Original Article

Overexpression of Human Epidermal Growth Factor Receptor 2 Protein in Urothelial Carcinoma of the Urinary Bladder in Ibadan: A Single-Institutional Experience

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Abstract

Background: Overexpression of human epidermal growth factor receptor 2 (HER-2) protein has been shown to have both prognostic and therapeutic values in several malignancies including urothelial carcinoma of urinary bladder (UCB). **Aims:** This study aimed to determine HER-2 protein overexpression and evaluate its correlation with clinico-pathological parameters in UCB. **Materials and Methods:** This was a descriptive-analytical study involving the immunohistochemical review of all histologically diagnosed urinary bladder malignancy in the Department of Pathology, University College Hospital, Ibadan, between January 2002 and December 2016. Urinary bladder malignancies whose tissue blocks could not be found and/or demographic data were not available were excluded. Immunohistochemistry analysis was done using rabbit anti-HER-2 antibody (Biocare) and American Society of Clinical Oncology/College of American Pathologists guidelines established for breast cancer were used for HER-2 status scoring. The statistical analysis was carried out usingthe Statistical Package for the Social Sciences, version 22 (SPSS Inc, Chicago, Illinois, USA). **Results:** HER-2 overexpression was found in four (6.3%) cases. All the HER-2-positive cases were males. One (2.9%) of the 35 high-grade UCB showed positive HER-2 overexpression, whereas three (10.7%) of the 28 low-grade tumor were positive for HER-2 protein overexpression.There was no statistically significant association between HER-2 protein overexpression and histological grades of UCB and muscle invasion by the tumor. **Conclusion:** Our study demonstrated the low percentage of HER-2 overexpression in UCB with no significant association with tumor grades and muscle invasion. Larger samples involving multiple centers can provide more robust information and further evaluate HER-2 overexpression in urothelial carcinoma in our environment.

Keywords: Human epidermal growth factor receptor 2 overexpression, Ibadan, immunohistochemistry, urothelial carcinoma of urinary bladder

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Introduction

Urinary bladder carcinoma is a common malignancy of the genitourinary tract. It is said to be the 10th most common cancer worldwide, accounting for 3.0% of all cancers, while being the 8th most common cancer in men, it is the 19th most common cancer in women.[1]. It accounts for the 11th and 18th cause of death globally with the mortality incidence of 3.2/10,000 and 0.9/100,000 among males and females, respectively.[1]

Among the different histological subtypes of urinary bladder malignancies, urothelial carcinomas of urinary bladder (UCB) account for a higher proportion.[2,3] These tumors can be classified into nonmuscle invasive (80%) and muscle-invasive (20%) types. The nonmuscle invasive UCB is associated with a recurrence rate of 50%–70% and 10%–15% of patients will progress to muscle-invasive disease over a 5-year period. The muscle-invasive types have higher recurrence rate (over 90%) and metastatic potential.

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Once UCB metastasizes, therapeutic options are limited and systemic chemotherapy provides only a small improvement in cancer-specific survival. There is increasing significant interest in targeted therapies for metastatic UCB. One of these important targets is human epidermal growth factor receptor 2 (HER-2) protein.[4]

HER-2 gene amplification and/or HER-2 protein overexpression have been long associated with tumors of solid organs including the breast. It has been demonstrated that HER-2 plays a role in the pathogenesis and prognosis of UCB.[5] The success of trastuzumab therapy in breast cancer has stimulated interest in exploring the potentiality of using this therapy for patients with urinary bladder carcinoma, and several studies including case reports have documented improved clinical outcomes with HER 2-targeted therapy.[4,5]

A few large studies have analyzed HER-2 overexpression in invasive UCB.[6] Most of the studies conducted supported the overexpression of HER-2 as a poor prognostic factor. Significant association between HER-2 overexpression and UCB recurrence and nodal metastasis has been reported.[7-9] Importance of HER-2 in UCB can then be summarized as prognostic and therapeutic values.

HER-2 status has been shown to vary between different ethnic or geographic populations and raises the hypothesis of a significant etiologic heterogeneity within UCB.[5,10,11] No known local study in our environment on the pattern of HER-2 overexpression in UCB. In this study, we used immunohistochemistry to determine HER-2 protein overexpression in UCB and to investigate its correlation with some clinicopathological parameters.

