EVALUATION OF HER-2/NEU EXPRESSION BY IMMUNOHISTOCHEMISTRY IN UROTHELIAL CARCINOMA OF URINARY BLADDER

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ABSTRACT

Objective: The objective of this study is to determine the HER-2/NEU expression by immunohistochemistry in Urothelial carcinoma of urinary bladder.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from 15th August 2011 to 14th August 2012.

Patients and Methods: Bladder cancer tissue specimens from 70 patients were selected in one year as per the inclusion criterion. Immunohistochemistry results were interpreted on light microscope using high power field objective and Her 2/ neu expression was recorded. Mean and standard deviation were calculated for quantitative variables. Frequencies and percentages were calculated for qualitative variables.

Results: Out of 70 cases of urothelial carcinoma, Her 2/ neu expression was found to be positive in 24 cases, out of which 5 were of low grade and 19 were of high grade while 16 were invasive and 8 were non invasive. The expression of Her 2/neu was detected in 16 out of 33 cases of invasive carcinoma (48.4%) and in 8 out of 37 cases of non invasive carcinoma (21.6%). As far as grade is concerned, Her 2/ neu was found to be positive in 5 out of 30 cases of low grade carcinoma (16.6%) and 19 out of 40 cases of high grade carcinoma (47.5%).

Conclusion: The expression of Her 2/neu has been shown to be related to the stage & grade of urothelial carcinoma. Her 2/neu expression is increased in high grade and invasive urothelial carcinoma. Molecular targeted therapy targeting Her 2/neu can be beneficial in patients after assessment of Her 2/neu expression.

Keywords: Her 2/neu, high and low grade, Invasive and non invasive, Urothelial carcinoma.

INTRODUCTION

Bladder carcinoma is the fourth most common malignancy in men and 10th most common malignancy in women in United States[1]. In developed countries, 90% of the bladder cancers are urothelial carcinoma[1]. Bladder carcinoma is more common in whites, with male to female ratio of 3:1, and median age at diagnosis is 68 years[1]. Different parameters determine the prognosis of bladder carcinoma, including stage, grade, patient`s age[2], lymph node status, HER 2/ neu and p53 expression[3].

The human epidermal growth factor receptor 2 (Her 2/ neu) or c-erbB-2 is an oncoprotein and belong to the Epidermal Growth Factor Receptor family[4]. It encodes for a tyrosine kinase transmembrane growth factor receptor[5]. The expression of Her 2/neu in bladder carcinoma appears to be an independent prognostic factor[6]. Molecular targeted therapy targeting Her 2/neu can be beneficial in patients after assessment of Her 2/neu expression[7]. The expression of Her 2/neu has been shown to be related to the tumor stage, lymph node involvement, and grade of the disease[8].

The expression of HER-2/neu can be analyzed using immunohistochemistry (IHC) or in situ hybridization (ISH) techniques, like fluorescent in situ hybridization (FISH) and more recently, chromogenic in situ hybridization (CISH) on formalin fixed paraffin embedded tissue sections[7]. Immunohistochemistry is simple, relatively inexpensive, and can be performed quickly with little technical difficulty[5].

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Literature shows different results in the incidence of expression of HER-2/ neu. In a similar study carried out in Egypt in 2010, HER-2/ neu expression was found to be significantly higher ($p < 0.01$) in invasive tumors (78.9%) as compared to non invasive tumors (43.3%) with high significance ($p < 0.01$)[4]. In another study at Glasgow, HER-2/ neu expression was observed with rates of 76% and 52% respectively for non invasive and invasive urothelial carcinomas[9]. These values were not significantly different ($p = 0.06$). There is no local study available on this subject. The rationale of current study is to determine HER-2/neu expression by immunohistochemistry in a subset of our population. The results of this study are likely to prove beneficial for the clinicians/oncologists to select the right patients for targeted therapy against Her-2/ neu receptor.

