

Her2neu expression in urinary bladder cancer: Histopathological, clinico-radiological and epidemiological aspects

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Abstract

Objectives: Her2/neu in bladder carcinomas as a prognostic marker has been widely studied, but with variable results. The potential for development of anti Her2/neu targeted therapy in Her2/neu positive tumours, warrants the need for a definitive conclusion on the Her2/neu status. Therefore, this study is undertaken to evaluate possible correlation between Her2/neu expression and various epidemiological, clinico-radiological and histological variables.

Materials and Methods: This prospective study was performed in the Pathology department, on 43 patients with bladder malignancies, diagnosed clinically in the Surgery Department at a tertiary care medical institute of Rohilkhand region. Formalin fixed TURBT specimens were processed as per protocol. Hematoxylin and Eosin stained sections were observed microscopically along with immunohistochemistry for Her2/neu, by three independent observers. χ^2 test with Yates correction was applied to evaluate possible association of Her2/neu with various variables. P value of <0.05 was considered statistically significant.

Results: Her2/neu positivity was seen in 41.86% of the cases. Her2/neu expression was found to be statistically significant with respect to histological grade ($p = 0.026$) and pathological stage ($p=0.025$). Statistically insignificant results were obtained for Her2/neu expression with respect to patient age, gender, history of smoking, presenting complaints and ultrasonographic findings.

Conclusion: A positive association between Her2/neu expression and tumour grade and stage suggests that Her2/neu status maybe helpful in formulating the treatment plan for patients of urothelial carcinoma. However, further studies with larger sample size and correlating Her2/neu expression with the patient survival are required to develop anti Her2/neu targeted therapy.

Introduction

Urinary bladder cancer ranks ninth in worldwide cancer incidence.¹ As per the Indian cancer registry, bladder cancer constitutes the eighth most common tobacco related malignancy.² Her2/neu has been widely studied as a prognostic marker in many malignancies. HER2 (also known as Neu, ErbB2) is a member of the epidermal growth factor receptor (EGFR; also known as ErbB) family of receptor tyrosine kinases, which influence the regulation of cellular proliferation, transcription, autophagy, apoptosis and chemotaxis.^{3,4} Ligand binding of these receptors causes its dimerization and activates the intracellular kinase domain, leading to phosphorylation of tyrosine residues and transduction of mitogenic signals.⁵

Antitumor antigen-specific immunotherapeutic strategies (tumor vaccines) and modulators of HER2/neu expression, such as inhibitors of HSP90, are advancing through clinical studies and have shown promising results.⁶ Thus, Her2/neu status of a tumor can be helpful in formulating the management plan and a predictor of prognosis, as seen in the case of breast malignancy where

Her2/neu targeted therapy has achieved wide acceptance. The success of anti Her2/neu targeted therapy in breast carcinomas has enthused interest in the role of this oncogene in tumor progression at other sites. Prevalence of Her2/neu in urinary bladder carcinoma and its prognostic significance carcinoma with respect to histopathological prognostic parameters such as tumor grade, tumor stage and tumor type is controversial, with several conflicting results in literature.⁵⁻⁹ The wide variability observed in the level of Her2/neu expression in various studies can be attributed to differences in techniques of interpretation (immunohistochemistry, FISH, BDISH), kits, methods, solutions, and subjective interpretation of staining result. Thus, the available literature in this field is extremely difficult to compare, and definite conclusions are hard to draw from the accumulated data.

Therefore, this study was undertaken to evaluate Her2/Neu protein expression in bladder cancers using immunohistochemistry and to determine its possible association/correlation with epidemiological, clinical,

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radiological and histopathological parameters of prognostic significance in patients of urinary bladder malignancies.

Materials and Methods

In this prospective study, 43 formalin fixed Trans-Urethral Resection of Bladder Tumor (TURBT) specimens from 2016 to 2018 were analysed in the Department of Pathology, at a tertiary care medical institute of Rohilkhand region, India, after obtaining clearance from the Institutional Ethics Committee.

Hematoxylin and Eosin staining was performed on 4 μ m thick sections. Glass slides containing tissue sections were rinsed into several jars filled with xylene, graded series of ethanol solutions, hematoxylin, acid alcohol, and eosin. The slides prepared from paraffin blocks of the specimens were stained with the conventional hematoxylin and eosin method and a preliminary scanning was done to include all well-preserved specimens and exclude all inadequate, autolyzed specimens, benign pathologies of the urinary bladder and specimens without detrusor muscle. All 43 cases of bladder malignancies enrolled in the study, were re-evaluated for histological diagnosis by three independent observers using WHO/ISUP 2004 classification.