Materials and Methods

This was a descriptive-analytical study of all histologically diagnosed urinary bladder malignancy in the Department of Pathology, University College Hospital (UCH) Ibadan, between January 2002 and December 2016. It was approved by Joint Institutional Review Board of University of Ibadan/UCH, Ibadan. Cases included in this study were all histologically diagnosed urinary bladder malignancies within the study period having complete clinicopathological information. Demographic and clinical data of all the cases were extracted from the records and files of the department.

Hematoxylin and eosin (H and E)-stained sections of cases of bladder malignancies were reviewed independently by two histopathologists to confirm the diagnosis, assess for muscle invasion and the grade of the tumor using World Health Organization guidelines. Muscle invasion was assessed in cystectomy specimens and biopsies with muscularis propria. The formalin-fixed paraffin-embedded tissue blocks of all cases of UCB that met criteria for immunohistochemistry were selected. The inclusion criteria included the presence of muscularis propria and adequate tissue for immunohistochemistry. The test was performed using rabbit anti-HER-2 antibody (Biocare) clone erb-2, reference number ACA342B, produced by Biocare Medical CA, USA at 1:100 dilution. The slides were also reviewed by the two pathologists. Known HER2-positive breast cancer tissue was stained for positive controls.

In accordance to previous HER-2 studies on UCB, the established American Society of Clinical Oncology/College of American Pathologists (ASCO-CAP) guidelines for HER-2 semiquantitative report in breast cancer was adopted for this study.[6] The level of HER-2 protein overexpression was assessed by evaluating only membranous staining. The semi-quantitation of the intensity and percentage of staining was done, and the score was on a scale of 0–3+. A score of 0 was defined as nonstaining or faint and incomplete staining in ≤10% of tumor cell, 1+ was defined as barely perceptible membrane staining in >10% of tumor cells, a score of 2 + was weak-to-moderate complete membrane staining present in >10% of tumor cells or complete and circumferential intense membrane staining ≤10% and a score of 3+ was regarded as strong complete membrane staining in >10% of tumor cells. A cytoplasmic or nuclear staining was considered nonspecific. Scores of 0 and 1+, 2+, and 3+ were categorized as negative, equivocal and positive HER-2 staining accordingly. Cases with score of 2+ would be validated by florescent *in situ* hybridization.[6,10,12]

Analysis was done using the Statistical Package for the Social Sciences, version 22 (SPSS Inc, Chicago, Illinois, USA). The associations between the variables were assessed by the Pearson's Chi-square test. Statistical significance was reached if a $P < 0.05$ was obtained.

Results

One hundred and forty urinary bladder malignancies were diagnosed at the Department of Pathology, UCH, Ibadan, between January 1, 2002 and December 31, 2016, of which urothelial carcinoma (UCB) constituted 111 (79.3%) cases, 14 (10%) were adenocarcinoma, and 13 (9.3%) were squamous cell carcinoma. There were two (1.4%) cases of embryonal rhabdomyosarcoma. Only 63 cases of the 111 UCB cases met the inclusion criteria for immunohistochemical evaluation of HER 2 protein [Table 1].

A total of 59 (93.7%) of the 63 UCB cases analyzed showed complete lack of staining corresponding to score 0, while 4 showed highly intense and complete membrane staining in ≥10% tumor cells corresponding to score 3+, giving the HER-2 overexpression percentage score of 6.3%. There was no case showing faint or incomplete staining [Tables 2 and 3, Figure 1]. All the HER-2 positive cases were males, although this was not statistically significant (P = 0.148). Only one (12.9%) of the 35 high-grade UCB showed positive HER-2 overexpression, while three (10.7%) of the 28 low-grade tumor were positive for HER-2 protein overexpression. There was no statistically significant association between HER-2 protein expression and histological grade of the tumor (P = 0.078). Three (7.1%)

Table 1: Gender distribution and its correlation with histological subtypes of urinary bladder malignancies

Histological types	Sex			*P*
	Female	Male	Total	
Adenocarcinoma	5 (3.6)	9 (6.4)	14 (10)	0.274
Embryonal rhabdomyosarcoma	0 (0.0)	2 (1.4)	2 (1.4)	
Squamous cell carcinoma	4 (2.9)	9 (6.4)	13 (9.3)	
Urothelial carcinoma	25 (17.9)	86 (61.4)	111 (79.3)	
Total	34 (24.4)	106 (75.6)	140 (100)	

$P<0.05$

Table 2: Human epidermal growth factor receptor-2 immunohistochemical status in urothelial carcinoma of urinary bladder

HER-2 overexpression	Frequency (%)
Positive	4 (6.3)
Negative	59 (93.7)
Total	63 (100)

