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ORIGINAL ARTICLE

The prognostic significance of HER2 expression in urothelial carcinoma

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Abstract

Introduction: Urothelial carcinoma poses a great challenge in disease management due to the high recurrence rate and a greater likelihood of disease progression. HER2 (human epidermal growth factor receptor 2) is one of the proteins variably expressed in urothelial carcinoma, prompting its investigation as a potential predictive marker. The aim of this study was to assess the HER2 status in urothelial carcinoma, its correlation with tumour grade, tumour stage, recurrence and progression. *Materials and Methods:* We retrospectively analysed 69 specimens of transurethral resection or cystectomy in patients with urothelial carcinoma. Immunohistochemistry for HER2 was performed and the expressions were correlated with tumour grade, tumour stage, presence of recurrence and tumour progression. Staining was evaluated according to the same criteria of breast cancer. Scores of 2+ and 3+ were considered positive. The data were analysed using the chi-square test with statistical significance set at $P < 0.05$. *Results:* Positive HER2 expression was found in 13 cases (18.8%). HER2 positivity was significantly associated with high-grade tumours ($P=0.005$). However, there is no significant association with tumour stage, recurrence or progression. *Conclusion:* HER2 is potentially a good immunohistochemical marker for identifying patients with higher-grade urothelial carcinoma and stratifying patients for future targeted therapy.

Keywords: HER2; immunohistochemistry; urothelial carcinoma; prognosis factor

INTRODUCTION

Bladder cancer, which constitutes one of the most common urological conditions, places a huge burden on the health system as it is the tenth most common cancer with 549,000 new cases worldwide.[1] In 2007, it was listed as the ninth most common cancer among Malaysian males with an incidence rate of 3.2%.[2] Urothelial carcinoma is the most common type of bladder cancer with the majority of them being non-invasive or early invasive (non-muscularis-propria invasive) at diagnosis. It is known to have a high recurrence rate with no reliable parameter predicting the risk of recurrence or progression.[3]

Nevertheless, clinical and morphological features have been associated with prognosis and predictive outcomes of urothelial carcinoma.[4] These include grade, stage, angiolymphatic invasion, variant histology, time to recurrence, response to intravesical therapy and early radical cystectomy. The more recent and ongoing revelation in molecular pathways of urothelial carcinoma has led to the identification of prognostic molecular and biomarkers among which HER2/neu is of interest.

HER2/neu (C-erbB-2), which is a member of the epidermal growth factor receptor (EGFR) family, is responsible for cell growth and proliferation by activating the tyrosine kinase pathway. A few studies have shown that HER2 over-expression is exhibited in tumour tissues of breast, colon, gastric, lung and bladder cancer.[5,6] It is known as a useful prognostic and therapeutic marker in breast cancer as well as advanced gastric cancer. A number of studies are now looking at HER2 status in urothelial carcinoma as an anticipated marker due to its potential as target for therapy and a predictive biomarker for disease progression. The modalities that

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have been used to study HER2 expression in bladder cancers include immunohistochemistry, fluorescence in situ hybridization, polymerase chain reaction and mutational analysis.[7,8]

Current treatment of urothelial carcinoma includes chemotherapy and immunotherapy. Urothelial carcinoma is a chemosensitive neoplasm but only a subset responds to current adjuvant immunotherapy, bacille Calmette-Guerin (BCG). One study demonstrated that HER2 negative urothelial carcinomas are less susceptible to recurrence or progression following adjuvant BCG.[9] Novel HER2 directed agents, either alone or in combination, are currently under investigation, making HER2 a potential predictive marker in selecting potential patients.[10-13] With more research looking into anti-HER2 as a treatment modality in urothelial carcinoma, it is imperative to collect data on HER2 expression and its correlation with the clinical or pathological features of urothelial carcinoma in Malaysia. The aim of this study was to assess the expression of HER2 in urothelial carcinoma and its association with tumour grade, stage and recurrence.

MATERIALS AND METHODS

Tissue samples

A total of 69 cases of primary urothelial carcinoma (biopsies and cystectomies specimen) from 2012 to 2017 were retrieved from the archives of the Histopathology Unit, Department of Pathology (Universiti Kebangsaan Malaysia Medical Centre, Cheras). This study was approved by the Universiti Kebangsaan Malaysia Research Ethics Committee (Reference number: JEP-2017-063). Clinicopathological parameters including grade, stage, recurrence, progression as well as receipt of chemotherapy/immunotherapy were reviewed from the patients' medical records, which included radiological findings for staging purposes. Cases with tumour other than urothelial carcinoma, cases of recurrence and prior chemotherapy were excluded from this study.

