Original Article

Human epidermal growth factor receptor 2/neu expression in urothelial carcinomas

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ABSTRACT

Introduction: Urothelial carcinomas of the bladder are more common in males, making them the sixth-most common cancer in men and the tenth-most common cancer overall, worldwide. Current guidelines do not recommend routine testing for human epidermal growth factor receptor (HER2/neu) expression on the biopsy specimens of patients with urothelial carcinoma. This study was aimed at determining the expression pattern of HER2/neu and its usefulness in muscle-invasive and nonmuscle-invasive urothelial carcinoma.

Methods: HER2/neu expression was assessed in 89 specimens of urothelial cancer by immunohistochemistry (IHC), and equivocal cases were subjected to fluorescent *in situ* hybridization (FISH).

Results: On IHC for HER2/neu, 17.9% (7/39) of the muscle-invasive bladder cancers (MIBCs) showed a 3+ expression, whereas 22% (11/50) of the non-muscle invasive cancers were positive with a score of 3+. A significant correlation between HER2/neu status and muscle invasion could not be established in the current study ($P = 0.74$, Fisher's exact test). Three cases of muscle-invasive (7.7%) and 2 cases (4%) among nonmuscle invasive cancers showed equivocal expression. All the cases with equivocal (2+) expression on IHC were subjected to FISH and none showed gene amplification on hybridization and were considered as negative.

Conclusion: Overexpression of HER-2/neu was seen in 17.9% of MIBCs and 22% of non-MIBCs. There are no norms for routine testing of HER2/neu expression in the biopsy specimens of urothelial carcinoma. There is an unmet need to establish guidelines for HER2/neu scoring, similar to that for breast and gastric cancers, to determine the proportion of positive cases and help in identification of those who may benefit from targeted therapies.

INTRODUCTION

In the current clinical scenario, the role of targeted treatment in the management of cancers is gaining importance. Human epidermal growth factor receptor (HER2/neu) has been extensively studied in the management of various cancers. It is a quintessential test for administering targeted therapy in carcinoma breast and is being increasingly used in gastric cancers as well with well established guidelines for reporting on immunohistochemistry (IHC) and by Fluorescent *in situ* hybridization (FISH) studies.

Molecular classification for urothelial carcinoma has also been established, and Kamoun *et al.* have proposed six classes: luminal papillary, luminal nonspecified, luminal unstable (LumU), stroma-rich, Basal/squamous, and neuroendocrine like. The highest copy number alterations were observed in the LumU subtype along with HER2 amplifications.[1]

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Depending on the stage, the treatment regimen for urothelial carcinomas is either platinum-based chemotherapy alone or surgical management, that is radical cystectomy with regional lymph node dissection following chemotherapy.[2] Many checkpoint inhibitor-based therapies have also been approved by the Food and Drug Administration in metastatic settings. HER2/neu is a regulator for cell growth and differentiation and is a potential therapeutic target in cancer treatment. Novel treatments to target patients with HER2/neu-positive urothelial carcinomas, such as antibody–drug conjugates (ADCs), have been developed recently.[3,4] However, HER2/neu testing has not been included in the standard clinical practice yet. We undertook this study to determine the expression of HER2/neu in muscle-invasive and nonmuscle-invasive urothelial carcinomas and to better understand its potential in newer treatment regimens against HER2/neu in patients with urothelial carcinoma.

PATIENTS AND METHODS

Prospective analysis of histologically confirmed 39 muscle-invasive urothelial bladder cancer (MIBC) and 50 non-MIBC (NMIBC) specimens received in the department as biopsy specimens after transurethral resection of bladder tumor or cystectomy over a period of 4 years was performed. Only the epithelial muscle-invasive and nonmuscle-invasive urothelial cancers were included in the study. All the patients who received chemotherapy were excluded from the study. This study was approved by the Institutional Ethics Committee (IEC number: AIIMS/IEC/2018/763). Clinicopathologic information, including age, gender, histological grade, tumor stage, variant of urothelial carcinoma, differentiation such as squamous/sarcomatous, lymphovascular invasion, and perineural invasion, was recorded.

