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Original Article

Pathology Section

Expression of GATA3, p63, E-cadherin and Her2Neu Immunohistochemical Stains in Urothelial Carcinoma and their Relationship with Histological Grading and Prognosis- A Cross-sectional Study

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ABSTRACT

Introduction: Urinary bladder cancer is the 10th most common cancer worldwide. Cystoscopy and biopsy/Transurethral Resection of Bladder Tumour (TURBT) are the best techniques for diagnosing and staging urinary bladder cancers. Urothelial Carcinomas (UC), particularly in transurethral resection biopsies, can pose diagnostic difficulties due to limited material. Immunohistochemistry (IHC) plays a valuable role in these settings, and many immunostains are being utilised for diagnostic evaluation. However, the relationship of these immunomarkers with histological grade and their prognostic utility has not been adequately explored.

Aim: To analyse expression of Her2Neu, E-cadherin, p63, and GATA3 in UC and its relation with histological grading and prognosis.

Materials and Methods: This was an observational cross-sectional study conducted in the Pathology Department of IPGMER and SSKM Hospital, Kolkata, West Bengal, India over two years (November 2020-October 2022). Samples of 100 bladder carcinoma patients with predominant TURBT specimens were included in the study. The histopathological reports and tumour grading were done according to the World Health Organisation (WHO) classification of urinary bladder tumours. The Formalin Fixed Paraffin-embedded (FFPE) sections of the tumour blocks were subjected to IHC staining, and the results were interpreted accordingly. Statistical analysis was performed with the help of the Statistical Package for Social Sciences (SPSS), IBM (version 25.0). Unpaired t-test and Z-test (Standard Normal Deviate) were used to test the significant difference between two proportions.

Results: The mean age of the study population was 59.69±14.53 years, and there was a male preponderance (male: female=3.54:1). Histopathological examination revealed 55% to be of low-grade morphology. Overall, 53% of cases were in T1 stage, and the rest were in T2 stage. On IHC analysis, E-cadherin showed a statistically significant decrease in intensity with increasing grade (p-value <0.001) and T stage of UC (p-value <0.001), but there was no statistically significant relationship between Her2Neu expression and tumour grade/stage (p-value 0.5764 and 0.5663, respectively). A statistically significant relationship was observed between GATA3 and p63 scores with the grade and T stage of the tumour, i.e., GATA3 positivity increased with increasing grade and T stage of the tumour (p-value <0.001 in both), and there was a loss of p63 with advancing grade and stage of the tumour (p-value <0.001 in both).

Conclusion: GATA3, p63, and E-cadherin can be used as prognostic markers in UCs. No significant relationship was found between Her2Neu expression and tumour grade in UC.

Keywords: Immunohistochemistry, Paraffin, Transurethral resection

INTRODUCTION

Urinary bladder cancer is the 10th most common cancer worldwide with an ever-increasing number of patients every year globally and in India as well [1]. A number of causative factors, including smoking and occupational exposure to aromatic amines, arsenic, phenacetin use, pelvic radiation, Schistosoma infection, and certain inherited cancer syndromes, have been implicated in the pathogenesis [2]. There is a male preponderance, and the mean age group is 60-70 years [2]. Early and accurate diagnosis by conventional histopathology as well as immunohistochemical markers helps in early intervention and modification in the disease course. Despite proven diagnostic utility, the prognostic role and clinicopathological relationship of these immunomarkers are still not properly established, and limited studies are available in the literature. Overexpression of Her2Neu is associated with poor prognosis in breast cancer. However, the clinical relevance of Her2Neu in bladder cancer remains ambiguous and under-investigated [3]. GATA3, as a sensitive and specific marker for UC, can be effectively used to exclude other genitourinary malignancies, such as prostate carcinoma and renal cell carcinoma, at the metastatic site. This marker can also be effectively used in predicting the probable grade and invasion in biopsy material with poor morphological characteristics, thereby helping in appropriate management in such cases [4]. The p63 immunostaining has utility in UC as it is one of the genes strongly deregulated in early stages of UC [5]. Different studies have shown that decreased E-cadherin expression is associated with an increased grade of UC and loss of adhesion molecule expression, thus helping in prognostication and severity of UC [6].

The aim of the study was to analyse the expression of Her2Neu, E-cadherin, GATA3, and p63 in UC and its relation with histological grading and prognosis.

