HER2 / neu Overexpression in Urothelial Carcinoma and Its Association with Tumour Grading - A Cross Sectional Study from a Tertiary Care Hospital in Kerala

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ABSTRACT

BACKGROUND

Human epidermal growth factor receptor-2 HER2 / neu, is a trans membrane tyrosine kinase receptor of epidermal growth factor receptor (EGFR) family and is involved in the pathogenesis of urinary bladder cancer. In this study we attempted to evaluate the HER2 / neu expression in urothelial carcinoma of bladder and its association with tumour grading.

METHODS

This was a cross sectional study with a sample size of 75**.** Routine 4 micrometre thick sections of formalin fixed paraffin embedded tissue blocks stained with haematoxylin & eosin were reviewed. Tumour grade was determined by using the World Health Organization (WHO) / International Society of Urological Pathologist criteria (ISUP). Immunohistochemistry was done by using HER2 / neu monoclonal antibody and its expression were observed. The membrane staining intensity and pattern were studied and scored.

RESULTS

In our study 75 cases of urothelial carcinoma were studied, of which 49 cases were papillary urothelial carcinoma low grade, 26 cases were papillary urothelial carcinoma high grade. Among these, 19 cases were infiltrating urothelial carcinoma. HER2 / neu positivity were observed in 27 (36 %) cases and overexpression in 8 (10 %) cases. Low grade urothelial carcinoma showed HER2 / neu positivity in 11 (22 %) cases and overexpression in 1 (2 %) case. High grade urothelial carcinoma showed HER2 / neu positivity in 16 (64 %) cases, among which 7 (28 %) cases showed overexpression. HER2 / neu positivity was seen in 13 (68 %) cases of infiltrating urothelial carcinoma with 4 (21 %) cases showing overexpression. A statistically significant difference in HER2 / neu expression was noted in high grade and invasive urothelial carcinoma compared to low grade and non-invasive urothelial carcinoma.

CONCLUSIONS

Urothelial carcinomas show overexpression of HER2 / neu and this over expression increases with increasing grade of tumour and muscle invasiveness.

KEYWORDS

Urothelial Carcinoma, HER2 / neu, Overexpression, Tumour Grade, Trastuzumab

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BACKGROUND

Bladder cancer is an aggressive disease and a leading cause of death worldwide.[1] Urinary bladder cancer is the fourth most common malignancy in men and the ninth most common in women. Eighty percent of urothelial carcinoma are superficial at the time of diagnosis with high survival rate. Approximately 10 to 15 % of superficial tumours progress to invasive stage. The invasive urothelial carcinoma is associated with a lower survival rate decreasing from 80 % to 50 %. According to the recent reports of the National Cancer Registry Programme, the overall incidence of the urinary bladder cancer in India is 2.25 % (per 100,000 annually), 3.67 % in males and 0.83 % in females. Despite the advances in cancer treatment, the disease continues as a big challenge to clinicians due to high recurrence rates and a great likelihood to progress to an aggressive, muscle invasive and metastatic disease. 50 – 70 % of newly diagnosed bladder cancers shows a tendency to recur within 5 years.[2] The most common prognostic markers of urothelial carcinomas are conventional clinic-pathologic parameters, such as tumour stage and grade, which are subject to considerable intra- and inter-observer variation. However, accurate estimation of biological behaviour of these tumours is important to select the appropriate treatment. Therefore, more reliable prognostic factors are needed, the prime interest being currently focused on protein over expressions and genetic markers.

Human epidermal growth factor receptor 2 is a trans membrane tyrosine kinase receptor of epidermal growth factor receptor family. In normal conditions, cells have low HER2 / neu protein, but this protein increases dramatically in cancer cells. Intrinsic tyrosine kinase activity of this protein involves cell proliferation and survival via the RAS-MARK pathway and has been shown that it is involved in the pathogenesis of urinary bladder cancer. A wide range of HER2 / neu overexpression from 6 to 80 % has been reported in urothelial carcinomas.[3] Its prognostic value and correlation with tumour stage and grade of urothelial carcinoma has extensively studied. Previous studies showed overexpression in 17 % to 76 % of non-invasive urothelial carcinomas and 23 % to 80 % of invasive carcinomas. It was found that HER2 / neu is overexpressed in a greater frequency in higher grades and stages of carcinoma. Also, HER2 / neu overexpression is associated with earlier tumour recurrence and decreased survival.[4] Trastuzumab is a recombinant monoclonal antibody which targets HER2 / neu protein by directly binding to the extracellular domain of HER2 / neu.