Immunohistochemistry

The IHC for HER2/neu was performed in all urothelial cancers using the ready-to-use HER2/Neu antibody (Rabbit monoclonal antibody, EP3 clone, PathnSitu, USA). Polymer-based technique with 3,3′-diaminobenzidine as the chromogen was used for the detection of the bound antibody (Leica Biosystems, Newcastle, UK).

The immunohistochemical expression of HER2/neu was scored according to the ASCO CAP 2018 guidelines for breast carcinoma from 0 to 3+. A score of 2+ was given for equivocal expression, and these cases were further subjected to FISH studies.

Fluorescent in situ hybridization

FISH was performed in cases with equivocal (2+) expression of HER2/neu on IHC. HER2/CEN17 dual-color probe kit (Kreatech ERBB2/CEN17 DC probe) was used. Formalin-fixed paraffin-embedded tissue sections of 5 μm thickness were used and an area in the IHC slide showing the strongest expression for HER2/neu in the maximum number of tumor cells was marked. The tissue from that area was lifted from the formalin-fixed paraffin blocks for the application of the probe. We evaluated 100 interphase cells in each case, and the ratio of ERBB2/CEP17 was calculated. Reporting was done as per the ASCO CAP 2018 HER2 testing by *in situ* hybridization guidelines.

Statistical analysis

All the cases were divided into two groups. Group 1 included muscle-invasive urothelial carcinomas. Group 2 included nonmuscle-invasive urothelial carcinomas. HER-2/neu positivity and other clinicopathological parameters were recorded for both the groups, and the *P* value was calculated using the Fisher's exact test. *P* value <0.05 was considered as statistically significant. Data analysis was performed by SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY, USA: IBM Corp.) software.

RESULTS

The present study included 89 cases of urothelial carcinoma comprising of 39 MIBC and 50 NMIBC. Most of the patients were in their sixth and seventh decades of life, with a male predominance (*n* = 80/89; 89.9%). There was no significant difference in the gender distribution between the two groups. 97% of the MIBCs (38/39) were high grade. Of the 50 NMIBCs, 23 were low-grade and 27 were high-grade tumors. The majority of the MIBC had pT2 stage (35/39; 89.7%) and perivesical soft-tissue extension was seen in three cases. Two of the 39 MIBCs showed pelvic lymph nodal metastases [Table 1].

On IHC [Table 2] for HER2/neu, 17.9% (7/39) of the MIBCs and 22% (11/50) of the NMIBCs showed a 3+ positivity. The mean age of HER2/neu-positive cases was 60 years in the MIBC group and 62 years in the NMIBC group. A statistically significant association between the age group and the HER2/neu status could not be found in either of

Table 1: Comparison of urothelial carcinomas with various clinicopathological parameters

Clinical Parameters	Muscle invasion	
	Invasive, *n* (%)	Noninvasive, *n* (%)
Sex		
Female	3 (7.7)	6 (12.0)
Male	36 (92.3)	44 (88.0)
Grade		
High grade	38 (97.4)	27 (54.0)
Low grade	1 (2.6)	23 (46.0)
Stage		
T1	0	50 (100.0)
T2	35 (89.7)	0
T3aN2	1 (2.6)	0
T4aNo	2 (5.1)	0
T4N2	1 (2.6)	0

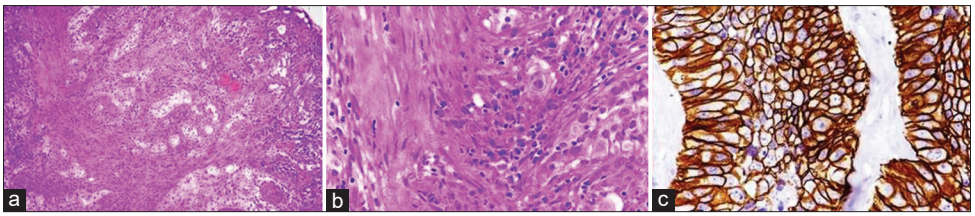

Figure 1: (a) High-grade muscle invasive urothelial carcinoma (H and E, ×100). (b) High power shows individual markedly pleomorphic tumor cells infiltrating into the deep muscle (H and E, ×400). (c) Complete, intense membranous staining (3+) of the tumor cells is seen with the antibody for Human epidermal growth factor receptor 2/neu (IHC, ×400)