Cohesive carcinomas with predominantly ordered architecture, minimal loss of polarity, mild nuclear pleomorphism, mild variation in nuclear chromatin, inconspicuous nucleoli and occasional mitotic figures were considered low grade. Discohesive carcinomas with predominantly disordered architecture, frequent loss of polarity, marked nuclear pleomorphism, marked variation in nuclear chromatin, prominent nucleoli and frequent mitotic figures were considered high grade.¹⁰ (Fig. 1)

Staging was done on the basis of depth of invasion. pTa being non-invasive, pT1 Lamina invasive and pT2 muscle invasive carcinomas.¹¹ (Fig. 1)

Immunohistological staining was further performed on formalin-fixed paraffin embedded tissue sections using antibodies against Her2. 4 μ m tissue sections were deparaffinised at 37°C for one day, followed by 58°C overnight and two changes of Xylene for 30 min each. Then, slides were rehydrated in a graded series of ethanol solutions. Antigen retrieval was done using microwave oven followed by two changes of TBS buffer for 15 minutes each. The slides were then treated with peroxidase block for 15 minutes to block nonspecific reaction with the other tissue antigens. After removing excess block, the slides were treated with primary antibody for 45 minutes. This was followed by three washes of TBS buffer, treatment with SS enhancer and then three changes of TBS buffer again. The slides were then treated with secondary antibody for 30 minutes. The slides were washed in TBS and stained with substrate - chromogen solution known as 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 5 minutes. The counterstaining was performed with hematoxylin for 1 minute and washed in water. The stained slides were immersed in graded series of ethanol and then, xylene to transparency and dehydration of tissues. Then slides were mounted to study under a microscope.

Her2/neu has a membrane staining that is scored based on complete or incomplete staining and the intensity of staining of the tumor cells. Score 0 presents no membrane staining or less than 10% of the tumor cells; score 1+ presents faint membrane incomplete staining in more than 10% of the tumor cells; score 2+ presents a weak or moderate complete membrane staining in more than 10% of the tumor cells; score 3+ presents a strong complete membrane staining in more than 30% of the tumor cells (Fig. 2). The same was applied in the present study. Grade 2+ and 3+ were considered positive.^{6,7} Adequate positive controls were used.

Data was analyzed using the SPSS (V.23). The association between Her2/neu expression with respect to tumour grade, type and pathological stage was assessed using χ^2 test. Yates correction with continuity was applied wherever applicable. P- value less than 0.05 was considered statistically significant at 95% confidence interval.

Results

In the present study 41.86% of the cases were Her2/neu positive (grade 2 and 3) and 58.14% of the cases were Her2/neu negative (grade 0 and 1) [Fig. 3]

Out of the 43 cases enrolled in the study, majority of them were above 60 years of age comprising 60.47% of the cases [Fig. 3]. The mean age of patients was 64.28 \pm 12.82 years, the age range being 32 to 88 years Highest frequency of Her2/neu positive cases was seen in the age above 70 years, comprising 33.33% (n=6) of all the Her2/neu positive cases. The association between Her2/neu expression and age was not found to be statistically significant (p=0.98) [Table 1].

In the present study, males outnumbered females, comprising 88.37% (n=38) of the cases [Fig. 4]. There was no statistical significant association between Her2/neu expression and gender of the patient (p=0.69). [Table 2]

In the present study, majority of cases observed were smokers comprising 67.44% (n=29) while 32.66% (n=14) were non-smokers. Her2/neu expression was higher in smokers comprising 83.33% of the cases. There was no statistical significant association between Her2/neu expression and history of smoking. [Table 3]

Hematuria was observed in all the enrolled cases. Hematuria was accompanied with other complaints in 11.63% of the cases. Her2/neu positivity was seen more frequently in patients with isolated hematuria, comprising 84.62% of the cases. There was no statistical significant association between presenting complaints and Her2/neu expression. [Table 4]

As seen on ultrasonography, majority of the cases presented as an irregular mass comprising 62.79% of all cases. Lateral wall was the most frequently involved site, comprising 34.88% of the cases. Equal frequency was seen in growths presenting with a mass less than 3 cm and between 3-7 cm in size, comprising 41.86% of cases each.