Table 3: Comparison of index study with previous studies on human epidermal growth factor receptor-2 overexpression

Studies' author	Year of study	Number of cases	Percentage HER-2 Overexpression (%)
Gardmark *et al*.	2005	90	80
Bolenz *et al*.	2010	198	27.8
Shawk *et al*	2013	32	62.5
Bellmont *et al*.	2015	111	22
(2 cohorts)		102	4
Lim *et al*.	2015	141	4.3
Goodman *et al*.	2016	27	74
Ochi *et al*.	2017	103	11.7
Index study	2019	63	6.3

of the noninvasive tumors showed HER-2 positivity while one (4.8%) of the invasive tumors showed similar finding. There was also no statistically significant association between HER-2 protein overexpression and muscle invasion by the tumour ($P = 0.417$) [Table 4].

Of the 111 UCB, 57 were high-grade tumors and 54 were low-grade tumors. While a higher proportion of males were low grades, about two-thirds of females were high grade. The overall male-to-female ratio was 3.44:1. There was no statistically significant association between gender and the histological grade ($P = 0.666$). Twenty (95.2%) of the 21 urothelial tumors that showed muscle invasion were high grade while about two-third of non-invasive tumors were low grade. There was statistically significant association between histological grade and muscle invasion by tumor ($P = 0.000$) [Table 5]. The age of patients with UCB ranged from 24 years to 91 years, with an overall mean age of 58.69 ± 17.79 years [Figure 2].

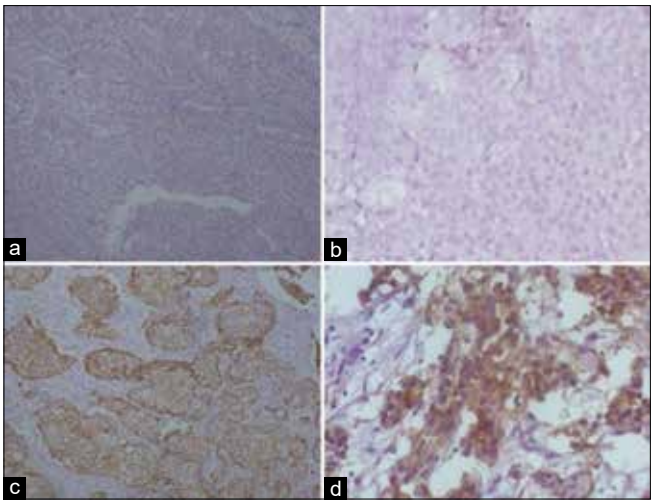

Figure 1: (a) Immunohistochemical staining of urothelial carcinoma of urinary bladder showing complete negative staining for human epidermal growth factor receptor 2 protein expression (×40), (b) immunohistochemical staining of urothelial carcinoma of urinary bladder showing complete negative staining for human epidermal growth factor receptor 2 protein expression (×100), (c) immunohistochemical staining of malignant mammary epithelial cells showing strong and complete membrane staining for human epidermal growth factor receptor 2 protein overexpression (control) (×100), (d) Immunohistochemical staining of the urothelial carcinoma of urinary bladder showing strong and complete membrane staining for human epidermal growth factor receptor 2 protein overexpression in >10% of the tumour population (×100)

Discussion

The guideline for HER-2 identification in breast cancers are well established according to the ASCO-CAP scoring guidelines, and this has been adopted for UCB.[13] Four (6.3%) of the 63 cases of UCB in this study showed strong expression (3+) in >10% of tumor cells and 59 (93.7%) showed no expression. All the 4 positive cases occurred exclusively in males. There were no local studies available on this subject to make comparison. There have been wide variations in the frequency of HER-2 overexpression in UCB, and it was observed that this variation is one of the highest in all human cancers.[14]

Several international studies over the years have observed varying the frequency of HER-2 overexpression. The number of cases that have been used in studies ranged from 27 to 198 and the percentage of HER-2 overexpression varied from as low as 4% to as high as 80%.[7,6,10,13,15,16] Although both the number of cases used in this study and the frequency of HER-2 overexpression were low, it was still within the values documented from previous studies.