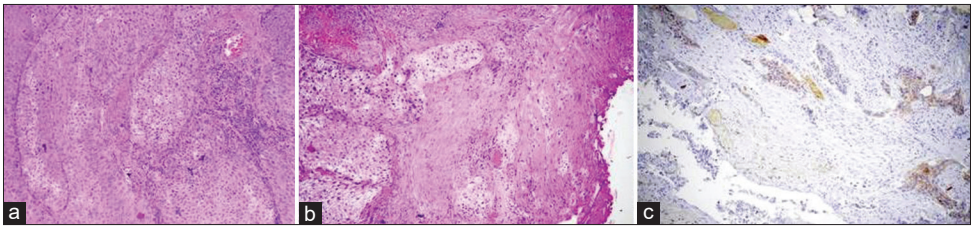

Figure 2: (a) Low-grade muscle invasive urothelial carcinoma (H and E, ×100). (b) Shows tumor cells infiltrating into the deep muscle (H and E, ×400). (c) Incomplete membranous staining in <10% of the tumor cells (1+) is seen with the antibody for human epidermal growth factor receptor 2/neu (IHC, ×100)

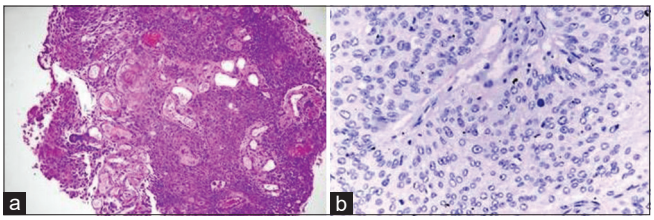

Figure 3: (a) Urothelial carcinoma with squamous differentiation (H and E, ×100). (b) No staining is seen with the antibody for Human epidermal growth factor receptor 2/neu (IHC, ×400)

Table 2: Distribution of human epidermal growth factor receptor-2/neu scoring immunohistochemistry

Score	Muscle invasive (*n*=39), *n* (%)	Nonmuscle invasive (*n*=50), *n* (%)
0	25 (64.1)	31 (62)
1+	4 (10.3)	6 (12)
2+	3 (7.7)	2 (4)
3+	7 (17.9)	11 (22)

the groups. In the MIBC group, all the 7 cases (100%) with a HER2/neu expression score of 3+ were males. Among the 11 cases which showed 3+ expression in the NMIBC group, 10 were males (90.9%). All the cases with positive HER2/neu expression among the muscle-invasive group were high-grade urothelial carcinomas [Figure 1]. Three cases with perivesical soft-tissue extension were immunonegative for Her2/neu. None of the low-grade tumors showed HER2/neu overexpression in this group [Figures 2 and 3]. In Group 2, 81.8% (9/11) of the cases were high-grade carcinomas. However, a significant correlation between the tumor grade and HER2/neu expression could not be established in the current study. Also, a significant correlation between HER2 expression and muscle invasions could not be established (P = 0.74, Fisher's exact test). Three cases of muscle-invasive (7.7%) and two cases (4%) of nonmuscle-invasive urothelial carcinomas showed equivocal (2+) expression and were subjected to FISH to evaluate for HER2 gene amplification [Figure 4]. None of the HER2 2+ tumors had HER2 gene amplification on FISH.

DISCUSSION

HER2/neu oncogene is located on chromosome 17q11-21 and codes for transmembrane growth factor receptors known as tyrosine kinase. Autophosphorylation of tyrosine kinase causes HER2/neu receptor activation which in turn leads to a cascade of reactions leading to activation of many intracellular proteins which in turn are responsible for cell proliferation, survival, and invasion.[5] Higher HER2/neu positivity has a proven prognostic role in patients with breast and gastroesophageal cancers.[6] The intensity and the pattern of staining determines the scoring of the expression pattern on IHC. *In situ* hybridization is utilised as a second-level technique in those with equivocal results.[7] Over the years, HER2/neu status has also been evaluated in patients with urothelial carcinomas and recently, promising results have been shown in clinical drug trials evaluating ADCs targeting the HER2/neu.[8]

