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Original Article

Study of HER2 Expression by Immunohistochemistry in Papillary Urothelial Carcinoma of Urinary Bladder and its Comparison in Low Grade and High Grade Histological Subtypes

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Abstract:

Introduction: HER2 protein expression in various carcinomas has a prognostic value. In urothelial carcinoma also, it has been found to be of prognostic and predictive importance. Like in other carcinomas, the search for HER2 expression has mainly focused on the utilization of the target therapies such as Trastuzumab against HER2 positive urothelial carcinomas.

Objective: Our objectives were to determine the frequency of HER2 expression in urothelial carcinoma, to identify the association between HER2 expressions with grade of the tumor and to compare the expression patterns/ score of HER2 in low grade and high grade urothelial carcinoma.

Material and Methods: This cross-sectional study was conducted in Department of Histopathology, Shaheed Zulfiqar Ali Bhutto Medical University, Pakistan Institute of Medical Sciences. Total 104 urothelial carcinoma cases of urinary bladder were selected. Of these, 52 cases were of low grade and 52 were of high grade. Immunohistochemistry for HER2 was applied on these cases and their subsequent expression and scores were noted.

Results: Of the 104 cases, 87.5% were males and 12.5% were females. Mean age was 58.8 years with standard deviation of 11.9 years. Lamina propria invasion was seen in 58 out of 104 cases. HER2 expression was seen in 42.3% of urothelial carcinoma. Forty eight percent of high grade and 36.5% of low grade cases showed HER2 positivity. Among cases without lamina propria invasion, 32.6% were HER2 positive, while among cases with lamina propria invasion, 50% were HER2 positive.

Conclusion: HER2 expression was significantly associated with high grade carcinoma and lamina propria invasion. No significant association was found between grade of tumor and score of HER2.

Key words: Papillary urothelial carcinoma, Urothelial carcinoma low-grade, Urothelial carcinoma high grade, HER2

Introduction

Urinary bladder is frequent site for development of urothelial malignancies. According to global cancer statistics, it is the ninth most commonly reported malignancy occurring worldwide, with its highest incidence occurring in Southern Europe being 36.7 per 100,000.[1]

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Throughout the world, male gender appears to be affected more as compared to female. In Pakistan, bladder cancer is the eighth most common malignancy and ranks as the fourth most frequent cancer in men.

A research carried out in the North West Pakistan stated a 5.4% prevalence of urinary bladder carcinoma with a 5.75% frequency among men and 1.61% among women.[2] About 90% of the malignant cancers of the bladder are Urothelial carcinoma. A study conducted in South Pakistan showed a high proportion of urothelial type i.e. 93.3%.[3] Another study conducted in Armed Forces Institute of Pathology (AFIP)

Rawalpindi revealed a 100% frequency of urothelial variety.[4]

most recent grading system is the World Health Organization (WHO) classification proposed in 2016. It has mainly divided the urothelial tumors into Non-invasive and Infiltrating types. Non-invasive (pTa) tumors have been further subdivided as Papillary and Flat lesions. By definition, invasive (pT1) carcinoma breeches the basement membrane, invades lamina propria or muscularis propria. Around 20% of low grade carcinomas are invasive while 95% of the invasive carcinomas are high grade.[5, 6]

Previously, surgical excision followed by chemotherapy had been adopted for treatment of invasive urothelial carcinoma. Due to improved understanding of the molecular genetics of tumorigenesis, the most recent advancements in the treatment of urothelial neoplasms have been the use of targeted therapies. One such therapy has been targeted against HER2.[7]

Human epidermal growth factor receptor type 2 (HER2) is a trans-membrane glycoprotein. It belongs to Epidermal Growth Factor Receptor (EGFR) family of tyrosine kinase receptors. Its regulatory gene (HER2 proto-oncogene) is located on chromosome 17q21. Different scoring systems have been used for the scoring of HER2 as detected by immunohistochemistry. Some of the studies have taken membrane staining score 0 and 1+ as negative while 2+ and 3+ positive.[8,9,10] The others have taken membrane staining score 0 and 1+ as negative, score 2+ as equivocal while 3+ as strongly positive. These studies suggest use of Fluorescent-in-situ-hybridization (FISH) for further verification of HER2 gene amplification.[11] Positivity of HER2 in urothelial malignancies has been reported in various studies ranging from 24% to 71% [12] with a higher percentage frequency in the high grade tumors as compared to low grade tumors.[10,11] HER2 positivity has been variably associated with earlier tumor recurrence, worsened pathological stage, high tumor grade and decreased survival.[13] In recent medical advancements, therapeutic agents directed against this protein are Trastuzumab, Lapatinib and Pertuzumab.[14]