The wide variations in the percentage of HER-2 overexpression in UCB have been attributed to a number of factors. Among these are the differences in the stages of tumor and lack of clear guidelines for assessing HER-2 status in urinary bladder cancer. This is because some studies have observed that higher stage tumors tend to stain more positively for HER-2 protein.[13]

There was no association between HER-2 overexpression and gender in this study as observed in another study.[17]

Table 4: Correlation of human epidermal growth factor receptor-2 overexpression with gender, histological types, histological grade of urothelial carcinoma of urinary bladder, and muscle invasion

	HER-2 overexpression			*P*
	Positive (%)	Negative (%)	Total	
Gender				
Female	0 (0)	17 (100)	17	0.148
Male	4 (8.7)	42 (91.3)	46	
UCB grade				
Low	3 (10.7)	25 (89.3)	28	0.078
High	1 (2.9)	34 (97.1)	35	
Muscle invasion				
Positive	1 (4.8)	20 (95.2)	21	0.417
Negative	3 (7.1)	39 (92.9)	42	

P<0.05. UCB: Urothelial carcinoma of urinary bladder, HER-2: Human epidermal growth factor receptor-2

Table 5: Correlation of histological grade of urothelial carcinoma with gender and muscle invasion

	Histological grade of UCB			*P*
	Low (%)	High (%)	Total	
Gender				
Male	45 (51.8)	41 (41.2)	86	0.666
Female	9 (36)	16 (64)	25	
Muscle invasion				
Positive	1 (4.8)	20 (95.2)	21	0.000*
Negative	27 (64.3)	15 (35.7)	42	

P<0.05. *Statistical significance. UCB: Urothelial carcinoma of urinary bladder

Low-grade tumors show higher positivity in our study. While only one (2.9%) of the high-grade tumors showed HER-2 overexpression, three (10.7%) of the low grade tumors showed HER-2 overexpression with no statistically significant association between percentage of HER-2 positivity and UCB grade. This lack of association between HER-2 and UCB grade has been documented in other studies.[18,19] However, most studies have shown that HER-2 overexpression significantly correlated well with tumor grade where higher percentage of HER-2 positivity occurred more in high-grade tumors.[9,20] These differences in other studies have been attributed to heterogeneity between antibodies, protocols, and the interpretations of HER-2 protein expression.[9]

Of the four cases of HER-2-positive cases in this study, 3 were non-muscle invasive (7.1% of all non-invasive), while 1 was muscle invasive (4.8% of all invasive) with no statistically significant association between HER-2 overexpression and muscle invasion. This was in contrast to what was obtained in most studies where higher HER-2 overexpression was significantly correlated with invasive UCB.[21,22] There was no possible explanation that could be adduced to this finding at the time of this study.

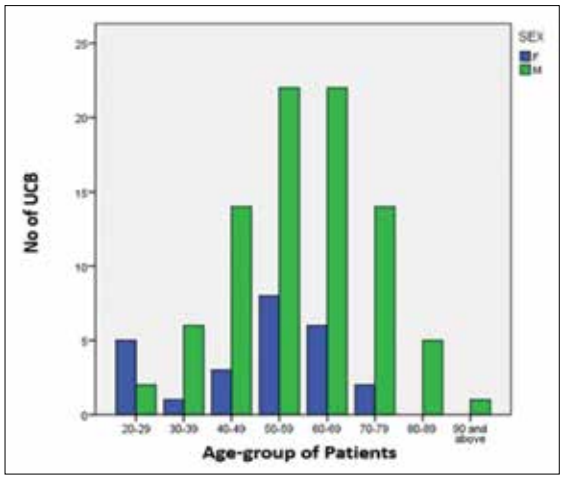

Figure 2: Clustered bar chart showing the age-gender distribution of urothelial carcinoma of urinary bladder

The age of patients with UCB ranged from the third decade to tenth decade. The peak age for females was in the sixth decade of life while there was bimodal peak ages in the males with the UCB most occurring in the sixth and seventh decades as earlier documented in literature.[23] There was no statistically significant association between age and UCB.

In our study, high-grade tumors relatively occurred more in females than in males with no statistically significant association. It was observed in a study that though there was a higher incidence of UCB in male, the prognosis was worse in females as high-grade UCB which portend worse prognosis occur relatively more in females than in male, as also observed in our study.[23] Almost all the UCB that showed muscle invasion (95.2%) were high-grade tumors with statistically significant association between histological grades and muscle invasion as previously documented in the literature.[24,25]

Conclusion

The percentage of HER-2 overexpression in UCB in this study was 6.3%. There was no statistically significant association between HER-2 overexpression and gender, grade of UCB and muscle invasion. Muscle invasion correlated well with high-grade UCB. With lack of similar local study prior to ours, further, possibly multicentre studies with larger sample size, possibly is advised to further evaluate HER-2 overexpression in urothelial carcinoma in our environment.

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Conflicts of interest

There are no conflicts of interest.

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