MATERIALS AND METHODS

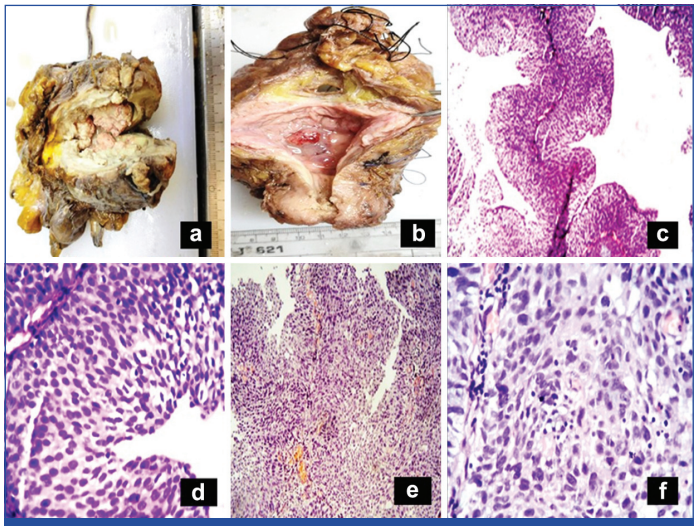
The present study was a observational cross-sectional conducted in the Department of Pathology in collaboration with the Department of Urology at IPGMER and SSKM Hospital, Kolkata, West Bengal, India over a peroid of two years from November 2020-October 2022. It was an observational cross-sectional study, The ethical

clearance was obtained from the Institutional Ethics Committee (IPGME&R/IEC/2021/018). Total 100 patients were chosen for the study. These patients underwent Transurethral Resection of Bladder Tumour (TURBT) (90%) or radical cystectomy (10%).

Inclusion and Exclusion criteria: The diagnosed cases of invasive UC of the bladder in TURBT samples and radical cystectomy specimens were included in the study. The exclusion criteria were: 1) Non neoplastic lesions of the bladder (inflammatory cases, etc.); 2) Bladder carcinomas other than UC, including metastatic carcinoma; or 3) Non representative samples of neoplasm of the bladder (extensive necrosis). In present study, most of the samples were from TURBT. Therefore, the adequacy of received samples was an important aspect for sample processing and reporting. However, those samples that were adequate for the study were included in the analysis.

Study Procedure

Whenever a TURBT or bladder resection specimen of a suspected case of bladder carcinoma with relevant clinical features and suspicious cystoscopy findings was received from the Urology Department, the patient was approached for necessary consent. All those samples underwent Histopathological Examination, and thus, 100 histologically proven cases of urinary bladder UC were included in the study. The following parameters were examined during histopathological assessment: cases of invasive urothelial bladder carcinoma (invasion of the basement membrane), presence of invasion of the lamina propria, invasion of muscularis propria, histological grade of the tumour, histological variant/divergent differentiation of invasive urothelial bladder carcinoma, tumour stage, necrosis, Lympho-vascular Invasion (LVI), Perineural Invasion (PNI) [Table/Fig-1 a-f]. Later, IHC staining for GATA3, p63, E-cadherin, and Her2Neu was performed on the formalin-fixed paraffin-embedded sections of the representative tumour blocks. Desmin IHC was done on challenging cases to pick up muscle invasion. According to the WHO classification of tumours of the urinary system and male genital organs 2016, the muscle-invasive (pT2), in-situ and lamina propria invasive tumours (pT1), the histological grading was determined based on striking nuclear pleomorphism with hyperchromasia, brisk mitotic activity, distinct squamoid appearance, and fused papillary structures or a sheet-like pattern [7].



[Table/Fig-1]: Gross and microscopic pictures of urinary bladder carcinoma. a,b) Showing a gross photograph of urinary bladder carcinoma; c,d) Showing low and high magnification views of a low-grade papillary UC (100X and 400X, Haematoxylin and Eosin (H&E)), and e,f) Showing low and high magnification views of a high-grade UC (100X and 400X, H&E).

The prognostic roles of E-cadherin, GATA3, p63, and Her2Neu were assessed by comparing their relationship with prognostic and predictive factors in bladder carcinomas, such as histological grading and tumour staging. Overall survival analysis was not conducted.

