SIGNIFICANCE OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR (HER2-NEU) MARKER IN UROTHELIAL CARCINOMA

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ABSTRACT

Objectives: To determine the value of immunohistochemical expression of Human Epidermal Growth Factor Receptor 2/ HER2-neu in urothelial carcinoma of urinary bladder.

Material and Methods: This was a cross –sectional descriptive study. It was conducted at histopathology section of Pathology Department Rehman medical institute and Khyber medical university Peshawar. Duration of this study was six months from January to June 2017. Sixty three (63) cases were selected by purposive sampling. Tissue samples from diagnosed cases of urothelial carcinoma urinary bladder were included. Cases of urothelial carcinoma from other sites were excluded. Paraffin embedded tissue blocks were cut and sections stained by H&E to evaluate histopathological features and tumor grades. Immunohistochemical expression of HER2 was determined applying standard techniques of immunohistochemistry.

Results: Out of total 63 cases, 44 were males and 19 were female with a mean age of 60.69 years (range 16-90 years). There were 38 High grade, 23 Low grade and 2 cases of PUNLMP. 36 cases were positive for HER2 expression in which 26(72.2%) were High grade, 9(25%) of Low grade and 1(2.7%) of PUNLMP (Papillary urothelial neoplasm of low malignant potential. High grade was more common in HER2 positive cases and this relationship was significant ($p<.05$). Among positive 36 cases, 19 cases of +3 score were High grade, 05 in Low grade and 01 in PUNLMP while score 2+ was in 07 cases of High grade and 04 were in Low grade. The relationship of HER2 score was not significantly related to grade of tumor ($p>.05$).

Conclusion: HER2 is highly expressed by high grades of urothelial carcinoma and this relationship is founded statistically significant between HER2 expression and tumor grades of urothelial carcinoma

Keywords: HER2, Urothelial carcinoma, Grades, Immunohistochemistry

INTRODUCTION

Malignancy of urinary is considered a 9th likely cause of carcinoma globally. its ratio among female to male is 1:3.5.[1]Morphologically speaking, urothelial carcinoma which arises in urothelial/ transitional epithelium makes 90% of all bladder cancers.[2] Pakistan carries a high burden of newly diagnosed cases of urothelial carcinoma with mortality rate of 3.8/100,000 in males and 1/100,000 in females.[1]

Etiologic factors of bladder carcinoma include smoking, industrial carcinogens as aromatic amines and aniline dyes, radiations, medications containing phenacetin compound and some chemotherapeutic drugs as cyclophosphamide, and genetic polymorphism. Chronic cystitis with schistosoma is implicated in development of squamous cell carcinoma in African countries.[3]

Urothelial carcinoma arises from the urothelial/ transitional epithelium of urinary bladder. 70-80% bladder tumor are superficial confined to mucosa and have good prognosis.[4]The resting 20-30% are invasive to muscles with worse outcome.[5]

Tumor Grade and stage are two important factors to determine prognosis, risk of recurrence and progression of disease. Molecular markers are involved in pathways of carcinogenesis and progression and their identification can aid into the prognosis.[6]

Oncogenes Her2neu (Human Epidermal growth Factor Receptor) belongs to the family of EGFR (epidermal growth factor receptor). It is located on chromosome 17.[7] Immune therapy with antibody directed against HER2 can be a potential therapy in bladder carcinoma esp. in advanced cases.[8] Her2 over expression and amplification is a common mechanism of oncogenesis in various tumors. Over expression of HER2 is studied in invasive breast carcinoma, gastric carcinoma, ovarian, endometrial, and esophageal and

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lung cancers. Urothelial carcinoma has a variable range of expression from 2-74% [8] which has a mixed association with prognosis of the carcinoma. It is related to aggressive behavior in some studies while other shows no association to prognosis.[9,10] HER2 targeted therapies have promising role in breast carcinoma.[8]

The aim of this study is to find out the importance of HER2 Neu marker expression in urinary bladder carcinoma and its relation with their histologic grades.

MATERIALS AND METHOD

This cross sectional analytical study was conducted at the histopathology section of Rehman medical institute H.abad Peshawar. Total 63 specimens were taken for this study. The sample size was calculated by non probability purposive sampling technique.

Where

Z= slandered deviation

D= margin of error

The 63 cases tissue specimens were taken for six month period from the month January to June 2017. Institutional based Ethical clearance was received to conduct the study. Tissue blocks of all diagnosed cases were collected.

Inclusion criteria

Purely positive urinary bladder carcinomas (urothelial carcinoma) were included.