In polypoidal lesions, Her2/neu positivity was more, comprising 55.56% of the cases. Highest number of Her2/neu positive cases were seen in cases presenting as

irregular masses, comprising 55.55% of the cases. Her2/neu positive cases were seen most frequently in cases involving the lateral wall of the bladder, comprising 38.88% of the cases. Her2/neu positivity was seen most frequently in lesions less than 3 cm, followed by lesions between 3 and 7 cm and lesions greater than 7 cm. There was no statistical significant association between Her2/neu expression and type, site or size of growth. [Table 5]

Fig. 5 represents the distribution of cases with respect to various histopathological parameters observed. Twenty three percent (n=10) of all urothelial carcinomas showed squamous differentiation. Most of the cases were high grade comprising 67.44%(n=29) of the cases while 32.56%(n=14) cases were low grade. pT2 stage was the most frequently seen stage, comprising 46.51%(n=20) of the cases. pT1 stage was seen in 44.19%(n=19) cases and pTa stage was seen in only 9.3%(n=4) cases.

Of the 43 cases analysed, Her2/neu positivity was seen in 41.86% (n=18) of the cases while 58.14% (n=25) of the cases were Her2/neu negative. Strong Her2/neu protein expression (3+) was observed in 11 of 43 cases (25.58%) while 7 cases (16.28%) were scored as 2+. A score of 0 and 1+ was seen in 20 (46.51%) and 5 (11.63%) cases, respectively. (Fig. 2)

Table 6 depicts the distribution and comparison of histological parameters observed with Her2/neu expression. Among Her2/neu positive cases, high grade carcinomas comprised 88.89% (n=16) of the cases while low grade

carcinomas comprised only 11.11% (n=2). The prevalence of Her2/neu positivity in low grade carcinomas and high-grade carcinomas was 16.67% and 44.83%, respectively. A statistically significant association was observed between the grade of urothelial carcinoma and Her2/neu expression (p= 0.026).

Her2/neu positive cases were seen slightly more in urothelial carcinomas than urothelial carcinomas with squamous differentiation, comprising 55.56% (n=10) and 44.44% (n=8) of the cases, respectively. Among urothelial carcinomas, Her2/neu positivity comprised only 43.47% (n=10) of the cases, while among urothelial carcinomas with squamous differentiation, Her2/neu positive cases were more frequent, comprising 80% (n=8) of the cases. There was no statistical significant association between Her2/neu expression and histological variant of urothelial carcinoma (p= 0.11).

Her2/neu positive cases were seen most commonly in pT2 stage, comprising 72.22% (n=13) of all cases followed by pT1 stage where positivity was 16.67% (n=3). Her2/neu positivity was least frequently seen in pTa stage, comprising 11.11% (n=2) of the cases. The frequency of Her2/neu positivity among individual stages was highest among cases of pT2 stage (65%) followed by stage pTa (40%) and pT1 stage (16.67%). A statistically significant association was observed between the pathological stage and Her2/neu expression (p= 0.025).

Table 1: Distribution and comparison of Her2/neu expression with respect to age

Age group	Positive		Negative		Yates χ^2 value	P value
	n	%	n	%		
<50	3	16.67	3	12	0.12	0.98
51-60	4	22.22	7	28		
61-70	5	27.78	7	28		
>70	6	33.33	8	32		
Total	18	100	25	100		

Table 2: Distribution and comparison of Her2/neu expression with respect to gender

	Positive		Negative		Yates χ^2 value	P value
	n	%	n	%		
Male	16	88.89	22	88	0.154	0.69
Female	2	11.11	3	12		
Total	18	100	25	100		

Table 3: Distribution and comparison of Her2/neu expression with history of smoking

Her2/neu expression	Positive		Negative		Yates χ^2 value	P value
	n	%	n	%		
Smokers	15	83.33	14	56	2.425	0.11
Non-smokers	3	16.67	11	44		
Total	18	100	25	100		

Table 4: Distribution and comparison of Her2/neu expression with presenting complaints

	Positive		Negative		Yates χ^2 value	P value
	n	%	n	%		
Isolated hematuria	16	84.62	22	89.29	0.154	0.69
Hematuria with other complaints	2	15.38	3	10.71		
Total	13	100	28	100		