Definition and diagnostic criteria

Grade and stage were determined according to the World Health Organization (WHO) classification and TNM staging system.[4] Recurrence was defined as the occurrence of a new tumour that is confirmed by biopsy three months after the first transurethral resection of bladder tumour, during follow-up.[14] Progression was defined as recurrence with invasion into the muscularis propriae or beyond or distant metastasis, and / or death due to the disease, during follow-up.[14]

Immunohistochemical Studies

HER2 immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tissue whole section slides using DAKO anti-HER2/neu (Code A0485, rabbit polyclonal; pre-dilution; Dako Denmark) antibody and its kit (EnVision FLEX Mini Kit High pH (Dako AS Plus) on an automatic immunostainer (BenchMark XT, Ventana Medical Systems), according to the manufacturer's instructions. Primary antibody omission was used for the negative control and breast cancer tissues with 3 different scoring (3 +, 2 + and 1 +) was used as the positive control.

Immunohistochemical analysis

IHC scoring was independently performed by two observers (one pathologist and one trainee) without prior knowledge of clinicopathological information. Whenever there was a discordant result, the slides were reviewed together and a consensus was agreed upon. The scoring was semi-quantitatively analysed based on the 2013 American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAPs) guidelines for scoring HER2 in breast cancer.[15,16] The 4 categories of scoring were as follows: 0, no staining; 1 +, incomplete membrane staining that is faint/barely perceptible in >10% of tumour cells; 2 +, incomplete and/or weak/moderate membrane staining in >10% of tumour cells; 3 +, circumferential, intense, complete membrane staining >10% of tumour cells (Figure 1). HER2 IHC score of either 2 + or 3 + was considered positive while negative HER2 expression were cases with HER2 IHC score of either 0 or 1 +.[17]

Statistical analysis

All data collected were tabulated accordingly and analysed using Statistical Packaged for the Social Science, (SPSS) software version 20 (IBM Corp., Armonk, NY, USA). The result was analysed statistically using the Chi-square test to test the significance between categorical variables. The p-value of <0.05 was accepted as statistically significant.

RESULTS

Clinicopathologic characteristics

A total of 69 cases of urothelial carcinoma were included in the study, comprising of 56 men (81.2%) and 13 women (18.8%) with a median