Exclusion criteria

Cases other than urothelial carcinoma like squamous carcinoma, adenocarcinoma, Neurendocrine carcinoma and sarcomatoid carcinoma etc were excluded. Tissue section with 2-4µm were taken by microtome from blocks. Slides were prepared and stained with basic H & E stain according to their standard protocol.[12]Histomorphological features of carcinoma studied and tumor was graded according to World Health Organization (WHO) classification of tumors grading 2004.[13]

By heating technique (microwave) the slides were deparaffinized and put in xylene solution for 4-5mints to carry out immunohistochemical study. Heat induced epitope retrieval method was used. Slides were put in EDTA solution and heated in microwave oven for 15-20 minutes at 140'c. on slides blocking reagent was used for 5minuts and washed with buffer saline. After that primary antibody DAKO was applied on the slides and incubated for 1 hour then secondary antibody FLEX/ HRP was applied and again incubated for half hour. At last DAB reagent was used to get a uniform color. Counterstaining was done with Hematoxylin solution. DPX medium was used for mountaining the slides, coverslips applied and labeled.[14] intensity of HER2NEU staining was scored by American college of pathologist as following.[15, 16]

STATISTICAL ANALYSIS

SPSS version 20 was used for analysis of data. The correlation between variables was calculated by chi-sq test and frequencies for variable were determined. Results are plotted in tables. Immunohistochemistry staining and scores are shown in figures.

RESULTS

The results of this study are described in tables and figures/ photographs.

DISCUSSION

Her2/ neu as an immunohistochemical marker is used for the grading and differentiation of breast carcinoma. It is considered for therapeutic purposes and targeted therapy directed against carcinoma in breast. It is also analyzed as prognostic and predictor marker for carcinoma breast and non breast carcinomas like ovarian, melanoma and esophageal carcinoma. Tyrosine kinase inhibitors (TKIs) are used against these tumors.[9]

In our study 57% expression of Her2/neu was seen which is almost similar to the study conducted by Alexa A et; al in 2010 with 53 % membranous expression of Her2/Neu in invasive urothelial carcinoma (TCC) cases.[18] The expression of epidermal growth factor receptor in malignant tumors of urinary bladder is variable from 3-75% in many different kinds of studies. Its role as a prognostic marker is debatable in urinary bladder carcinoma.[17] According to most of the studies its expression shows poorly differentiated carcinoma, high grade, violent behavior and ominous prognosis[18,19]

A study conducted in 2016 by Nejdadi et; al founded the same expression of HER2 with score +3 in both low grade and high grade urothelial carcinoma but founded more cases of +2 score in low grade in comparison with high grade tumors.[19 in] another study 51% significant expression of Her2/Neu was seen conducted by Khalid Gehani et al in 2012. He also observed 44.4% cases expressed in low grade and 66.6 expresses in high grade but this association was considered insigniganct.[20]

Ismail NEH conducted a study in 2015 concluded that high grade urothelial carcinoma expresses Her2 with score+3 but in his study he also determind that there was no variation in low grade carcinoma with score +3 (5.9%) and score +2 (5.9%) respectively.[21]

In our study Her2 neu expression was 39% in low grade and 68.4% in high grade, this correlation was founded significant statistically. Similar expressions were observed by Ismail NEH in 2015 with 62.8% in high grade tumor and in low grade cases 11.8%. in 2015 study conducted by Jamal N et;al also noted 16.6%

Table 1.1 Cases with Gender and Age wise Distribution

Gender	No. of cases	Frequency	Age range with mean
Female	19	30%	16-70(54.3)
Male	44	63%	21-90(62.5)
Total	63	100%	60.69(16-70)

Table 2.1 Cases with Grade wise distribution of Her2/Neu Expression

Grade	Negative	Positive	Total
PUNLMP	50%(1)	50%(1)	3%(2)
Low Grade	60.9%(14)	39%(9)	36.5%(23)
High Grade	31.6%(12)	68.4%(26)	60%(38)
Total	43%(27)	57%(36)	100%(63)

PUNLMP: Papillary urothelial neoplasm of low malignant potential
P value: P=.025 (P<0.05) which was considered significant.