Table 5: Distribution and comparison of Her2/neu expression with ultrasonographic findings

Ultrasonographic findings	Histological grade	Positive		Negative		Yates χ^2 value	P value
		n	%	n	%		
Site	Base	3	16.67	4	16	1.27	0.86
	Lateral wall	7	38.88	8	32		
	Posterior wall	4	22.22	2	8		
	Posterolateral wall	2	11.11	6	24		
	Superior wall	2	11.11	5	20		
	Total	3	16.67	4	16		
Greatest dimension	<3	9	50	9	36	0.566	0.75
	3 to 7	6	33.33	12	48		
	>7	3	16.67	4	16		
	Total	9	50	9	36		
Type of growth	Polypoid	5	27.78	4	16	0.452	0.79
	Irregular mass	10	55.55	17	68		
	Diffuse thickening	3	16.67	4	16		
	Total						

Table 6: Distribution and comparison of histological parameters with Her2/neu expression

		Positive		Negative		Yates Chi-square value	P value
		n	%	n	%		
Histological grade	Low	2	11.11	12	48	4.914	0.026
	High	16	88.89	13	52		
	Total	18	100	25	100		
Histological variant	Urothelial carcinoma	10	55.56	23	92	2.425	0.11
	with squamous differentiation	8	44.44	2	8		
	Total	18	100	25	100		
Pathological stage	pTa	2	11.11	3	12	7.353	0.025
	pT1	3	16.67	15	60		
	pT2	13	72.22	7	28		
	Total	18	100	25	100		

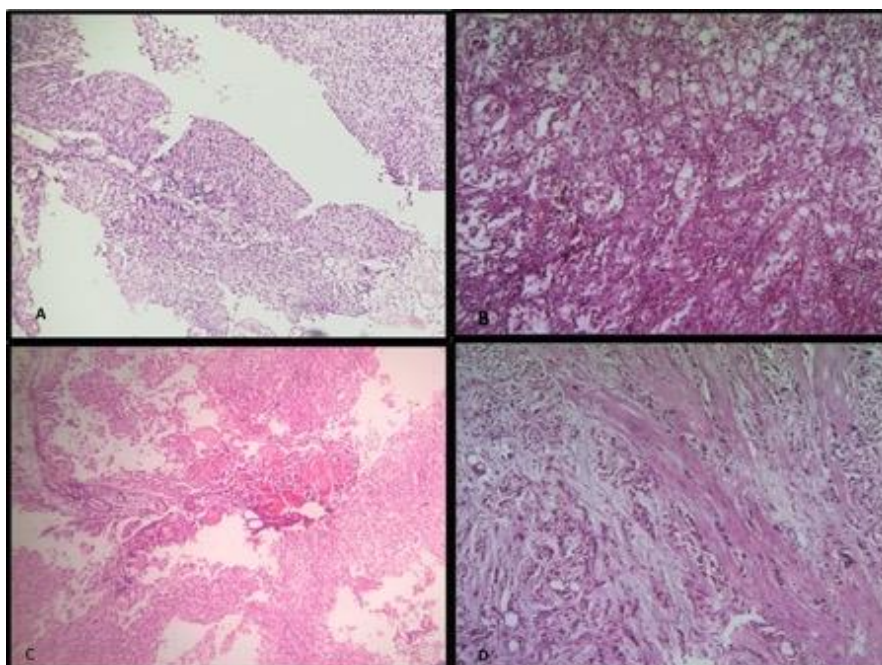


Fig. 1: (A): Low grade urothelial carcinoma; (B): High grade urothelial carcinoma; (C): Lamina invasive urothelial carcinoma, Stage pT1; (D): Muscle invasive urothelial carcinoma, Stage pT2. (H&E, 10x)