The stratification of patients according to HER2 status in urothelial carcinoma may be helpful in optimizing individualized treatment, as patients showing HER2 positivity might potentially benefit from target therapy against HER2. Old-age patients, patients with co-morbids and in patients having advanced metastatic disease in which surgical therapy may have limited benefits may be the beneficiaries.

This study focuses on expression of HER2 in urothelial carcinoma and comparing the expression patterns of HER2 in low grade and high grade urothelial carcinoma in our population.

Objectives

To determine the frequency of HER2 overexpression by immunohistochemistry in papillary urothelial carcinoma.

To analyze the association of HER2 overexpression with histological grade of urothelial carcinoma .

3. To statistically compare the scores of HER2 between two groups of papillary urothelial carcinoma identified as High grade and Low grade within the study duration.

Methodology

Ethical approval was taken from Ethical Review Board (ERB) committee.All urinary bladder biopsies and TURBT specimens received in the Department of Pathology, Pakistan Institute of Medical Sciences from May 2017 and July 2019, were collected. Patients' data and registration number with relevant details were recorded.

After fixation of the specimen in 10% formalin, it was submitted in tissue cassettes. Tissue was then processed in automated tissue processor, LIECA TP-1020, followed by cutting of 3 to 4 micron tissue sections. The sections were then mounted on glass slides and staining of the tissue with Hematoxylin and Eosin (H&E) in a tissue stainer Shandon Varistan 24-4 was done.

The slides were examined under Olympus CX 22 LED series microscope by post-graduate resident along with supervisor and diagnoses were recorded. Fifty two cases each of low grade papillary urothelial carcinoma and high grade papillary urothelial carcinoma were taken for study, making a total of 104 cases.

Three to four micron sections of the selected 104 blocks were prepared. Immunohistochemistry were applied for HER2.

The slides stained were examined under light microscope. HER2 positivity was interpreted as brown coloured staining in the cell membrane of tumor cells. Scoring and interpretation was done according to the criteria recommended by American Society of Clinical Oncology (ASCO) [14] (Table 1).

Table 1: Scoring criteria for HER2 in urothelial carcinoma

HER2 Expression	Score	Description
Negative	0	No membrane staining OR Incomplete and faint membrane staining within ≤ 10% of tumor cells
	1+	Incomplete and faint membrane staining within >10% of tumor cells
Equivocal	2+	Circumferential and moderate membrane staining within >10% of tumor cells OR Circumferential and intense membrane staining within ≤ 10% of tumor cells
Positive	3+	Circumferential and intense membrane staining in >10%of the tumor cells

All data was assembled on a Master chart and data analysis was done by using SPSS version 21.
Qualitative variables such as gender, histological grade, lamina propria invasion and HER2 expression were expressed as frequencies.
For age, mean and standard deviation were calculated. Patients were categorized into two groups i.e. less than or equal to 60 years and greater than 60 years. Second, the patients were distributed into six categories according to age range i.e. 30-39 years, 40-49 years, 50-59 years, 60-69 years, 70-79 years and 80-89 years.
Chi square test was applied for analyzing the association of HER2 expression with histological grade, lamina propria invasion with histological grade, HER2 expression with lamina propria invasion and score of HER2 with histological grade.
In addition, association of HER2 expression with age groups and gender was also determined.
p value of less than 0.05 was considered significant.