Interpretation of IHC: The E-cadherin rabbit monoclonal antibody of the EP6 clone was used, with colon cancer tissue serving as the positive control. For Desmin, a mouse monoclonal antibody of subclass-IgG1/k was used, and skeletal muscle tissue was used as the positive control. The rabbit monoclonal antibody SP3 was used for Her2Neu interpretation, with a case of breast carcinoma known to be Her2Neu positive used as the positive control. Negative control was achieved by omitting the primary antibody. The GATA3 mouse monoclonal antibody L50-823 was used, with a known case of bladder transitional cell carcinoma serving as the positive control. For p63, the rabbit monoclonal anti-p63 antibody was employed, and prostate tissue was taken as the positive control. The Her2Neu IHC scoring patterns as per [Table/Fig-2] were followed.

Score	Her2Neu interpretation
0	Negative
1+	Negative
2+	Equivocal
3+	Positive

[Table/Fig-2]: Her2Neu interpretation by IHC method [8].

For Her2Neu, both the membranous pattern of staining in the percentage of tumour cells and intensity were considered [8]. For GATA3 and E-cadherin, the following scoring patterns as outlined in [Table/Fig-3] were followed.

Interpretation		Score				
		0	1	2	3	4
Percentage score	GATA3	0%	1-10%	11-50%	51-80%	81-100%
	E-cadherin	0-9%	10-24%	25-49%	50-74%	≥75%
Intensity score	GATA3 and E-cadherin	No staining	Weak staining	Moderate staining	Strong staining	

[Table/Fig-3]: GATA3 and E-cadherin interpretation by IHC method [9,10].

For E-cadherin, a membranous staining pattern was considered positive, and for GATA3, nuclear staining was considered positive [9,10]. The final immunoreactivity scores were calculated by multiplying the staining intensity score and percentage score, with the highest value being 12. Based on the degree of staining of E-cadherin, the cases were classified into three groups: Group-I (negative)-score 0-2, Group-II (weakly positive)-score 3-6, Group-III (strongly positive)-score ≥7 [10]. For GATA3, the final immunoreactivity scores were further subdivided into four groups: Group-I (negative)-score 0-1, Group-II (weakly positive)-score 2-4, Group-III (moderately positive)-score 5-8, Group-IV (strongly positive)-score 9-12 [9].

For p63, the nuclear staining pattern of tumour cells was considered, and the intensity of staining corresponded to negative, weak, moderate, and strong intensity. The percentage of tumour cells was scored as follows: 0-no reactivity, 1-less than 10% of cancer cell nuclei positive, 2-10-25% positive, 3-25-50% positive, 4-50-75% positive, 5-75-90% positive, and 6-more than 90% of tumour cell nuclei positive [11].

STATISTICAL ANALYSIS

Statistical analysis was performed with the help of IBM SPSS software (version 25). Using this software, basic cross-tabulation, inferences, and associations were conducted. The unpaired t-test was used to test the association of different study variables. The Z-test (Standard Normal Deviate) was used to test the significant difference between two proportions. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 100 cases of invasive urothelial bladder carcinoma were studied, with the most common age group being 51-70 years (44%

of cases), and the mean age was 59.69±14.53 years, ranging from 25-82 years. There were 78 male cases and 22 female cases, indicating a male predominance (78%) with a male to female ratio of 3.54:1. Detailed histories were taken from the patients regarding pesticide and aniline dye exposure, but a positive history of such exposures was absent. Addiction was identified as a major risk factor, with 60 patients being addicted, primarily to smoking. In addition to smoking, other forms of tobacco exposure such as oral tobacco and alcohol intake were also present. Patients typically presented with mixed clinical features, with the most common symptom being haematuria. 56% of patients presented with painless haematuria, and 15% presented with haematuria associated with dysuria, resulting in 71% of patients presenting with haematuria. Dysuria was the next most common symptom, with 20% presenting with isolated dysuria and 15% with dysuria associated with haematuria. Other symptoms included urgency (3%) and nocturia (6%).

In the study, the most common specimen type was only TURBT (Transurethral Resection of Bladder Tumour) in 90% of cases, while 10% were radical cystectomy specimens. 74% of samples were greater than 5cc during gross processing. Of the cases, 45% belonged to high-grade tumours and 55% belonged to low-grade tumours. Depth of invasion was greater than 1.5 mm in 54 cases and less than 1.5 mm in 46 cases. 53% of cases were of T1 (low) stage, with the remaining 47% at a higher stage (T2). Among these patients, 38% had a carcinoma in situ component.

In the study, 16% of cases had Lymphovascular Invasion (LVI), while the majority (84%) had no LVI. All cases of LVI were associated with high-grade urothelial carcinoma, and no low-grade cases showed the presence of LVI. 5% of patients had both LVI and Perineural Invasion (PNI).