PATIENTS AND METHODS

The study was conducted in the department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, after approval by the ethical review committee of the institute. Total duration of the study was one year. Research work was carried out from 15th August 2011 to 14th August 2012. Total seventy (70) cases of Urothelial carcinoma were studied, as per inclusion criteria. All the cases of Urothelial Carcinoma from both genders and all ages were included in the study, fulfilling the inclusion criteria. The samples containing poorly fixed and scanty tumour tissue were excluded. The study design was cross sectional.

All the formalin fixed paraffin embedded (FFPE) tissue sections of Urothelial Carcinomas were cut at 3-5 µm thickness. Endogenous peroxidase activity was blocked by incubating in 0.5% hydrogen peroxide in methanol. Antigen retrieval was done by heat mediated method using 750 W domestic microwave. Later on primary antibody of Her-2/neu (Clone by NOVACASTRA's) was instilled followed by incubation in avidin-biotin complex.

Interpretation of Results

Results of immunohistochemistry assay were interpreted manually using 4 point scale, as follows:[6,9]

- No membrane staining.
- 1+ Faint, partial membrane staining.
- 2+ Weak complete membrane staining in more than 10% of tumor cells.
- 3+ Strong complete membrane staining in more than 10% of tumor cells.
- A reaction of 2+ was undertaken as borderline, 3+ as positive and 0 and 1+ as negative[6,9].

RESULTS

A total of seventy (70) patients with Urothelial Carcinoma were studied. Sixty eight were males (97.14%) and 2 were females (2.8%) with male to female ratio of 35:1. The mean age of patients was 62.14 years with standard deviation of 9.49. Their ages range from 43 to 85 years with a median age of 60 years.

Histologically, 40 carcinoma were of high grade (57.14%) and 30 were of low grade

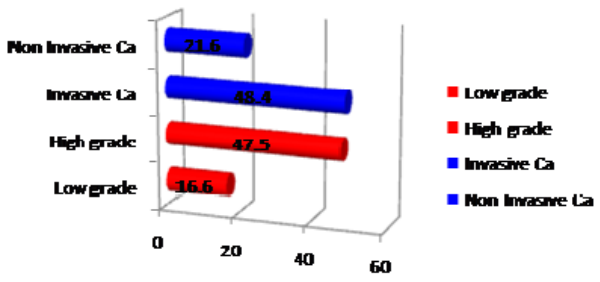

Figure: Expression of Her 2/neu according to the grade and invasiveness of Urothelial carcinoma.

(42.85%). Total 37 were non invasive (52.85%) and 33 were invasive (47.14%). Her 2/neu expression was found to be positive in 24 cases, five cases (16.6%) were of low grade and nineteen cases (47.5%) were of high grade (Fig). Total sixteen were invasive and eight were non invasive. The expression of Her 2/neu was detected in 16 out of 33 cases of invasive carcinoma (48.4%) and in 8 out of 37 cases of non invasive carcinoma (21.6%)

indicated in Fig. Table 1 and 2 compare the results from various studies with the present study.

DISCUSSION

Her 2/neu evaluation is a new emerging prognostic factor for the urothelial carcinoma. Its expression can be assessed by Immunohistochemistry, In situ hybridization techniques & PCR etc. Among these techniques, immunohistochemistry is reasonably good, reliable & cost effective. On the basis of Her 2/ neu positivity, an additional treatment modality can be introduced in the form of targeted therapy against Her 2/neu receptor. Her 2/neu positivity is scored on immunohistochemistry according to the percentage of the tumor cells, showing immunoreactivity for Her 2/neu antibody. More than 10 % complete membranous staining of tumor cells is taken as positive[6,9].