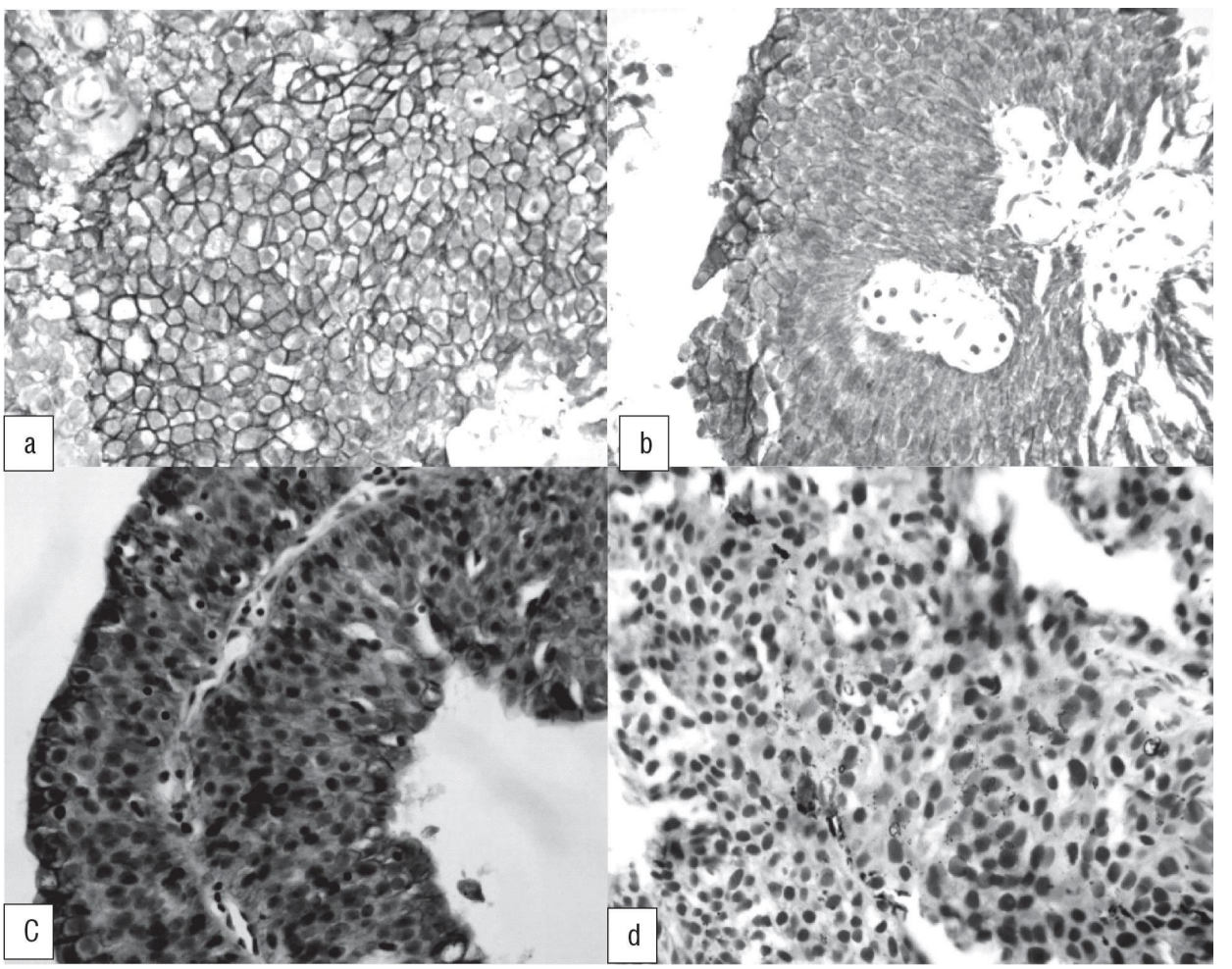

Figure 01: IHC staining shows HER2 expression as membranous staining of cells
(a) Score 3+ staining pattern
(b) Score 2+ weak/moderate membrane staining in >10% cells
(c)Score1+ with incomplete, weak/faint and partial membrane staining in >10% cells
(d) No staining score0

expression for low grade and 47.5% for high tumors respectively.[21, 22]

Further investigations by more advanced techniques as FISH or flow IHC are needed to verify HER2 status especially in those of equivocal cases. Stage of carcinoma was not studied in detail and the relationship with HER2 must be determined properly which is important in prognosis. Immune mediated treatments against HER2 are already working in breast carcinoma and this gives hope to urothelial carcinoma once HER2 status is established accurately.

CONCLUSION

From the results it has been concluded that high grade urothelial carcinoma has significant positive association with HER2/Neu expression. This expression will aid help in the targeted therapy of urothelial carcinoma. Its association with its histologic grades required more studies and florescence insitu hybridization technique (FISH) for categorization.

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