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The Frequency of Her2/Neu Immuno Histo-Chemical Expression in Bladder Urothlial Carcinoma: A Study At CMH Quetta

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ABSTRACT

Introduction: Patients with invasive urothelial carcinoma that expresses HER2/Neu have a dismal prognosis, and studies over the last decade have established a consistent staining pattern for HER2/Neu expression in urothelial bladder cancer.
Aims & Objectives: The goal of this study is to quantify the prevalence of Her2/Neu immunohistochemical expression in bladder urothelial cancer.
Place and duration of study: Research design: descriptive, cross-sectional, May 15–November 14, 2017. Combined Military Hospital, Quetta, Pathology Department.
Material & Methods: Both male and female patients aged 20-60 with bladder urothelial cancer were included. Patients who have had an immunohistochemistry report before or who are undergoing radiation treatment were excluded. Her2/Neu positivity/negativity was determined via immunohistochemical processing.
Results: The participants' ages varied from 20 to 60, with an average age of 44,16 +/- 8,27 years. Her2/Neu overexpression was seen in 42.70 percent of cases of urothelial carcinoma of the bladder, and males made up just one-quarter (1.6:1) of the 89 patients studied.
Conclusion: Her2/Neu overexpression was shown to be common in this research of bladder cancer (urothelial carcinoma).

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

INTRODUCTION

Cancer of the urinary bladder ranks fourth among males and ninth among females. At the time of diagnosis, the prognosis is good for the most majority of urothelial bladder cancers (80%)[1]. Alternatively, the survival probability drops from 80% to 50% for the invasive variant. Most metastases occur in the liver, lungs, and bones; however, lymph nodes are not the only potential secondary locations. When diagnosed, aggressive or metastatic cancer has already spread in as many as 30 percent of individuals[2].

According to the American Cancer Society, only around 10%-15% of superficial tumours progress to the invasive stage. One person's danger level may seem high to another, depending on their age and educational level. The extracellular domain of HER2, also known as the human epidermal growth factor receptor 2, is substantially similar to the epidermal growth factor binding domain of the epidermal growth factor receptor[10]. Twenty-five to thirty percent of invasive breast tumours have amplifications or over expressions of the HER2/Neu proto-oncogene[10]. So search for HER2 expression has mainly focused on the utilization of the target therapies such as Trastuzumab against HER2 positive urothelial carcinomas and other organ systems like in breast and esophagus[4].

As stated before overexpression of ERBB2 and ERBB2 DNA amplification are often linked in 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]. HER2/Neu over expression has been associated to a poorer prognosis in various studies, but only in those with invasive malignancies; this staining pattern has been known in urothelial cancers for a decade. In one study, 35.59% of patients tested positive for Her2/Neu using immunohistochemistry.

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 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]

Objectives We sought to investigate the association between Her2/Neu expression and epithelial cell proliferation and differentiation control in urothelial carcinoma of the bladder in order to ascertain the prevalence 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 will provide data specific to the study area and 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. Long-term patient follow-up and the determination of a correlation between HER2/Neu expression and tumour grade, stage, and lymph node status are beyond the scope of this research.

MATERIAL AND METHODS

This descriptive, cross-sectional research was carried out between May 15 and November 14, 2017, at the Combined Military Hospital in Quetta.

The number of participants in the study was calculated using the formula $n = Z^2P (1-P) / d^2$, with the parameters z = 1.96, p = 35.59%.9, and d = 10%. A total of 89 patients were used as the sample size. We employed a non-random, sequential sampling method.

Inclusion Criteria:

We included male and female patients aged 20-60 with urothelial carcinoma of the bladder.

Exclusion criteria:

Patients with a prior immunohistochemistry report were not included since duplicating those tests would have diminished the study's cost-effectiveness.

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 affect the expression of Her2/Neu

DATA COLLECTION:

Any biopsy samples meeting the inclusion criteria that were submitted to the Department of Pathology at the Combined Military Hospital in Quetta were included. In this case, the consultant pathologist assessed the immunohistochemical data for overexpression (positive/negative) of Her2/Neu after receiving written permission from the patient. Expression of Her2/Neu was noted as per following scoring system and only membranous staining was considered significant:

- 0 = Insufficient membrane staining, or fewer than 10% of cells.
- 1+ = Over 10% of cells show partial membrane staining.
- 2+ = Over 10% of cells show only weak, circumferential membrane staining, while fewer than 30% show strong membrane staining.
- 3+ = The membranes of more than 30% of the cells stain intensely.

In this study, a score of 3+ on the immunohistochemistry test for Her2/Neu over expression was deemed positive. Cases carrying 2+ score were considered equivocal, to be confirmed on FISH, which is beyond scope of this study.

All this data was recorded on a predesigned proforma.

STATISTICAL ANALYSIS:

The statistical analysis was performed in SPSS 20.0. We portrayed the ages and durations of patients using means and standard deviations. Overexpression of Her2/Neu was also provided as a frequency and percentage, as were gender and urinary bladder cancer stages (I, II, III, IV).

Stratifications and a post-stratification Chi square may be used to handle effect modifiers including gender, age, and cancer stage (I, II, III, or IV). A P value of 0.05 or less was considered significant in this study.

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)	Overexpression 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.

On the basis of their cancer stage, patients are shown in Fig-1.