Trastuzumab act synergistically in breast carcinoma with agents more commonly used in treatment of transitional cell carcinoma such as cisplatin with minimal additional toxic side effects, suggesting it may be of value in treatment of transitional cell carcinoma.[5] With respect to the potential application of HER2 / neu over expression in the treatment of transitional cell carcinoma both protein over expression and gene amplification may predict the clinical response to treatment with trastuzumab. The different techniques available for assessment of the HER2 / neu status from tissue sections and the most widely used techniques are immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Compared to other methods IHC is inexpensive in terms of reagents and equipment. The prognostic significance of HER2 / neu were intensively studied and show several conflicting results in the literature. But different studies were conducted using different clones of antibodies for IHC and also different criteria for IHC positivity based on cytoplasmic and / or membrane staining pattern were used. The success of trastuzumab therapy in breast carcinoma patients made interest in exploring the potential of this therapy for patients with urothelial carcinoma. Early studies of the expression of HER2 / neu protein in bladder carcinoma found a correlation between increased HER2 / neu expression with tumour grade and stage. Some studies suggested HER2 / neu overexpression as an independent variable in determining patient survival. Goal of our study is to know the expression of HER2 / neu in bladder transitional cell carcinoma patients by using immunohistochemical methods and to evaluate the association of HER2 / neu overexpression with tumour grading in invasive and non-invasive bladder carcinoma.

Objectives

1. To study the expression of HER2 / neu in bladder transitional cell carcinoma (TCC) patients by using immunohistochemical methods.
2. To evaluate the association of HER2 / neu overexpression with tumour grading.

METHODS

This was a cross-sectional study conducted at Department of Pathology, Govt. Medical College, Trissur, from January 2016 to July 2017.

Inclusion Criteria

Biopsy and cystectomy specimens received during the study period along with retrospective blocks from previous one year, which are diagnosed histologically as urothelial carcinoma were included in the study.

Exclusion Criteria

Biopsy specimen diagnosed histologically as urothelial carcinoma but material inadequate for IHC were not included in the study.

Routine 4 micrometre thick sections of all formalin fixed paraffin embedded tissue blocks stained with haematoxylin – eosin was reviewed. Tumour grade determined by using the WHO / International society of urological pathologist criteria. Immunohistochemistry was done by using poly L lysine coated slides. Antigen retrieval was done using multi epitope antigen retrieval system. Slides were stained with HER2 / neu monoclonal antibody and its expression in urothelial carcinoma specimens were observed. The staining

was interpreted as a scoring system considering the membrane staining intensity and staining pattern.

Sample Size

Calculated using the formula

$$Sample\ Size\ =\ 4pq/d^2p = prevalence$$

$$q\ =\ \{100-p\}\,d\ =\ 20\,\%\,p$$

As per study conducted by Latif et al. prevalence, P is taken as 57 (HER2 / neu overexpression is present in 57 % transitional cell carcinoma).

Hence, sample size = 75. Since, this number of sample size was not obtained during study period, all the cases of urothelial carcinoma received in pathology department during the study period were included in this study. (Universal sampling technique).

Scoring System for IHC Staining

According to scoring system by Jalali Nadousan et al.[6]
Score: Zero-membrane staining observed in less than 10 % of tumour cells or no staining.

1 + partial membrane staining in more than 10 % of the tumour cells and membrane staining not circumferential.

2 + circumferential weak to moderate staining observed in more than 10 % of the tumour cells.

3 + strong circumferential membrane staining observed in more than 10 % of tumour cells.

Both score 2 + and 3 + are taken as positive and score 3+ was considered as overexpression.

Statistical Analysis

The collected data was entered in Excel sheets and analysed using the SPSS software and the chi-square was used to compare variables. P value of < 0.05 was considered as statistically significant in the tests for correlation.

RESULTS

Of the 75 cases, 48 were transurethral resection of bladder tumour (TURBT) specimens, 24 were biopsies, 5 cases were cystectomy specimens. The age of patients ranged from 45 to 85 years. The most common age groups were between 61 - 70 years and mean age was 65 years. Majority of patients were males. There were 69 males and 6 females. Of the 75 cases, 56 cases were noninvasive and 19 cases were invasive. 49 cases were low grade papillary urothelial carcinoma and 26 were high grade papillary urothelial carcinoma. Of the 75 cases, 29 cases were Her 2 positive.

