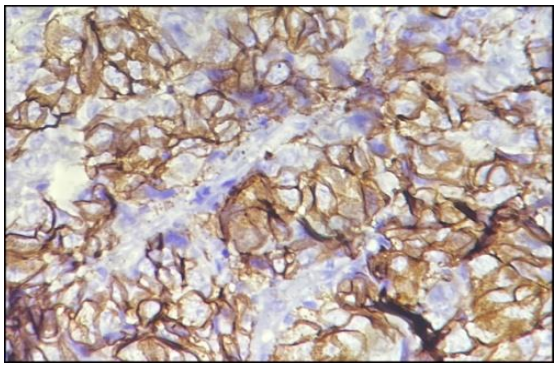

Figure 3: IHC score 3+.

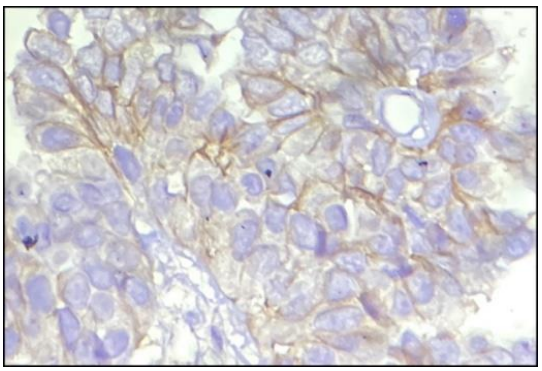

Figure 4: IHC score 2+.

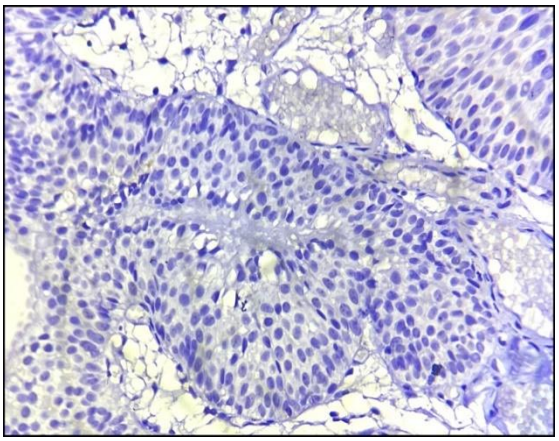

Figure 5: IHC score 0.

DISCUSSION

In our study it was seen that the majority of high-grade invasive urothelial carcinomas (82.6%) showed positive HER2/NEU expression. HER2/NEU expression increased with increasing grade with a statistically significant p-value of <0.0001. Concordant findings were also seen by Kumar S et al who found HER2/NEU positivity of 75.9% in high grade tumors and 63% low grade tumors.[13] These results were also similar to study conducted by Danaei S et al in 2018 who observed HER2/NEU expression of 48.9% (23/47 cases) in high grade urothelial carcinoma and 27.4% in low grade urothelial carcinomas.[17] Agarwal M et al reported HER2/NEU expression of 88.89% in high grade urothelial carcinomas and 11.11% in low grade carcinomas.[18]

The present study also suggested that HER2/NEU expression gets upregulated from non-invasive to invasive lesions. Statistically significant correlation (p value-0.000) of HER/2NEU expression was seen with histological type as shown in Table 3. Concordant result was seen by El Ochi RM et al in 2017 and Charfi S in 2013 who reported statistically significant association between tumor type and HER2/NEU expression with a p-value of 0.003 and 0.002 respectively.[14,19]

HER2/NEU is a transmembrane tyrosine kinase receptor that is responsible for cell growth and proliferation. Many studies have shown that its expression in urothelial carcinomas ranges from 9% to 80% on immunohistochemistry. The variability of expression is attributed to many factors like tumor heterogeneity, type of specimen i.e biopsy or cystectomy, different histological types, different antibodies used for IHC, and different protocols for IHC.16 In our study we included cases with 2+ score as positive since FISH was not available for confirmation. Similar methodology was adopted by Kumar S, El Ochi MR and Ibrahim BB in their respective studies.[13-15]

CONCLUSION

In the present study, majority of high grade invasive urothelial carcinomas showed positive HER2/NEU expression. It was observed that HER2/NEU expression increased with increasing grade with a statistically significant p-value <0.001. Thus, it is concluded that patients with high grade urothelial carcinoma may benefit from HER2/NEU targeted therapy, however further studies are required.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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