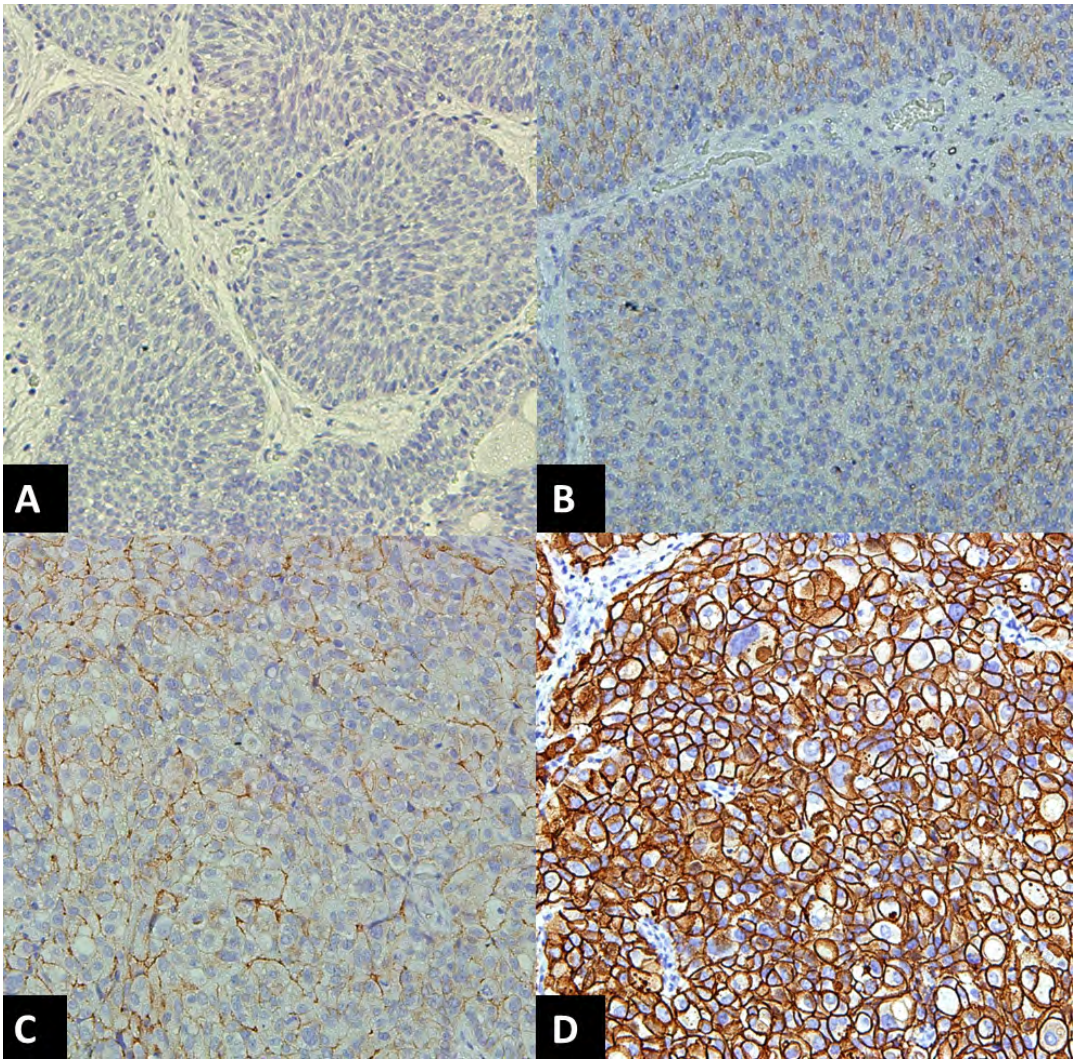

FIG. 1: Urothelial carcinomas with A. HER2 score 0 (x200), B. HER2 score 1+ (x200), C. HER score 2+ (x200) and D. HER2 score 3+ (x200)

age of 68 years (range 20 to 89 years) (Table 1). There were 35 cases (50.7%) of low-grade tumours and 34 cases (49.3%) of high-grade tumours. Pathologic stage consisted of pTa in 18 patients (26.1%), pT1 in 38 patients (55.1%), pT2 in 11 patients (15.9%) and pT3 in 2 patients (2.9%). Tumour recurrence was found in 52 patients (75.4%) while tumour progression in 16 patients (23.2%).

Immunohistochemical studies of HER2 expression
The majority of cases showed a negative HER2 expression (56 out of 69 cases; 81.2%) with positive HER2 expression (score 2+ and 3+) only seen in 13 out 69 cases (18.8%). The correlation between HER2 expression and clinicopathological features were illustrated in Table 2. HER2 was more prevalent in the high-grade tumours (11 out of 34 cases; 16.0%) as compared to the low-grade tumours (2 out of 35 cases; 2.9%). HER2 positivity was significantly associated with high-grade tumours ($P = 0.005$). There was no association found between HER2 expression and gender ($P = 0.045$), age ($P = 0.154$), pathological stage ($P = 0.615$), recurrence ($P = 0.419$) or progression ($P = 0.472$).

DISCUSSION

Urothelial carcinoma has a worldwide regional variation in terms of incidence and mortality.[1,3] A few studies conducted in the Asian region have demonstrated that bladder cancer incidence is highest among male with age at presentation between 60-65 years old with urothelial

Table 1: Clinicopathologic characteristics of 69 patients with urothelial carcinoma

Characteristics	No. of cases (%)
Gender	
Male	56 (81.2%)
Female	13 (18.8%)
Age	
Range	20 to 89 years
Mean	65.9 years
Median	68 years
Tumour grade	
Low grade	35 (50.7%)
High grade	34 (49.3%)
Tumour stage	
Ta	18 (26.1%)
T1	38 (55.1%)
T2	11 (15.9%)
T3	2 (2.9%)
Recurrence	
Absent	11 (15.9%)
Present	52 (75.4%)
Unknown	6 (8.7%)
Progression / Metastasis	
Absent	53 (76.8%)
Present	16 (23.2%)

carcinoma being the most common histological type.[18-20] Our findings exhibited similar clinicopathologic characteristics.

Histopathology is the gold standard for diagnosing and staging of urothelial carcinoma. Based on the WHO classification, invasion to sub-epithelial connective tissue is considered as pT1 while muscularis propria invasion is pT2.[4] Even though the majority of urothelial carcinomas are non-muscularis propria-invasive when first diagnosed (pT1), the high recurrence rate and unpredictable tumour progression poses a challenge to clinicians in terms of treatment management.[3,4] The current study also demonstrated that the majority of cases are those in the pT1 stage, which are non-muscularis propria-invasive.