The majority (94 cases) showed no PNI, while only six cases showed PNI, all of which were observed in high-grade tumours.

In present study, 74% of cases showed positive Her2Neu expression, while 26% showed negative Her2Neu expression. Additionally, 17% showed a score of 0 (negative), 9% showed a score of 1+ (negative), 55% showed a score of 2+, and 19% showed a score of 3+. There was no statistical relationship between HER2 Neu score and the grade of UC (p=0.5764). The relationship between Her2Neu expression and tumour stage (T stage) was also not statistically significant (p=0.5663) [Table/Fig-4].

Parameters		Her2neu scores				p-value
		Negative	1+	2+	3+	
Histological grade	High	15	0	14	16	0.5764
	Low	2	9	41	3	
Tumour stage	T2	15	0	16	16	0.5663
	T1	2	9	39	3	

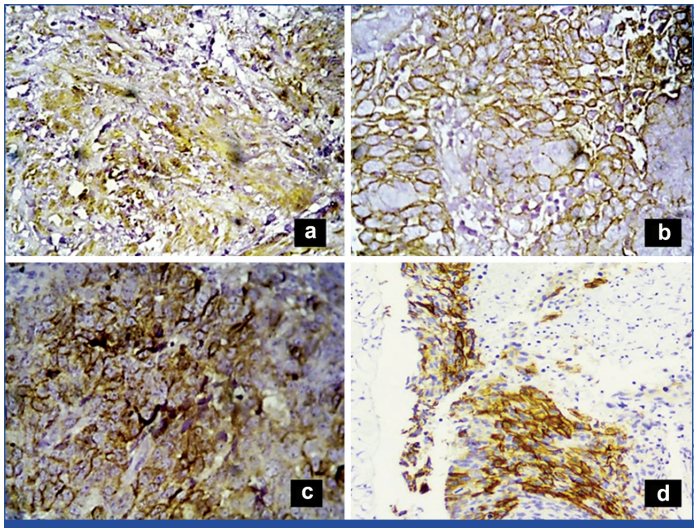
[Table/Fig-4]: Distribution of Her2neu scores according to histological grade and tumour stage.

The E-cadherin values of high-grade and low-grade UC patients were compared using an unpaired t-test with two tails, resulting in a p-value of <0.001, which was statistically significant. Similarly, when comparing the E-cadherin values in patients with T1 and T2 stages of UC, the p-value was <0.001, also showing statistical significance [Table/Fig-5]. Therefore, with an increase in grade and T stage, there was a decrease in E-cadherin expression score. One UC case with 3+ Her2Neu positivity and two UC cases with total E-cadherin scores of 12 and 2 are shown in [Table/Fig-6a,d].

In the study, the scores of GATA3 in high-grade and low-grade tumours respectively were as follows: negative (0 cases in HG/13 cases in LG), weakly positive (26 cases in HG/27 cases in LG), moderately positive (0 cases in HG/4 cases in LG), strongly positive (19 cases in HG/11 cases in LG). This clearly indicated that GATA3 score increased with the increasing grade of the tumour, which was

Parameters		E- cadherin Scores			p-value
		Group-I (score 0-2)	Group-II (score 3-6)	Group-III (score ≥7)	
Histological grade	High	20	10	15	<0.001
	Low	3	3	49	
Tumour stage	T2	20	10	17	<0.001
	T1	3	3	47	

[Table/Fig-5]: Distribution of E-cadherin scores according to histological grade, invasiveness and tumour stage.

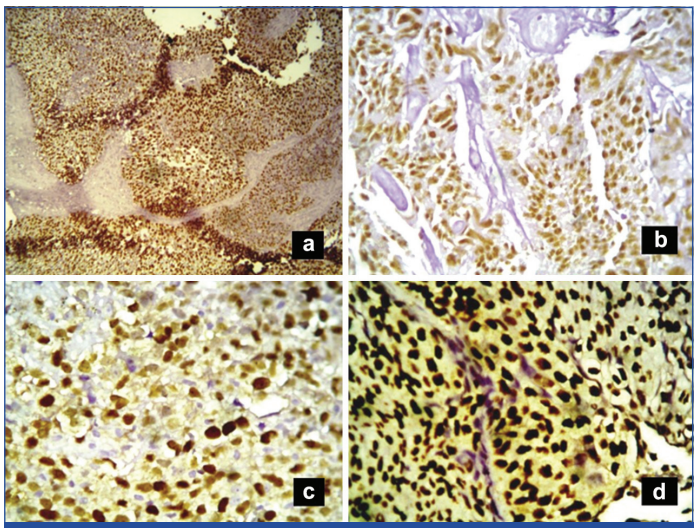


[Table/Fig-6]: a) High magnification view showing desmin positive muscle in a muscle invasive bladder carcinoma (400X); b,c) High magnification view showing E-cadherin positivity (12 and 2 score, respectively) (400X); d) High magnification view showing Her2Neu 3+ bladder cancer (400X).