There are no local studies available on the subject. As far as international studies are concerned, in one study conducted in Egypt by Hammam et al in 20105, total 220 patients, including both benign and malignant urological lesions, admitted in urology department Theodor Bilharz Research institute were studied.5 In malignant group, Her 2/neu 1+ positivity was found in 30% cases, 2+ positivity was found to be in 40.9% & 3+ positivity in 20.09%. Her 2/neu over expression was found to be significantly higher in high grades of urothelial carcinoma (63.6%) as compared to low grades (56%) with ($p < 0.01$), also Her 2/neu expression was significantly high in invasive tumors, e.g. 78.9% as compared to 43.3% in non invasive tumours with high significance ($p < 0.01$). Her 2/neu

Table-1: Comparison of results of Her 2/neu positivity in High grade and Low grade urothelial carcinoma.

Study	Her 2/neu expression in High Grade urothelial carcinoma	Her 2/neu expression in Low Grade urothelial carcinoma
Kolla et al (2007)7	71%	32%
Hommam et al (2010)5	63.6%	56%
Present study	47.5%	16.6%

Table 2: Comparison of results of Her 2/ neu positivity in invasive and non invasive urothelial carcinoma.

Study	Her 2/neu expression in invasive urothelial carcinoma	Her 2/neu expression in non invasive urothelial carcinoma
Latif (2003)2	52%	76%
Hommam et al (2010)5	78.9%	43.3%
Present study	48.4%	21.6%

The literature shows great variability in the results regarding expression of Her 2/neu in urothelial carcinoma. Her 2/neu overexpression was first described in bladder carcinoma in 1990 by Zhau et al[10]. Since then various studies show great variation in the Her 2/neu expression in urothelial carcinoma, ranging from 2 to 71%[10,11]. This heterogeneity in results is attributable to various factors including stage of disease under study, definition of Her 2/neu positivity, type of specimens (fresh/ formalin fixed) and clones of antibodies used in the analysis.

immunoreactivity was more in urothelial carcinoma (72.2%) as compared to squamous cell carcinoma (40%)[5].

In another study, conducted at India by Kolla in 20077, Her 2/neu expression was evaluated in 90 patients, who were selected retrospectively and had undergone cystectomy. The study revealed that Her 2/neu was significantly related to tumor stage, grade of the disease as well as lymph node involvement. The study showed Her 2/neu expression in 71% cases

of high grade as compared to 32% cases of low grade tumors with (p = 0.001). Kaplan-Meier curves showed a worse disease related survival period for patients with Her 2/neu positive urothelial carcinoma as compared to Her 2/neu negative urothelial carcinoma. In this study, Her 2/neu status was identified as an independent prognostic factor for disease related survival in a multivariate analysis.

In another study by Latif in 2003 at Glasgow[2], patients with tumors that had progressed from superficial disease (pTa/ pT1) to muscle invasive disease (pT2) were selected. pTa/ pT1 and pT2 tumors from the same patient were compared to assess Her 2/neu expression during the disease progression from non invasive to invasive disease. Her 2/neu expression was found to be 76% in non invasive urothelial carcinoma, while 52% in invasive urothelial carcinoma. This study results suggest that over expression of Her 2/neu occurs prior to and persist with the onset of invasive disease[2].

In a study carried out by Eissa S et al in 2005 in Egypt[12], no significant correlation was found between expression of Her 2/neu and stage or grade of bladder carcinoma, although it was related with the lymph node status of the tumour.

In another study by Christopher LA Coogan in 200313, 54 patients of urothelial carcinoma were selected randomly from 1998 to 2000. The study showed 14% grade 1 tumors, 12% of the grade 2 tumors and 48% of grade 3 tumors were positive for Her 2/neu. Furthermore, it showed that 17% of stage Ta / T1 and 44% of the stage T2/ T3 were reactive for Her 2/neu.

CONCLUSION

The present study shows that Her 2/neu expression is significantly related to the grade and extent of invasiveness in urothelial carcinoma. Her 2/neu can be used as an independent prognostic factor in urothelial carcinoma. Molecular targeted therapy in the form of Transtuzumab can be instituted as an additional treatment tool in the patients with urothelial carcinoma.

Conflict of Interest

This study has no conflict of interest to declare by any author.

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