Results

Total 104 cases of urothelial carcinoma of the bladder were taken over a period of 26 months. These comprised of 52 high grade and 52 low grade cases.
Of 104 cases, 91 (87.5%) patients were male, while 13 (12.5%) patients were female.
The minimum age was 30 years and the maximum age was 85 years. The mean age was 58.81 years, with standard deviation of 11.9 years. Out of the 104 patients, 56 were of age ≤60 years while 48 were of age >60 years. Lamina propria invasion was seen in 58 cases (55.7%). Association of lamina propria invasion with high grade carcinoma was significant (p=0.006)
Out of the total 104 cases, 42 (40.4%) were negative for HER2 expression, 18 (17.3%) were equivocal, while 44 (42.3%) came out to be positive (Fig.1).

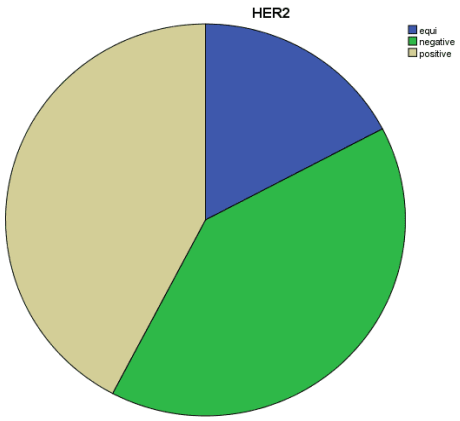


Figure 1: Pie chart showing percentage frequency distribution of HER2 status in urothelial carcinoma (n=104)

Of the 52 high grade cases, 25 (48%) were HER2 positive, 12 (23.2%) were equivocal and 15 (28.8%) were negative, while out of the 52 low grade cases, 19 (36.5%) cases were HER2 positive, 6 (11.5%) were equivocal and 27 (52%) were negative. The association of HER2 expression with grade of the tumor was significant using Chi square test (Table 2).

Table 2: Association of HER2 expression with histological grade of tumor

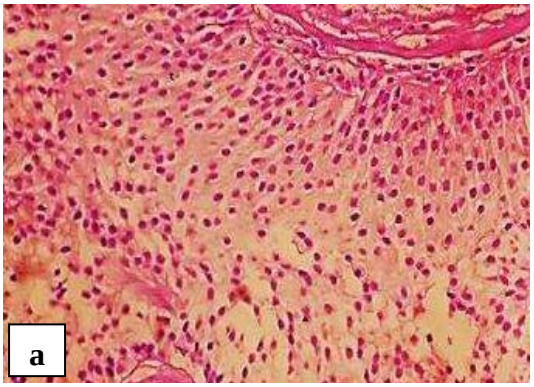
Histological grade	HER2			Total	
	Negative No. (%)	Equivocal No. (%)	Positive No. (%)		
High	15 (28.8)	12(23.2)	25 (48)	52	p=0.04
Low	27 (52)	6 (11.5)	19 (36.5)	52	
Total	**42**	**18**	**44**	**104**	

Out of the 46 cases without lamina propria invasion, 15 (32.6%) were HER2 positive, 7 (15.2%) were equivocal and 24 (52.2%) were negative. Of the 58 cases with lamina propria invasion, 29 (50%) were HER2 positive, 11 (19%) were equivocal, while 18 (31%) were HER2 negative (Fig. 2). The p value was significant (p=0.03)

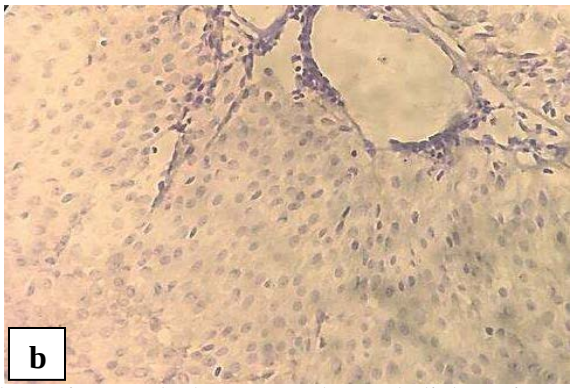
Of total 104 cases, the highest frequency of cases i.e. 42.3% showed score 3+. Of the 52 high grade cases 25 (48%) showed score 3+, 12 (23%) showed score 2+, 8 (15.4%) showed score 1+ and 7 (13.6%) showed score 0. Of the 52 low grade cases, 19 (36.5%) showed score 3+, 18 (34.6%) showed score 0, 9 (17.4%) showed score 1+ and 6 (11.5%) showed score 2+.There was no significant association of HER2 score with the grade of tumor (p=0.052).

