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Frequency of Her2/Neu Immuno Histochemical Expression in Bladder Urothelial Carcinoma: A Study At CMH Quetta

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ABSTRACT

Introduction: The staining pattern of HER2/Neu expression in urothelial bladder cancer has been established for more than a decade, and literature review of the past reveals a dismal prognosis in Her2/Neu expressed invasive urothelial carcinoma.
Aims & Objectives: To determine the frequency of immuno histochemical expression of Her2/Neu in urothelial carcinoma of bladder.
Place and duration of study: This descriptive, cross-sectional study was performed at the Department of Pathology, Combined Military Hospital, Quetta from 15th May 2017 to 14th November 2017.
Material & Methods: A total of 89 patients with urothelial carcinoma bladder, from 20 to 60 years of age, irrespective of the gender were included. Patients with already immuno histochemistry report and taking radiotherapy were excluded. The immuno histochemical processing was done and results were interpreted for expression (positive/negative) of Her2/Neu receptor.
Results: The participants in this research ranged from 20 to 60 years old with an average age of 44.16± 8.27 years & male to female ratio of 1.6:1.38. One in every four (1.6:1) of the 89 (42.70 percent) individuals studied with urothelial carcinoma of the bladder cases were found to have over expression of Her2/Neu receptor.
Conclusion: This study concluded that frequency of over expression of Her2/Neu in urothelial carcinoma of bladder is very high.

Keywords: Ectopic urothelial carcinoma, immuno-histochemistry, Her2/Neu, overexpression

INTRODUCTION

Urinary bladder cancer is the fourth most frequent cancer among men and the ninth most common among women. 80% of urothelial bladder malignancies are superficial and have a favourable prognosis at the time of diagnosis.[1] the invasive form, on the other hand, has a lower survival rate, falling from 80% to 50%. The liver, lungs, and bones are the most frequent sites of metastasis, although lymph nodes are not the only possibility. As many as 30 percent of cancer patients are already invasive or metastatic at the time of diagnosis.[2]

The American Cancer Society notes that ten to fifteen percent of superficial tumours become invasive. Depending on their age and grade, the threat posed to a particular person may vary from one observer to the next.[3] The human-epidermal-growth-factor receptor 2 (HER2, also known as Neu, ErbB-2, and p185HER2) is a trans-membrane glycoprotein with an intracellular tyrosine kinase activity and an extracellular domain very similar to those of the epidermal-growth-factor-binding domain of the epidermal-growth-factor receptor.[10] The HER2/Neu proto-oncogene is amplified and/or overexpressed in approximately 20 to 25% of invasive breast cancers.[10] Search for HER2 expression has mainly focused on the development of the target therapies such as Trastuzumab against HER2 positive urothelial carcinomas and other organ systems like in breast and esophagus.[4]

As stated earlier overexpression of ERBB2 and ERBB2 DNA amplification are often linked to cases of breast cancer. Patients with intermediate

levels of HER2 protein expression, which may be identified via fluorescence in situ hybridization, may benefit from HER2-targeted treatment (FISH).[5] The staining pattern of HER2/Neu expression in urothelial tumours has been recognised for a decade, and HER2/Neu overexpression has been linked to a worse prognosis in several studies, but only in individuals with invasive malignancies. Her2/Neu immunostain positivity was found in 35.59 percent of patients in research. Nucleic acid-binding domains of the epidermal-growth-factor receptor (HER2) have been shown to be remarkably similar to those of the transmembrane glycoprotein known as p185 HER2 and Neu.[10] Although the poor prognosis linked with HER2/Neu overexpression has been shown in many studies[11,12,13] It is debatable whether or not HER2/Neu is an accurate predictor of responsiveness to hormone treatment or chemotherapy.[14,15,16]

As many as 45 percent of patients with metastatic breast cancer have HER2/Neu protein broken and released into the bloodstream, where it may be identified by ELISA.[17] Increasing levels of HER2/Neu in the blood have been linked to the progression of metastatic illness and a poor response to chemotherapy and hormone treatment.[18]

We set out to investigate the relationship between Her2/Neu expression and epithelial cell growth and differentiation control in urothelial carcinoma of the bladder to determine the frequency of immune histochemical expression of Her2/Neu in urothelial carcinoma of the bladder in the local population. Aside from adding to the body of knowledge already available, our study focused to provide data specific to the study area. Keeping in view past literature, patients with urothelial carcinoma who have over-expressed Her2/Neu can be identified and referred to medical oncologist to be treated with target therapies i.e., trastuzumab and thus they can be followed in long term to establish prognostic significance of Her2/Neu expression in future studies. This study aims to establish frequency of HER2/Neu immune histochemical expression in urothelial carcinoma.