also statistically significant (p-value <0.001). The GATA3 scoring was also compared with the UC tumour stages (T stage), showing that higher stages were associated with increased GATA3 scores (p-value <0.001) [Table/Fig-7]. Two urothelial carcinoma cases with total GATA3 scores of 12 and 6 are shown in [Table/Fig-8a,b].

Parameters		GATA3 scores				p-value
		Negative (score 0-1)	Weakly positive (score 2-4)	Moderately positive (score 5-8)	Strongly positive (score 9-12)	
Histological grade	High	0	26	0	19	<0.001
	Low	13	27	4	11	
Tumour stage	T2	0	23	0	24	<0.001
	T1	13	30	4	6	

[Table/Fig-7]: Distribution of GATA3 scores according to histological grade and tumour stage.



[Table/Fig-8]: a) Low magnification view showing GATA3 positivity (score 12, 100X); b) High magnification view showing GATA3 positivity in bladder carcinoma (6 score, 400X), and c,d) High magnification view showing p63 positivity (3 and 6 scores, 400X).

In the study, a statistically significant relationship was observed between p63 score and the grade of the tumour (p=<0.001). As the grade of the tumour increased, the p63 score declined, indicating a loss of p63 with the advancing grade of the tumour. p63 scores also had a statistically significant relationship with tumour stages (T stage), suggesting that p63 expression is mostly limited to the low-grade and lower-stage UCs [Table/Fig-9]. Two UC cases with p63 scores of 3+ and 6+ are shown in [Table/Fig-8c,d].

Parameters		p63 scores							p-value
		Negative	1+	2+	3+	4+	5+	6+	
Histological grade	High	0	0	22	8	6	1	8	<0.001
	Low	5	0	32	9	0	9	0	
Tumour stage	T2	0	0	19	11	6	3	8	<0.001
	T1	5	0	35	6	0	7	0	

[Table/Fig-9]: Distribution of P63 scores according to histological grade and tumour stage.

DISCUSSION

Urinary bladder carcinoma is a common multistage progressive malignancy, ranking 10th in worldwide cancer incidence and responsible for significant mortality and morbidity. In the past few decades, many studies have explored the prognostic value of various biomarkers involved in the molecular pathogenesis of bladder cancer [8-11].

In the present study, of the total 100 cases of invasive urothelial bladder carcinoma, the most common age group was 51-70 years (44% of cases), with a mean age of 59.69±14.53 years, a median age of 60.50 years, and ranging from 25-82 years. According to WHO 2016, the median patient age at diagnosis is 65-70 years [2]. The present study was consistent with previous studies regarding the age distribution of bladder carcinoma, with mean ages ranging from 55.9 to 68.32 years [4,8,11-15]. In present study, out of 100 cases, 78 were males and 22 were females. Thus, a male predominance (78%) was observed, with a male to female ratio of 3.54:1. The study was in line with available literature regarding the sex distribution of urothelial bladder carcinoma, all of which found male preponderance [4,8,11-17].

Total 71% of patients presented with haematuria, with dysuria being the next most common symptom. These results were similar to previous studies which also showed haematuria as the most common symptom [4,12,13,18]. In present study, the most common specimen type was only Transurethral Resection of Bladder Tumour (TURBT) in 90% of cases, while 10% were radical cystectomy specimens. The study findings were consistent with those of Wang L et al., where TURBT specimens constituted about 86% of the sample size, and Asmi ATS and Madathiveetil S reported 64% of samples as TURBT [17,18].