Out of the 44 positive cases, 36 (82%) were male and 8 (18%) were female. Out of the 18 equivocal cases, 17 (94.5%) were male and 1 (5.5%) was female. Out of the 42 negative cases, 38 (90.5%) were male and 4 (9.5%) were female. There was no significant association of HER2 expression with gender (p=0.29)

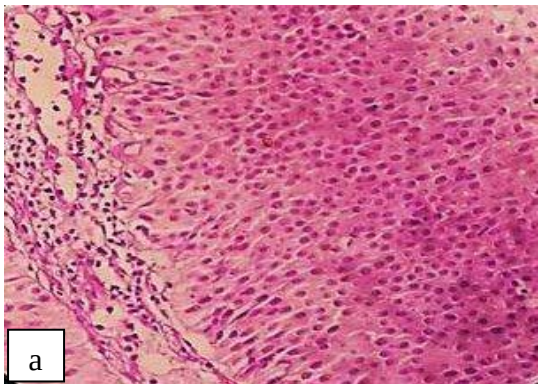
By categorizing the patients into age groups, we found that in age group ≤60 years 24/56 (43%) showed HER2 positivity, while in age group >60 years, 20/ 48 (41.7%) showed HER2 positivity. There was no significant association of age group with HER2 expression (0.66).



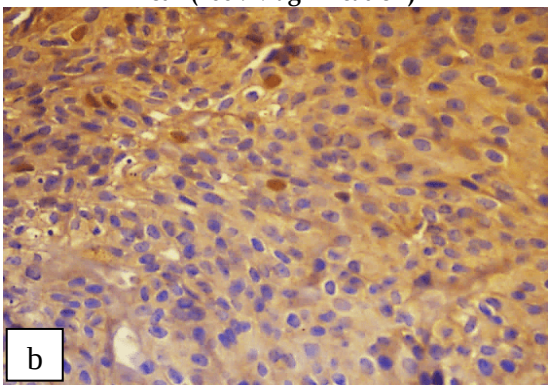
Photomicrograph 1a: Low Grade Urothelial Carcinoma; H&E (400x Magnification)



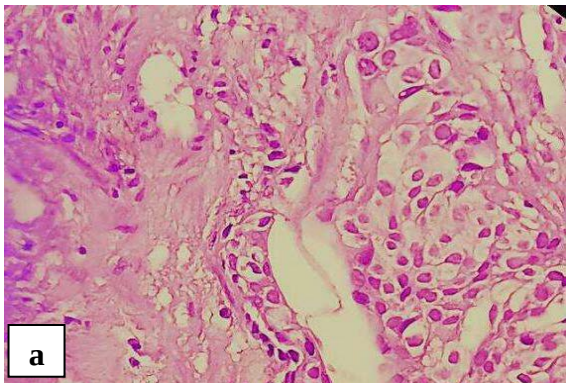
Photomicrograph 2b: Low Grade Urothelial Carcinoma; HER2 stain negative, Score 0 (400X magnification)



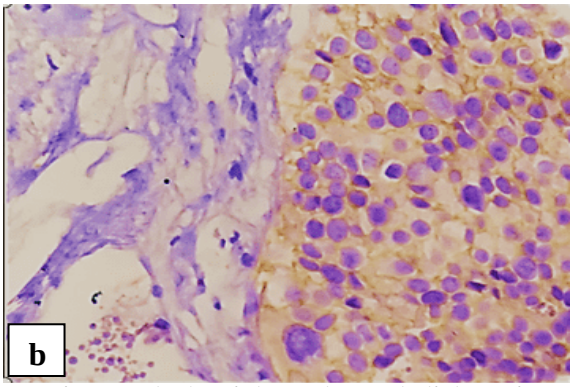
Photomicrograph 2a: Low Grade Urothelial Carcinoma; H&E (400x Magnification)