MATERIAL AND METHODS

This descriptive, cross-sectional study was conducted in the Department of Pathology, Combined Military Hospital, Quetta, from 15th May 2017 to 14thNovember 2017. Sample size was determined by the formula= $Z^2P (1-P) / d^2$, Where z=1.96, p=35.59%.[9] and d=10%. Thus sample size n = 89 patients. Non-probability, consecutive sampling technique was used.

Inclusion Criteria:

All patients with urothelial carcinoma bladder from 20-60 years of age with both genders were included in our study.

Exclusion Criteria:

Following patients were excluded from the study:

Patients already having immuno histochemistry report, as repeating immuno histochemistry in such cases could have reduced cost effectiveness of the study.

Patients showing equivocal staining for HER2 expression i.e., 2+, to be confirmed on FISH were excluded, as performing FISH in such cases is beyond scope of this study. Patients on radiotherapy as it affects the expression of Her2/Neu

Data Collection:

Biopsy specimens presented to the Department of Pathology, Combined Military Hospital, Quetta, fulfilling the inclusion criteria was selected. After taking informed written consent, the immuno histochemical processing was done and results were interpreted by the consultant pathologist for overexpression (positive/negative) of Her2/Neu. Expression of Her2/Neu was noted as per following scoring system and only membranous staining was considered significant:

- 0 = No membrane staining or less than 10% of cells.
- 1+ = Partial membrane staining in more than 10% of cells.
- 2+ = Weak, circumferential membrane staining in more than 10% of cells, or intense membrane staining in less than 30% of cases.
- 3+ = Intense membrane staining in more than 30% of cells.

Her2/Neu overexpression was considered positive if immuno histochemistry score was 3+otherwise taken as negative. Cases carrying 2+ score were considered equivocal, to be confirmed on FISH, which was beyond scope of this study.

All this data was recorded on a predesigned proforma.

Statistical Analysis:

SPSS 20.0 was used for the statistical analysis. Mean and standard deviation were used to represent age and length of illness. Gender, stage of urinary bladder cancer (I/II/III/IV) and overexpression of Her2/Neu (Positive / negative) were presented as frequency and percentage.

Effect modifiers such as gender, age, and stage of cancer (I/ II / III / IV) were managed by stratifications and a post-stratification Chi square. A P value of 0.05 or less was considered significant.

RESULTS

Participants in this study varied in age from 20 to 60, with an average of 44.16 years and 8.27 months. Among the 89 patients, 55 were men and 34 were women, with a ratio of 1.6:1. As shown in Table-1.

Age (in years)	No. of Patients	%age
20-40	27	30.34
41-60	62	69.66
Gender		
Males	55	61.80
Females	34	38.20

Table-1: Age& Gender distribution for both groups (n=89).

- **Mean ± SD = 44.16 ± 8.27 years**

Patients were ill for an average of 5.53 months on average, according to the data shown in Table-2.

Duration of disease (months)	Total (n=79)	
	No. of patients	%age
≤6 months	69	77.53
>6 months	20	22.47
Mean ± SD	5.53 ± 1.06 months	

Table-2: Distribution of patients according to duration of disease.

The stratification of over expression of Her2/Neu by age and gender is shown in Table-3.

Age (years)	Over expression of Her2/Neu		p-value
	Positive	Negative	
20-40	09 (33.33%)	18 (66.67%)	**0.239**
41-60	29 (46.77%)	33 (53.23%)	
Gender			
Male	21 (38.18%)	34 (61.82%)	**0.273**
Female	17 (50.0%)	17 (50.0%)	

Table-3: Stratification of over expression of Her2/Neu with respect to age & Gender groups.

Based on their cancer stage, patients are shown in Fig-1.