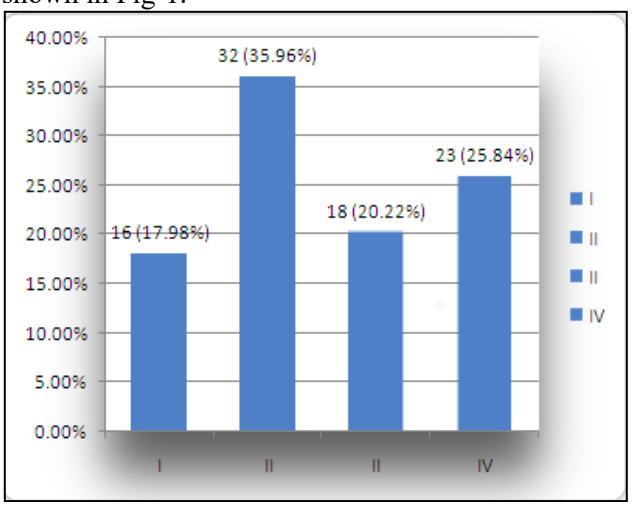

Fig-1: Distribution of patients according to stage of carcinoma (n=89)

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

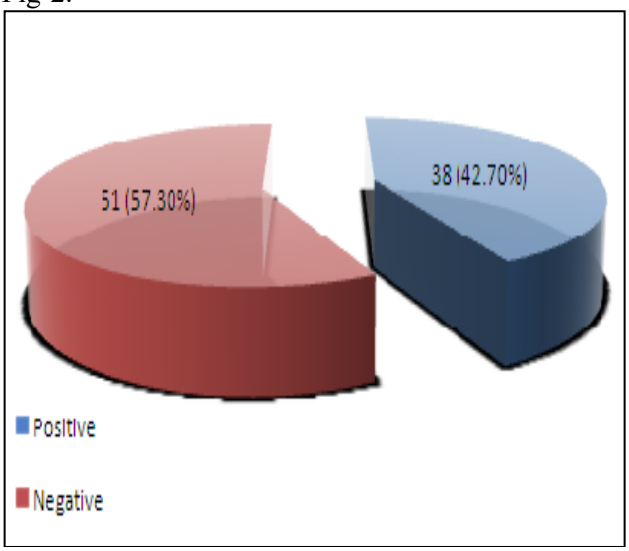

Fig-2: Frequency of overexpression of Her2/Neu in urothelial carcinoma of bladder (n=89)

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.

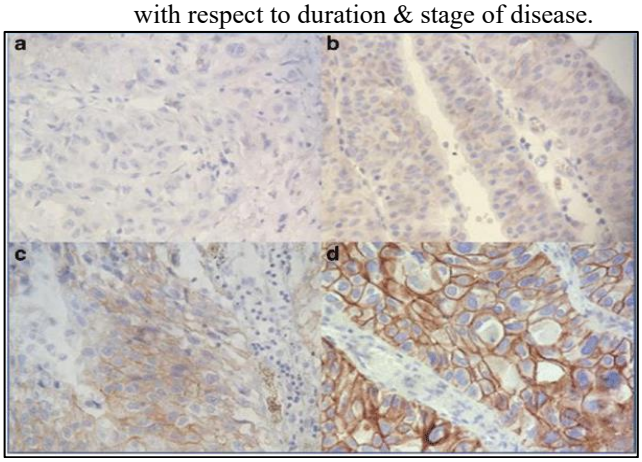

Fig-3: In urothelial bladder cancer, a score of 0 indicates no HER2 expression. There was just little staining for HER2, hence the score was 1+. 2+ for moderate HER2 staining. Three-plus positive staining for HER2

DISCUSSION

The intrinsic tyrosine-kinase activity of the HER2 gene helps the body accomplish a physiological function—cell proliferation. Family relatives of the EGFR include EGF2, NEU, HER3, and ERBB2. It's also known as the NEU gene for abbreviation's sake. 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 for one in four. Her2/Neu was overexpressed in 38 out of 100 cases of bladder urothelial cancer (42.70 percent). 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 about whether HER2 expression corresponds with overall survival in UC[29, 30]. According to the aforementioned research, HER-2 overexpression was found in 23 of 39 tumours (59% of the total), however this difference was not statistically significant. Over-expression of HER-2 was linked to advanced tumour status (p=0.011). Squamous cell carcinomas (SCCs) lack HER-2 expression, but TCCs and ACs express it at greater levels than SCCs (SCC). There was no correlation between patients' ages or genders and HER-2 expression levels[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 tumor growth and recurrence, according to the Olsson study[14]. HER2-IHC analysis was carried out using entire tumor sections in compliance with the ASCO/CAP recommendations for breast cancer, according to Olsson and colleagues[36].

IHC and FISH were recently employed by Chen et al[37]. in a similar work to assess the HER status of NMIBCs on TMA. Testing for HER2 status may be helpful in identifying NMIBC patients who require careful monitoring since it was identified in 9% of persons with high-grade NMIBC. The Hercep Test, developed by Krueger et al., was utilised to identify HER2 gene amplification in patients who had radical cystectomy for muscle-invading malignancy[38].

HER-2 overexpression was not linked to tumor stage in high-grade bladder tumours. Pathological staging and tumor 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.

To our knowledge a long term follow up in patients taking targeted therapies for HER2 may substantiate its prognostic significance, and lack of such data is limitation of our study. Besides that a similar approach for HER2 equivocal cases to be confirmed on FISH is necessary to add statistical significance to the data available.

CONCLUSION

This research indicates that urothelial carcinomas have an exceptionally high rate of Her2/Neu overexpression. Keeping in view past data and findings of this study we recommend Her2/Neuimmuno-staining for these high-risk patients on a frequent basis in order to substantiate the prognostic significance of HER2, as to tailor targeted therapies for individual patients and thus reduce community mortality and morbidity.

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