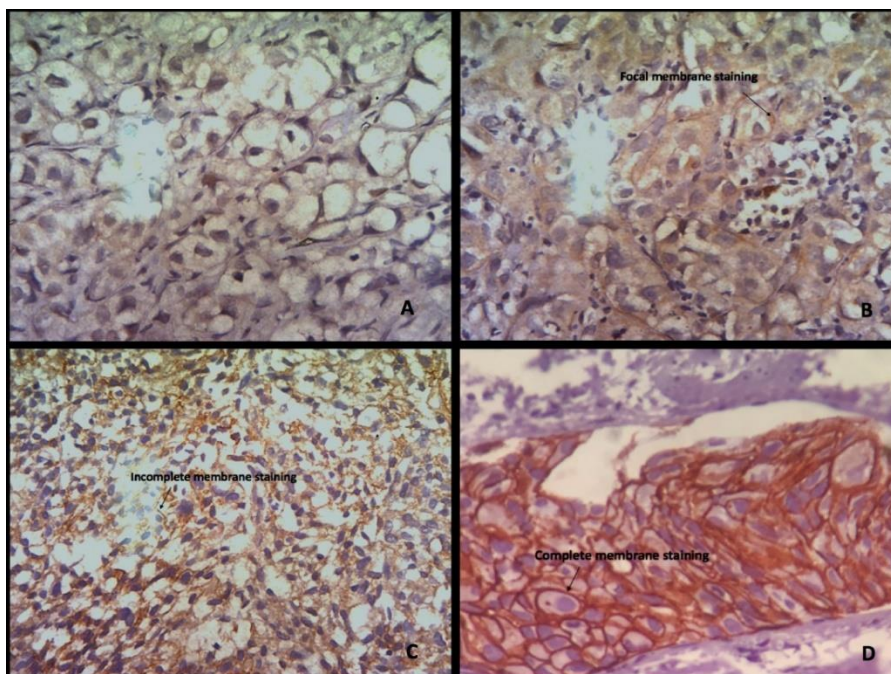


Fig. 2: HER-2 expression in four primary tumors detected by immunohistochemistry; (A): No staining or membrane staining observed in <10% of tumor cells (HER-2 score 0) (40x); (B): Faint or barely perceptible membrane staining detected in >10% of tumor cells: cells are stained only in part of their membrane (HER-2 score 1+) (40x); (C): Weak to moderate, complete membrane staining observed in >10% of tumor cells (HER-2 score 2+) (40x); (D): Strong complete membrane staining observed in >10% of tumor cells (HER-2 score 3+(40x).

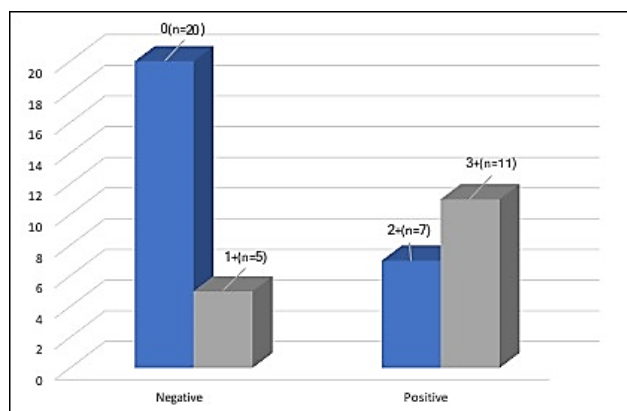


Fig. 3: Frequency distribution of Her2/neu positive (grade 2+,3+) and negative (grade 0,1+) cases.