	HER 2 Grade 1	HER 2 Grade 2	HER 2 Grade 3	HER 2 Grade 4	Total
Low grade	16	19	11	3	49
High grade	7	4	8	7	26
Total	**23**	**23**	**19**	**10**	**75**

Table 1. Distribution of Low Grade and High-Grade Urothelial Carcinoma

Grade	1 No (%)	2 No (%)	3 No (%)	4 No (%)
Low	16 (32.7 %)	19 (38.8 %)	11 (22.4 %)	3 (6.1 %)
High	7 (26.9 %)	4 (15.4 %)	8 (30.8 %)	7 (26.9 %)

Table 2. HER2 neu Expression

Chi square value 9.18, P value .027

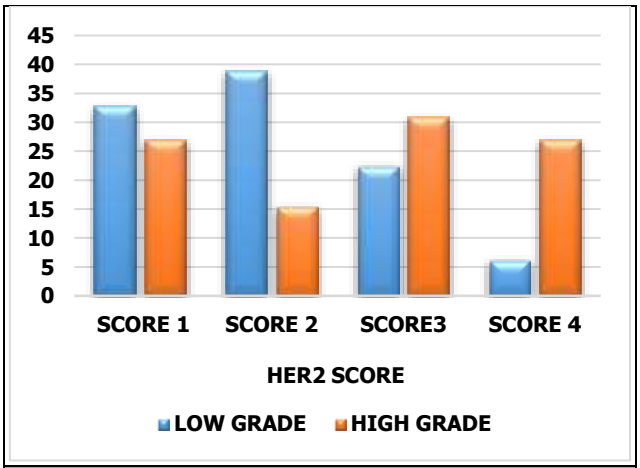

Figure 1. HER2 / neu Expression

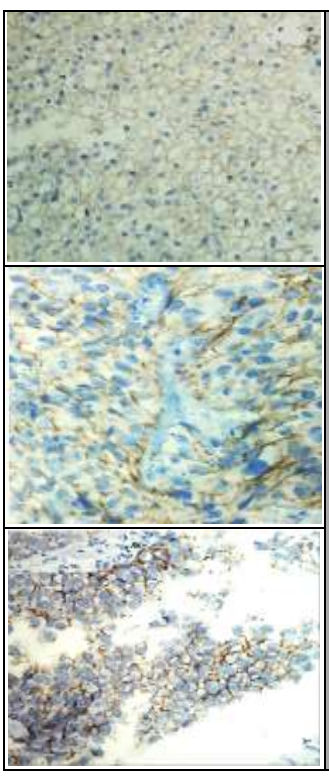

Figure 2. Grade 1, 2, 3 Positivity for HER2 neu Immunohistochemistry

Majority of patients were in the age group of 61 - 80. Most of the patients were males (92 %) with a male to female ratio of 11.5:1 similar to other studies which showed male preponderance.[8] Out of the 75 cases studied, 50 cases were papillary urothelial carcinoma low grade, 25 cases were papillary urothelial carcinoma high grade and 19 cases were infiltrating urothelial carcinoma. The most frequent symptom was haematuria (60 %) both in low grade and high-grade tumours. Among the 75 cases, 48 cases were TURBT specimens and remaining cases were bladder biopsy or cystectomy specimens. In this study HER2 positivity were observed in 27 cases (36 %) and overexpression in 8 cases (10 %). Out of 50 low grade urothelial carcinoma 11 cases (22 %) showed HER2 / neu positivity and only 1 case (2 %) showed overexpression. In 25 cases of high-grade tumours

16 (64 %) cases were HER2 positive, among which 7 (28 %) cases showed overexpression of HER2 / neu. Among the 19 cases of infiltrating urothelial carcinoma 13 (68 %) cases showed HER2 / neu positivity and 4 (21 %) cases showed overexpression.

DISCUSSION

Bladder cancer has various molecular alterations and complex biological pathways which regulate cellular events, such as proliferation, differentiation, angiogenesis, metastasis and apoptosis. The discovery of new biological markers may lead to the improvement of clinical prediction, personalisation of therapeutic approaches and reduction of risk of progression in patients with bladder cancer. Many studies evaluated various markers involved in the regulation of the cell cycle, among which the human epidermal growth factor receptors have a promising therapeutic target. Activation of the HER2 / neu receptor following auto phosphorylation of the tyrosine kinase residues leads to the activation of a cascade of intracellular proteins. Its activation increases the mitotic activity and metastatic potential of the cell which leads to oncogenic transformation. Several studies have reported overexpression of HER2 / neu in transitional carcinoma of bladder and the overexpression seems to be correlated with early recurrence, higher grade and worse prognosis.[7]

In low grade urothelial carcinoma most of the cases showed score 1 and score 0 staining but high grade and invasive cases showed score 2 and score 3 in most of the cases. Similar to the study by Slim Charfi et al., in our study also tumours with score 3 + were considered as Her2 overexpression. Tumours scored 2 + should not be considered as overexpressing HER2 without demonstration of HER2 gene amplification by in situ hybridisation technique. According to various literatures majority of cases scored 2 + are not amplificated by in situ hybridisation.