HER2/neu status in urothelial carcinomas is a subject of interest for many pathologists and clinicians alike. However, the wide variability in the reported expression patterns of HER2/neu in the various studies makes it is difficult to determine whether HER2/neu has a significant contribution in the management of urothelial carcinomas or not.[9] In the Indian population, Kumar *et al*.[10] showed that 28/40 cases (70%) of muscle-invasive urothelial cancer had HER2/neu overexpression by IHC. Skagias *et al*.[11] showed that HER2 protein was overexpressed in 41/80 patients (51.25%) by IHC, demonstrating that advanced staged carcinomas had a higher expression of HER2/neu;

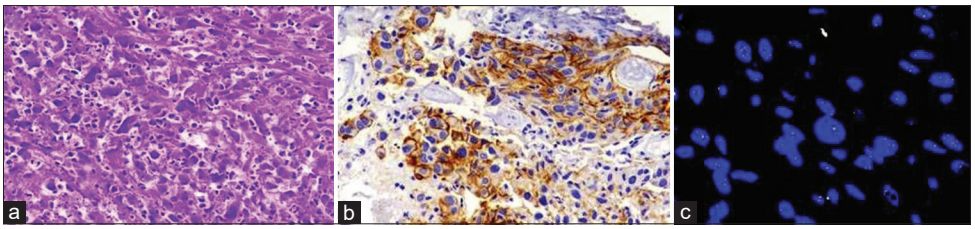

Figure 4: (a) Urothelial carcinoma with sarcomatoid differentiation (H and E, ×400). (b) Complete, membranous staining in <10% of the tumor cells (2+) is seen with the antibody for HER2/neu (IHC, ×400). (c) Fluorescent *in situ* hybridization with dual probe assay does not show any amplification of signals (probe used, human epidermal growth factor receptor 2/neu gene orange, chromosome 17 green). FISH: Fluorescent *in situ* hybridization

tumor stage (P = 0.032) and tumor grade (P = 0.0001). Jimenez *et al*.[12] found overexpression of HER2/neu by IHC in 28% (22/80) of the muscle-invasive urothelial carcinomas. Kolla *et al*.[13] showed HER2/neu overexpression in 55.6% of the muscle-invasive urothelial carcinomas. Agrawal *et al*., in 2020, showed HER2/neu overexpression in 9/25 (36%) cases of muscle-invasive urothelial carcinomas by IHC in the Indian population.[14] In the present study, 7 (17.9%) specimens of MIBC showed 3+ HER2/neu positivity. Ethnicity and geographical location of the patient subsets may have a role in explaining the difference in the HER2/neu expression noted in the present study. Besides, the use of different antibody clones for IHC, and the variations in the technique of the procedure may also be a contributing factor. Larger multicenter studies are required to overcome such limitations.

Among NMIBC, 22% (11/50) of the specimens showed 3+ HER2/neu positivity, however, a significant correlation between tumor grade and HER2/neu expression could not be established in the current study. A previous study by Pietzak *et al*. used next-generation sequencing analysis on specimens of nonmuscle-invasive urothelial carcinoma and showed that the higher-grade tumors were consistently enriched with HER2/neu.[15] In our study, five cases (from both the groups) with equivocal HER2/neu expression on IHC were subjected to dual probe assay by FISH which did not show any amplification. This is similar to the study by Agarwal *et al*.,[14] wherein none of the 2+ cases in their study showed HER2/neu amplification.

Even though the proposed molecular classification focuses only on MIBCs, HER2/neu is a potential marker to be included in the antibody panel for molecular subtyping of urothelial carcinomas. Furthermore, it can be used as a biomarker of circulating tumor cells and can be assessed through liquid biopsies in advanced cases. To better understand the role of this biomarker in newer treatments such as antibody drug combinations, urothelial carcinoma specific criterias for defining HER2/neu positivity are required, as currently, the guidelines developed for breast and gastric cancers are being used for urothelial carcinomas also.

There are no separate guidelines for grading HER2/neu expression in urothelial carcinomas; hence, the grading system recommended for breast cancer was relied on, which was a limitation of this study and another limiting factor was the low caseload.

CONCLUSION

Strong immunohistochemical expression of HER-2/neu was observed in 17.9% of MIBC and 22% of NMIBC. Nevertheless, HER-2/neu overexpression did not show a significant correlation with conventional clinicopathologic parameters and muscle invasion.

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