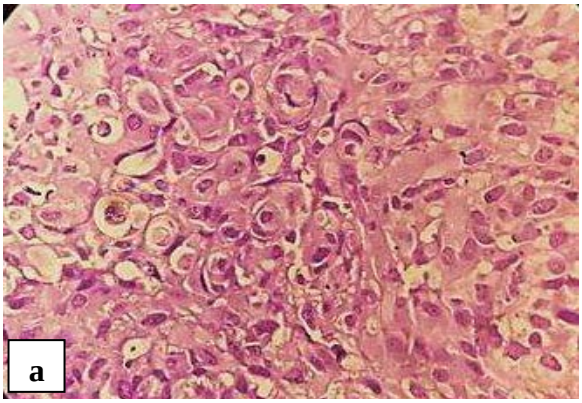
Photomicrograph 2b: Low Grade Urothelial Carcinoma; HER2 stain negative, Score 1+, incomplete and faint membrane staining by IHC (400X magnification)



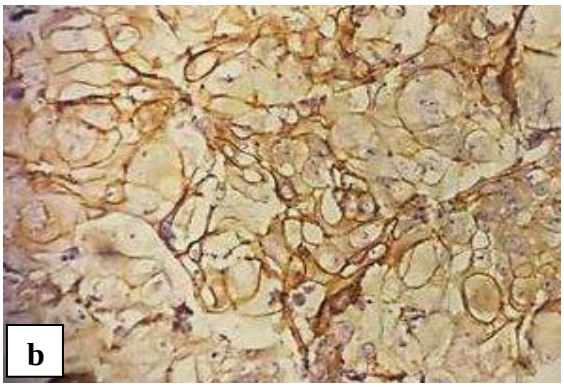
Photomicrograph 3a: High Grade Urothelial Carcinoma with Lamina Propria Invasion; H&E (400x Magnification)



Photomicrograph 3b: High Grade Urothelial Carcinoma; HER2 stain positive, Score 2+, circumferential membrane staining by IHC in 15% tumor cells with moderate staining intensity (400X magnification)



Photomicrograph 4a: High Grade Urothelial Carcinoma; H&E (400x Magnification)



Photomicrograph 4b: High Grade Urothelial Carcinoma; HER2 Stain Positive, Score 3+, circumferential membrane staining by IHC in 70% tumor cells with strong staining intensity (400X magnification)

Discussion

Urothelial carcinoma is more predominant in the male population. This has also been stated by Dobruch et al. in a collaborative review conducted in Europe.[15]Male to female ratio was 7:1 in our study. Malats et al. in 2015 by a systemic review from Spain have shown a male to female ratio of 3:1.[16] The reason for a greater male predominance in our study may be due to a easier access of male patients to heath care facilities in our part of the world. A study by Mubarak et al. done in Southern Pakistan has reported male to female ratio of 5.3:1, which is comparable with our results.[3]

Mubarak et al. have shown mean age of 57.5±8.6 years for urothelial carcinoma in southern Pakistan. [3]In 2015, Ahmed et al. in a study from central Punjab Pakistan found that mean age in males was 57.5±12.9 years and mean age in females was 63.5±13.6 years. [4]Jawad et al. in a study conducted in Iraq reported a mean age of 58.72±1.6 years and age range of 30 to 80 years. [17]

In our study, age range 50 to 59 years showed maximum number of cases of urothelial carcinoma i.e. 29 (27.9%). Suryalakshami et al. showed in a study from India, that peak incidence of urothelial carcinoma was between 61 and 70 years.[18] Nedjadi et al. conducted a study in Saudi Arabia , found a greater percentage (56%) of patients with urothelial carcinoma in age group >60 years as compared to age ≤60 years (44%).[19] In a study conducted in China, Ding et al. also found a higher percentage frequency (52.1%) of urothelial carcinoma in higher age group.[20] Both these findings are in contrast to our study. The reason may be difficulty in access or reluctance of the elderly patients to seek medical help in time.

In our study lamina propria invasion was significantly related to high grade tumors. Humphrey et al. have claimed that >95% of invasive carcinomas are high grade[5] as muscle invasive carcinomas were also included in that study, while our study had cases with lamina propria invasion only.