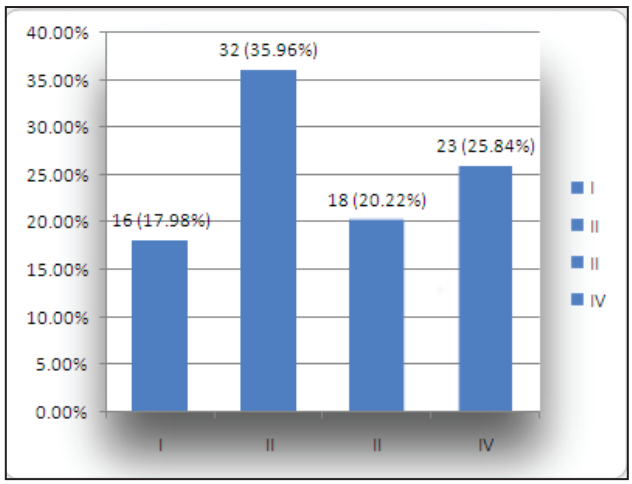

Fig-1: Distribution of patients according to stage of carcinoma (n=89)

38 (42.70%) of individuals with bladder urothelial cancer had over expressed Her2/Neu as shown in Fig-2.

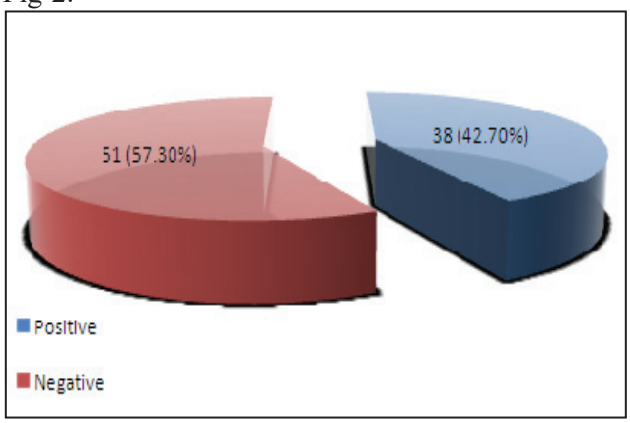

Fig-2: Frequency of overexpression of Her2/Neu in urothelial carcinoma of bladder (n=89)

The over expression of Her2/Neu is based on sickness duration. Over expression of Her2/Neu dependent on tumor stage is shown in Table-4.

Duration of disease (months)	Over expression of Her2/Neu		p-value
	Positive	Negative	
≤6	24 (34.8%)	45 (65.2%)	**0.005**
>6	14 (70.0%)	06 (30.0%)	
Stage of carcinoma			
I	09 (56.3%)	07 (43.8%)	**0.686**
II	13 (40.6%)	19 (59.4%)	
III	07 (38.9%)	11 (61.1%)	
IV	09 (39.3%)	14 (60.9%)	

Table-4: Stratification of over expression of Her2/Neu with respect to duration & stage of disease.

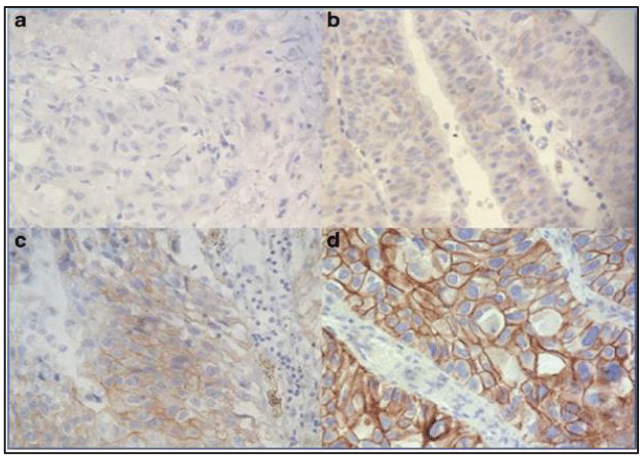

Fig-3: HER2 expression in urothelial bladder carcinoma: a HER2 negative staining scored as 0. b HER2 weak staining scored as 1+. c HER2 moderate staining scored as 2+. d HER2 positive staining scored as 3+