In our study we found a statistically significant difference in HER2 / neu expression in high grade urothelial carcinoma compared with low grade carcinoma (P value = 0.027). Also, HER2 / neu expression was higher in invasive urothelial carcinoma than non-invasive urothelial carcinoma (P value = 0.027, chi square value 9.18). HER2 / neu overexpression was detected only in the high grade and invasive tumours. Our findings are similar to the studies of Gorgoulis et al., Moch et al., Moriyama et al., Kruger et al., Coogan et al., and Atis G et al., Ghada A. et al., and Shawkya A. et al.[9]

In one of the largest series (1005 cases) reported by Lae et al. HER2 overexpression was 9.2 %, which was lower than that reported in many studies in the literature (23 - 80 %) This observation is similar to our study which showed over expression in 10 % cases.[10] This variability may be due to the difference in immunohistochemistry assays, related to the heterogeneity between kits, antibodies, protocols, interpretations of staining or cut-off values. Various studies use various scoring systems and there is no consensus on the definition of HER2 / neu overexpression in bladder carcinoma.

Several studies compared IHC to FISH results of HER2 in bladder cancer.[11] Sauter et al. in his study of 141 cases reported overexpression in 43 % of cases, but only 7 % of them showed gene amplification. Similar results have been reported by Zhau et al. in his study and this discordance is more frequent than in breast cancer.[12] This can be explained by the several mechanisms which leads to protein expression (translocation, mutation), the bias in sampling, cross-reactivity of antibodies, or by inter-observer variation. Therefore, they suggest that the decision algorithm used in breast cancer can also be used in bladder carcinoma; i.e., in urothelial cancer also patients with strong IHC should be selected as over expressive and in weak or moderate positivity FISH or chromogenic in situ hybridization (CISH) analysis become necessary.

Bellmunt J et al. in their study evaluated patients with primary bladder tumours from two cohorts (Spain and Greece), who treated with platinum-based chemotherapy. Patients were tested for HER2 / neu status by immunohistochemistry, fluorescence in situ hybridisation, DNA copy number, mRNA expression, and mutation status. ERBB2 mutation was determined by hotspot sequencing and mRNA expression was assessed by Nano String counting. 22 % of Spanish and 4 % of Greek cohorts had 3 + HER2 staining by IHC. FISH amplification was seen in 20 % of Spanish and 4 % of Greek cohorts. The study showed that HER2 / neu positive tumours expressed higher levels of HER2 / neu mRNA than HER2-negative tumours ($P < 0.001$). But they could not find out a significant association of HER2 overexpression or amplification in the primary tumour with overall survival in patients with metastatic urothelial carcinoma. They suggested that HER2 / neu positivity rates can vary between different populations.[13]

Ross and colleagues reported the results of next-generation sequencing in 35 patients with urothelial carcinoma.[14] In this study genomic alterations in ERBB2 was seen in two (6 %) patients; one patient with gene amplification and the other with mutation (S310F). The Cancer Genome Atlas Project (TCGA) performed an integrated analysis to characterise molecular alterations in 131 patients with high-grade urothelial carcinoma. In this study mutations identified in 32 genes. Mutations or amplifications in ERRB2 were seen in 9 % of patients. Some of these molecular alterations were similar to those found in the TCGA for breast cancer, suggesting that these two tumours may share pathways for tumour progression.[15]

Several studies showed that HER2 overexpression is predictive of bladder cancer-related death in patient with invasive tumours. B. Kolla et al. in his study observed a significantly high disease-free survival in HER2-negative patients compared to HER2 / neu positive patient. But Rafeael et al. reported that there is no significant difference in survival between HER2 / neu-positive and negative patients. Molecular alterations in HER2 / neu occur in up to 20 - 30 % of bladder cancer patients and the alterations are most commonly amplifications, although mutations occasionally occur. As a result, multiple HER2 targeting-agents, with varying mechanism of action, have been tested in advanced urothelial bladder cancer with modest results.

Recently, an anti-HER2 antibody (trastuzumab) was proposed as therapeutic tool in invasive urothelial carcinoma of the bladder which showed a response rates ranging from 3 % to 63 %.[16] All these results suggest that assessment of HER2 overexpression by immunohistochemically has an important role in evaluation of patients with bladder carcinoma.

CONCLUSIONS

A statistically significant difference in HER2 / neu expression was noted in high grade and invasive urothelial carcinoma compared to low grade and non-invasive urothelial carcinoma. Similar to previous studies, our study results also suggest that urothelial bladder carcinoma shows HER-2 overexpression in significant proportion of cases and its expression is more in high grade and invasive cases. So, it could be used as bad prognostic factor in urothelial bladder carcinoma with regard to grade. And patients with HER2 / neu positive urothelial carcinoma may benefit from adjuvant HER2 / neu targeted therapies.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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