HER2 was expressed in 42.3% of urothelial carcinomas in our study. The frequency of expression of HER2 in urothelial carcinoma has a variable range in literature due to the different scoring criteria used by different studies, as well as whether or not FISH analysis was used. American Society of Clinical Oncology (ASCO)recommended that the equivocal positivity of HER2 be confirmed by FISH analysis for therapeutic purpose.[14] Similar to our study, taking only 3+ score as positive and 2+ as equivocal, Danaei from Iran have reported HER2 overexpression in 27.4% urothelial carcinomas by IHC, the equivocal cases were not confirmed by FISH.[21] Hammam et al. from Egypt, reported HER2 overexpression in 27% of the urothelial carcinomas by IHC, however, the number of positive cases increased to 33% after confirmation by FISH. [11] In contrast to our study, taking both 2+ and 3+ scores as positive, Kumar et al. from India and Ismail et al. from Egypt have reported a positivity of 70% and 48.3% respectively. [10]

The prognostic parameters used in this study were grade of the urothelial carcinoma and lamina propria invasion.

The association of HER2 expression in this study with high grade of the tumor came out to be significant. Jamal et al. from Army Medical College, Rawalpindi reported that 47.5% of high grade carcinomas and 16.6% of the low grade carcinomas were positive for HER2.[22] Similar has been reported by Jawad et al. from Iraq[17], Hammam et al. and Ismail et al. from Egypt [10,11] and Charfi et al. from Tunisia.[23] This relation is evidently helpful in the fact that target therapies with anti-HER2 agents may be more efficiently applied in the tumors of high grades which have a higher potential to become metastatic. As opposed to our study, Kumar et al. from India and Nedjadi et al. from Saudi Arabia, suggested that there was no significant association between grade of tumor and HER2 expression.[12, 19] The difference may be due to variation in scoring criteria.

Association of HER2 expression with lamina propria invasion was significant in our study. Similar to our study, Ding et al. presented a significant difference of HER2 expression between Ta and T1 urothelial tumors.[20] In contrast; Kumar et al. showed that no significant difference. [12]

Statistically, there was no significant association between histological grade of tumor and score of HER2. This was also studied by Jawad et al, who concluded that maximum number of high grade tumors showed score 3+ and maximum number of low grade tumors showed score 1+. [17] Kumar et al. and Alexa at al. showed that the intensity of HER2 expression increases with grade of the urothelial carcinoma.[9,12] The difference of these results from our study might be due to differences in pre-analytical techniques involved in immunohistochemistry and also, some degree of inter-observer variability in the diagnosis of low grade and high grade carcinoma, especially due to changing classification over the years. [5, 24, 25]

The association of clinical parameters , age and gender of the patients, was not significant. Danaei et al. showed that males showed 91.8% positivity for HER2 in urothelial carcinoma, while females showed 8.2%.[21] This difference in percentage might be due to the difference in sample size. Like our study, Ismail et al.[10], Jawad et al. [17]and Soria et al. [26]did not find any correlation between gender and HER2 expression.

Age range 50-59 and 60-69 years showed maximum number of HER2 positive cases, a greater percentage of younger age group showed HER2 positivity, but the association was statistically insignificant. Nedjadi et al. and Ding et al. found a higher frequency of HER2 positive cases in older age groups but the association between age and HER2 expression were insignificant, similar to our study. [19,20]The possible reason for a greater expression of HER2 in younger age group in our study might be due to a greater number of younger age group patients recruited in our sample.

Conclusion

HER2 was expressed in 42.3% of urothelial carcinoma. There was a significant association of HER2 expression with high grade urothelial carcinoma. No significant difference in expression scores of HER2 between high grade and low grade urothelial carcinoma was found. HER2 expression was significantly higher in T1 urothelial carcinomas as compared to Ta. No significant association of HER2 expression with gender and age of the patient was identified.

Limitations and Recommendations

The score 2+ was taken as equivocal, not confirmed by gene analysis due to constraints of resources.

Confirmation of HER2 status, especially for equivocal cases, by FISH for gene amplification should be done in view of better patient stratification for potential anti-HER2 therapy.

Conflict of Interest: Authors declare no conflict of interest.

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KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

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