DISCUSSION

One of the physiological functions of cell proliferation is supported by HER2 gene intrinsic tyrosine kinase activity. The NEU, HER3, and ERBB2 genes, as well as EGF2, are all members of the EGFR family. Cancer cell proliferation, angiogenesis, and metastasis have been linked to HER2 over-expression in a variety of malignancies.[19] High levels of HER2 overexpression have been found in invasive breast, gastric, and colonic carcinomas, and bladder cancers.[20] Up to 34% of bladder cancer tumors express the HER2 gene, making it one of the most prevalent HER genes.[21]

Her2/Neu overexpression in bladder cancers was investigated in this study. The study's participants ranged in age from 20 to 60 on average. Study participants had a male-to-female ratio of 1.6:1 or one in four. Her2/Neu was overexpressed in 38 out of 100 cases of bladder urothelial cancer (42.70 percent). In our study, the risk of overexpression of Her2/Neu receptors was higher as the disease progressed but not at the time of initial assessment in Bladder Urothelial Carcinoma as this determination was done after the carcinoma had developed. A study found that 35.59 percent of subjects had Her2/Neuimmuno staining.[9] 40% of instances of micro-papillary UC were found to have ERBB2 extracellular domain mutations, according to a recent study.[24]

Certain UC patients with extracellular domain mutations may benefit better from ERBB2 kinase inhibitors, which could lead to new therapy choices. There are two possible mechanisms for UC HER2 overexpression, DNA amplification and/or protein overexpression.

It is possible that HER2 is both a marker for a more aggressive disease and a therapeutic target. Another study, however, found no evidence of a connection between the two.[27, 28] However, prior investigations have found inconsistent results regarding whether HER2 expression corresponds with overall survival in UC.[29, 30]According to the results of the study indicated above, HER-2 overexpression was observed in 23 of 39 tumors (or 59% of the total), however the difference was not statistically significant. A correlation between HER-2 overexpression and tumour stage was found (p=0.011). HER-2 expression is higher in TCC and AC than in squamous cell carcinomas (SCC), but not in SCC (SCC). HER-2 overexpression was not linked to the gender or age of patients.[31]

According to a single study, HER2/Neu overexpression was detected in 81% of cases and 67% of metastases in metastatic urothelial bladder tumors.[32] Based on these findings; anti-HER2 medication should be employed in these situations. To make conclusions about upper urinary tract cancers, there are just a few research on a small number of cases.[33, 34]

No correlation was detected between these cancers and survival in a 2002 analysis of 61 patients, according to Fontana LO et al.[35] As of yet, no published studies have associated HER2/Neu overexpression and survival. While HER2 status was found in 12.4 percent of T1 bladder cancers, there was no correlation between HER2 status and outcomes, such as tumour growth and recurrence, according to the Olsson study.[14] HER2-IHC analysis was carried out using entire tumour sections in compliance with the ASCO/CAP recommendations for breast cancer, according to Olsson and colleagues.[36]

Chen et al.[37]recently used IHC and FISH to determine the HER status of NMIBCs on TMA in a similar study. HER2 gene amplification was found in 9 percent of individuals with high-grade NMIBC, indicating that testing for HER2 status may be useful in identifying NMIBC patients who need close monitoring. In patients who underwent a radical cystectomy for muscle-invading cancer, the Hercep Test established by Krueger et al was used to detect HER2 gene amplification.[38]

HER-2 overexpression was not linked to tumour stage in high-grade bladder tumours. Pathological staging and tumour grade were not connected to HER-2 in two more trials.[39] Bladder cancer patients with HER-2 expression had no connection

with stage or grade.[40] According to the research, there was no statistical difference between the TCC and SCC groups.

Limitations:

Long term follow up was'nt in our research ambit to substantiate prognostic significance of Her2/Neu expression in patients taking targeted therapies for HER2. Further another confirmational approach for HER2 equivocal cases as FISH was not undertaken.

CONCLUSION

Keeping in view past data and findings of this study wherein Her2/Neu was overexpressed in 42.7% cases of bladder urothelial cancer, we recommend Her2/Neu immunostaining for high-risk patients. This should be performed frequently to substantiate the prognostic significance of HER2 and to tailor targeted therapies for individual patients and thus reduce community mortality and morbidity.

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