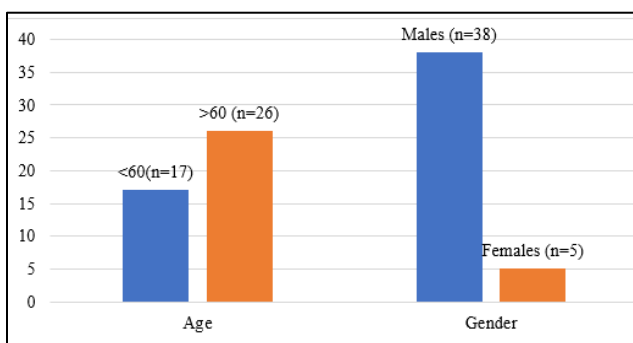


Fig. 4: Distribution of cases with respect to age and gender

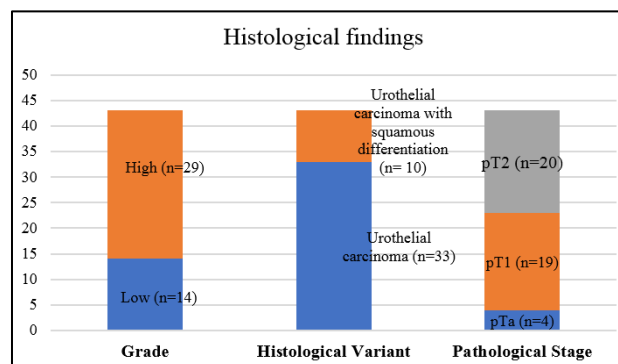


Fig. 5: Distribution of cases based on histological findings

Discussion

In this study, 43 cases of urothelial carcinoma were analysed and an attempt was made to determine the association between Her2/neu expression in urothelial carcinoma with respect to age, gender, clinic-radiological findings and histopathological parameters of prognostic significance, such as grade, type and stage. Urothelial carcinoma was universally observed in all the cases and majority of the cases were high grade, belonging to pathological stage pT2. Her2/neu expression was found to be statistically significant with respect to tumour grade and pathological stage.

The current study did not observe any statistically significant result between Her2/neu expression and age; and although published material on the above relationship shows almost similar results,^{12,13} it is limited. Therefore, studies

with larger sample population exploring the strength of association between the two are warranted for a definitive conclusion. However, the demographic profile pertaining to age of patients in the study is largely similar to those reported in previous studies.

This study observed a broad age range, which was 32 to 88 years, similar to those reported by Biswas et al¹⁴ (30 to 81 years) and Chinnasamy et al¹⁵ (26 to 84 years). Latif et al⁹ and Gupta et al¹⁶ reported a slightly higher age range of 43 to 92 years and 18 to 90 years respectively.

However, the mean age of presentation was 64.28 ±12.82 years which was almost similar to the studies by Kunze et al.,¹⁷ Kucuk et al.,¹⁸ Chinnasamy et al.¹⁵ and Gupta et al.¹⁶ who reported a mean age of 60.2 ± 4.4 years, 69.2 for males and 69.3 for females; 66.1 years and 61.5 years respectively.

The present study further supports the view that urothelial cancers are more prevalent in higher age groups, which has been explained by various authors, based on molecular studies. The processes interact at multiple levels; for example, tumour protein 53 (p53)—a tumour suppressor—is involved in both cancer and aging: alteration of the p53 gene (*TP53*) is the most frequently encountered mutation in human cancers (including bladder cancer), and the efficiency of the response to p53 has been found to vary according to age.¹⁹ Gu et al²⁰ also reported that low p53 expression, which is associated with increasing age, predisposes to higher bladder cancer risk.

Gender predilection for males and cigarette smoking are established inter-related risk factors for urothelial carcinomas.¹⁷ The observations in this study, also support the above stated fact with a 7.2 times more common prevalence in males than females and majority of patients being smokers (68.29%); an observation similar to Biswas et al¹⁴ (75%) and Kucuk et al¹⁸ (85.3%) These observations can be explained primarily on smoking and exposure to occupational hazards being more common in males, and secondarily on the basis of differences in the cellular metabolism of carcinogens in the two gender groups. For example, gender-related differences in a variety of hepatic pathways could lead to differences in the degradation of carcinogens, resulting in differential exposure of the urothelium to carcinogens. In particular, differential expression of isoforms of the enzyme uridine 5'-diphospho glucuronosyltransferase (UGT), which is involved in aromatic amine metabolism in the liver, may be a key mediator in this process.²¹ Smoking has been reported to substantially increase the risk of bladder cancer, as concluded by Zeegers et al.²² The urine of cigarette smokers is mutagenic²³ and it has been suggested that the quantities of 2-naphthylamine found in cigarette smoke might be responsible for the bladder tumour.²⁴

With respect to urothelial carcinomas and Her2/neu positivity, higher expression of Her2/neu was seen in smokers and male gender, however the current study did not observe a statistically significant association with smoking, similar to Kumar et al⁷ (p= 0.096); and gender, similar to Shawkya et al,⁵ Danaei¹³ and Nedjadi et al¹² (p= 0.272, 0.33

and 0.19 respectively). With the available inadequate literature and limited sample sizes in the published studies it may not be prudent enough to derive any definite conclusion which therefore warrants follow-up studies with adequate sample sizes.

Several studies in the past have been undertaken to evaluate the presenting features and radiological findings in urothelial carcinomas; however, Her2/neu expression in urothelial carcinomas and its association with various clinical and radiological presentations remains a largely understudied subject.