The management of urothelial carcinoma relies heavily on clinicopathological parameters such as grade, stage and history of prior recurrence. These parameters have been shown to assist in prognostic assessment.[21] As mentioned earlier, patients' outcome is unpredictable despite having similar grade or stage.[22] The difficulty in predicting which patient would recur, progress to later-stage or respond to available therapy has led to studies for prognostic biomarkers, in which HER2 has emerged as a key player.

Table 2: Correlation between Her2/neu protein expression patterns and clinicopathological features of urothelial carcinoma

Features	No. of cases (%)	HER2 expression		*P* value
		0/1+	2+/3+	
Gender				0.045
Male	56 (81.2%)	48 (69.6%)	8 (11.6%)	
Female	13 (18.8%)	8 (11.6%)	5 (7.2%)	
Tumour grade				0.005
High grade	34 (49.3%)	23 (33.3%)	11 (16.0%)	
Low grade	35 (50.7%)	33 (47.8%)	2 (2.9%)	
pT stage				0.615
Ta	18 (26.1%)	16 (23.2%)	2 (2.9%)	
T1	38 (55.1%)	30 (43.5%)	8 (11.6%)	
T2	11 (15.9%)	8 (11.6%)	3 (4.3%)	
T3	2 (2.9%)	2 (2.9%)	0	
Recurrence				0.419
Absent	11 (15.9%)	8 (11.6%)	3 (4.3%)	
Present	52 (75.4%)	44 (63.8%)	8 (11.6%)	
Unknown	6 (8.7%)	4 (5.8%)	2 (2.9%)	
Progression / Metastasis				0.472
Absent	53 (76.8%)	44 (63.8%)	9 (13.0%)	
Present	16 (23.2%)	12 (17.4%)	4 (5.8%)	

This is largely due to the benefit that HER2-positive breast carcinoma patients received from HER2-targeted therapy as well as the current development of anti-HER2 therapy for urothelial carcinoma.

Studies have shown HER2 as one of the proteins that are heterogeneously expressed in urothelial carcinoma. HER2 status in urothelial carcinoma has been reported to vary from 9% to 80% in regards to protein overexpression and 0% to 32% in relation to gene amplification.[14] HER2 protein overexpression was only present in 18.8% of our studied population. The varying levels of HER2 expression presented in different studies may be attributed to urothelial carcinoma heterogeneity and other confounding variables such as type of specimen (biopsies versus cystectomies, primary versus metastatic lesion), different histological type, different antibodies and protocols in immunohistochemical staining, method of assessment (whole sections versus tissue microarray), stage of the disease (superficial versus muscle-invasive) as well as subjective scoring protocols with ambiguous cut-off values.[23-26]

Nevertheless, many studies have shown promising results supporting the role of HER2 expression as a prognostic marker for urothelial carcinoma. One study, comprising of 198 samples, found a significant correlation between HER2 expression with lymphovascular invasion.[27] Other studies had also revealed a significant correlation with primary and metastatic tumour,[11] tumour grade and tumour stage.[9,28,29] Furthermore, a good prognostic value of HER2 was demonstrated in a recent systematic review and meta-analysis.[30] Despite similar significant correlation with disease progression and overall survival,[15,22,27,31] contrary results were seen in a few other studies.[32,33] Our cohort exhibited a correlation between positive HER2 expression only with higher tumour grade, but not with tumour stage, risk of recurrence or progression. The selected characteristics of HER2 and its association with predictor and prognostic parameters in recent and the present study have been summarised in Table 3.

Evaluation of HER2 is not only dependent on the immunohistochemical study with many studies proceeding with further HER2 gene amplification study via FISH/CISH or PCR FISH for borderline positive IHC cases (cases with a score of 2 +). One study showed higher rate of amplified cases were found at ISH compared to IHC, thus concluding immunohistochemistry alone may underestimate the number of amplified cases.[24] The limitation of our study is that we did not perform any molecular studies to assess concordance with gene amplification due to financial constraint.

In the absence of standardised HER2 scoring in urothelial carcinoma, our study along with many other urothelial carcinoma studies adopted the widely used HER2 evaluation for breast cancer.[15,22] However, there still are inconsistencies among studies in terms of cut-off value for the percentage of tumour cells with HER2 positivity.[14,22,27,32] This lack of definition on overexpression may contribute to the variable correlation of HER2 expression and the pathologic parameters. An agreed-upon definition of HER2 positivity as well as the best method to evaluate HER2 in urothelial carcinoma should be established prior to its study as a prognostic marker.