Hematuria, whether microscopic or gross, has been known to be the most common presenting symptom of urinary bladder cancers, as also seen in the present study. The origin of hematuria in bladder cancer is from direct haemorrhage of the tumour, however minor it may be.²⁵

Among the 43 cases, hematuria was present in all cases universally, while 12.2% (n=5) of the cases also had other complaints like increased urgency, frequency and pain abdomen. This finding is almost similar to the findings of Murta et al.²⁶ Kumar et al⁷ and Gupta et al.¹⁶ who reported hematuria in 80%, 98% and 97% of the cases, respectively. Similarly, Biswas et al,¹⁴ also reported hematuria as the commonest presenting complaint, in their study.

Radiological evaluation of the 43 urothelial carcinoma cases revealed lateral wall of the bladder as the most common site of involvement and majority of lesions presenting as irregular masses, mostly less than 7 cm in greatest dimension. In contrast to the above findings, Biswas et al.¹⁴ reported posterior wall to be the most frequently involved site (53%) followed by lateral wall (43%).

The current study observed no statistical significance between Her2/neu expression and presenting complaints (p= 0.69) as well as radiological findings, with respect to size (p=0.75), type of growth (p=0.79) and site of involvement (p=). To the best of our knowledge no other study has yet been undertaken to evaluate the above-mentioned relationship and therefore, to draw an unambiguous conclusion further research with adequate sample sizes is essential.

An attempt was made in the present study to evaluate the association of Her2/neu expression in urothelial carcinomas with histological parameters. Among the 43 cases, urothelial carcinoma was the only histological type observed. This finding is similar to that of Biswas et al.¹⁴ and Matalka et al.²⁷ who reported urothelial carcinoma in 100% and 95.7% of all cases. In the present study, 21.95% of cases had squamous differentiation. However, 56.3% of cases studied by Ahmed et al²⁸ had squamous differentiation. No statistical significant association was observed between Her2/neu expression and histological variant.

High grade carcinoma was the most frequently encountered grade in this study, similar to that reported by Ahmed et al.²⁸ and Murta et al.²⁶ On the other hand Biswas et al.¹⁴ and Matalka et al.²⁷ reported a higher frequency of low grade carcinoma comprising of 58% and 60% of the

cases respectively. Majority of the cases belonged to pT2 stage comprising of 46.21% (n=20) cases.

A statistical significant association was found between Her2/neu expression with respect to histological grade and pathological stage. Shawkya et al⁵ and Danaei et al¹³ reported a similar significant association with histological grade (p=1.27) and pathological stage (p<0.001 in both). In studies by Nedjadi et al¹⁴ and Gehani et al²⁹ a statistical significant association was observed between tumour stage and Her2/neu expression (p=0.002 and 0.011), but Her2/neu expression and tumour grade were not found to be significantly associated (p= 0.62 and 0.21). However, Kumar et al⁷ reported that there was no statistically significant correlation between tumour grade as well as stage and Her2/neu expression

In breast, ovarian, prostate, pancreatic and liver malignant tumors, HER2/neu overexpression is associated with bad prognosis.³⁰ Positive HER-2/neu status has been linked with aggressive tumour behavior and resistance to cytotoxic and endocrine therapies.³¹ This could explain the higher frequency of Her2/neu positive cases in high grade carcinomas and carcinomas of the pT2 stage.

Statistically insignificant results are obtained for various clinic- epidemiological parameters, ultrasonographic findings and histological variants of urothelial carcinoma, and the present study cannot definitely comment on their probability of association with Her2/neu expression because of their small individual sample size; and therefore, this necessitates follow-up studies with a larger sample size for definite conclusion.

It is necessary to carry out further studies on malignant urinary bladder neoplasms with larger sample size and correlating Her2/neu expression with the clinico-pathological prognostic parameters and patient survival. This will provide an opportunity for development of herceptin targeted therapy for urinary bladder carcinoma.

Conclusion

In the current study, 43 cases of urothelial carcinoma were analysed for Her2/neu expression. A positive association was observed between Her2/neu expression and tumour grade as well as pathological stage. This suggests that assessment of Her2/neu status maybe helpful in formulating the treatment plan for patients of urothelial carcinoma. The development of target therapies using anti-HER2 and the identification of patients who may benefit from those therapies need further studies.

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