CONCLUSION

In conclusion, our study parallels with most studies in terms of urothelial carcinoma demography and HER2 protein expression in high-grade tumours. HER2 expression might provide additional prognostic information for patients with urothelial carcinoma. A consensus of HER immunohistochemistry scoring algorithm is pivotal to ensure it is a good first line biomarker to determine subsequent patient selection for molecular studies and eventually patient selection for targeted treatment.

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Availability of data and materials

The raw data of the clinical demographics and HER2 immunohistochemistry results are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: The ethical approval was obtained from the Institutional Review Board, Universiti Kebangsaan Malaysia Medical Research Ethics

Table 3: Characteristics of selected recent studies of HER2 expression in urothelial carcinoma and its association with important predictor or prognostic parameters

References	No of cases (M/F)	Age	Methods	HER2 IHC positive rates (cut-off value)	HER2 correlation with parameters
Bolenz *et al*. (2010)	198 (156/42)	NR	IHC	27.8% (10%)	LVI (P = 0.026), Recurrence (P = 0.003) Mortality (P = 0.004)
Lae *et al*. (2010)	1005	NR	IHC/ FISH	9.2% (10 to 30%)	NR
El Gehani (2012)	39 (33/6)	64.5 (mean)	IHC	59%	Tumour stage (P = 0.011)
Olsson *et al*. (2012)	201 (167/34)	NR	IHC	12.4% (30%)	Not associated with recurrence or progression
Hegazy *et al*. (2015)	88	NR	IHC	NR (20%)	Tumour grade (P = 0.025) Recurrence and progression (P = 0.025)
Lim *et al*. (2015)	141 (122/19)	68.9 (mean)	IHC/ FISH	4.3% (50%)	PFS (P = 0.031)
Hammam *et al*. (2015)	33 (23/10)	NR	IHC/ FISH	27%	Tumour grade (P <0.01) Tumour stage (P <0.01)
Millis *et al*. (2015)	441	NR	IHC/ FISH/ mutational analysis	10% (10%)	NR
Nedjadi *et al*. (2016)	160 (133/27)	70 patients younger than 60	IHC/ BDISH	60%	Tumour stage (P = 0.002), Tumour grade (P = 0.03), Lymph node metastasis (P = 0.04) DSS (P = 0.02)
El Ochi *et al*. (2017)	103 (96/7)	63 (mean)	IHC	11.7%	Tumour grade (P = 0.003) Tumour stage (P =0.015)
Moktefi *et al*. (2018)	188 (157/31)	62 (mean)	IHC/ FISH	56%	10-year OS (P = 0.24) Worse outcome associated with positive cases
Agrawal *et al*. (2020)	93	NR	IHC/ FISH	29% (10 to 50%)	PFS (P <0.05)
Franceschini *et al*. (2020)	61 (50/11)	72	IHC/ CISH/ FISH	6% (10%)	NR
Moustakas *et al*. (2020)	48 (45/3)	68	IHC	12.5% at diagnosis 25% post intravesical therapy (10%)	Recurrence free survival (P = 0.02)
Grigg *et al*. (2021)	149 (122/27)	NR	IHC	10.6%	NR
Mohanty *et al*. (2021)	78 (54/24)	59	IHC/ FISH	28.2% (10%)	Tumour grade (P <0.05) Tumour stage (P <0.05)
Current study	69 (56/13)	65.9 (mean)	IHC	18.8%	Tumour grade (P = 0.005)

Abbreviations: NR: not recorded; IHC: immunohistochemistry; FISH: fluorescence in-situ hybridisation; LVI: lymphovascular invasion; OS: overall survival; PFS: progression-free survival; DSS: disease-specific survival.

Committee prior to the commencement of the study (Reference no: JEP-2017-063).

Authors'contribution: All authors confirmed their contribution to the paper as follows: study conception and design (N Rosli, N Abd Shukor); data collection (N Rosli, M Mahasin, MF Mohd Saleh); analysis and interpretation of results (N Rosli, N Abd Shukor); Draft manuscript preparation (N Rosli, M Mahasin, MF Mohd Saleh, N Abd Shukor). All authors reviewed the results and approved the final version of manuscript.

Conflict of interest: The authors declare no conflict of interest.

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