In present study, the majority (55 cases) belonged to low-grade tumours, while (45 cases) belonged to high-grade tumours. Features like necrosis, LVI, and PNI were noted in high-grade tumours only. These findings were consistent with the results of Asmi ATS and Madathiveetil S, where the majority of cases (66.67%) were low-grade tumours and 33.33% were high-grade tumours [17]. Depth of invasion was defined as the distance from the deepest level of invasion to the reconstructed mucosal surface. A 1.5 mm depth of invasion predicted advanced-stage bladder carcinoma at cystectomy with a sensitivity of 81%, a specificity of 83%, and positive and negative predictive values of 95% and 56%, respectively. Total 95% of patients with a depth of invasion of 1.5 mm at TUR specimens had advanced-stage (pT2) bladder carcinoma at cystectomy [19]. In practice, authors measured the depth of invasion by calculating the number of fields examined multiplied by the field of vision of the measuring lens. Total 54% patients had a depth of invasion >1.5 mm, while 46 (46%) patients had <1.5 mm.

In the present study, authors used desmin to confirm muscle invasion by UC. Non-muscle Invasive Bladder Carcinoma (NMIBC) and Muscle-invasive Bladder Carcinoma (MIBC) were classified based on desmin IHC. Council L and Hameed O; Kamela NN et al., Saha K et al., showed the diagnostic utility of Desmin in diagnosing muscle involvement in tumours [20-22].

In present study, out of 100 cases of bladder carcinoma, 53% were of T1 (low) stage, while the remaining 47% were of the higher stage (T2). Among these patients, 38 patients had a carcinoma in situ component. Nedjadi T et al., studied 76% of tumours belonging to tumour stage T1/T2 and 24% belonging to T3/T4 [8]. Thakur B et al., found 52.7% of their urothelial bladder carcinomas were of pT1 stage and 26.36% of cases were of pT2 stage [14]. Agarwal H et al., found 32.4% of their tumours belonged to T1 stage and 47.2% belonged to T2 stage [4]. Agarwal M et al., observed that the pT2 stage (46.51%) was the most frequently seen stage, with the pT1 stage seen in 44.9% of cases [15]. Ibrahim BB et al., observed 63.3% high (T3/T4) stage tumours and 36.6% low (T1/T2) stage tumours as they studied all radical cystectomy specimens [16]. The study followed the findings of Nedjadi T et al., and Thakur B et al., but was discordant with the findings of Agarwal H et al., Agarwal M et al., and Ibrahim BB et al., [4,8,14-16].

In the study, 16 cases had LVI, while the majority (84%) had no LVI. All LVI was present in high-grade tumours. 5% of patients had both LVI and PNI. The present study had similar findings as Agarwal H et al., Nedjadi T et al., Kumar M et al., [4,8,13]. In present study, the majority (94%) showed no PNI, while a minor 6% showed PNI. All PNI were also observed in high-grade tumours only. 5% of patients had both LVI and PNI. Kumar M et al., reported PNI in urothelial tumours and its absence in 70% of urothelial tumours [13]. Agarwal H et al., observed the presence of PNI in 8% of cases and its absence in the majority (91.89%) of cases [4]. Ibrahim BB et al., observed PNI in 36.7% of cases, with the majority (63.3%) showing no PNI [16].

In the study, 74% of cases showed positive Her2Neu expression, while 26% of cases showed negative Her2Neu expression. Additionally, 17% showed a 0 score (negative), 9% showed a 1+ score (negative), 55% showed a 2+ score (positive), and 19% showed a 3+ score (positive). The percentage of Her2Neu positivity in various studies done earlier ranged from 36% to 70% [12,13,15-17,19,23-25]. In present study, authors did not find any statistical relationship between Her2 Neu score and tumour grade (p=0.5764) or tumour stage. The present findings were similar to those of Li Y et al., Jawad NA et al., Kumar M et al., Agarwal M et al., Asmi ATS and Madathiveetil S, Stephan A et al., Kumar S et al., Hegazy R et al., El Moneim HMA et al., Abd El-Fattah GA et al., and El Gehani K et al., [9,12,13,15,17,23-28]. Asmi ATS and Madathiveetil S found a significant correlation between Her2Neu status and tumour grade (p-value 0.027) [17], but Stepan A et al., found no statistical relationship between Her2Neu immunoexpression and tumour stage (p-value 0.07) [23].

The study compared the E-cadherin value of high-grade and low-grade UC patients by the application of an unpaired t-test with a two-tailed p-value of <0.001, which was statistically significant. Similarly, by comparing the E-cadherin values in patients with T1 and T2 stages of UC, the p-value was <0.001, which was statistically significant. Popov Z et al., (p-value <0.0001 for tumour stage and <0.001 for tumour grade), El Nemr Esmail RS et al., (p-value <0.001 for stage), Griffiths TRL et al., Hasan AA et al., (p-value <0.001 for stage), Favaretto RL et al., (p-value <0.001 for both tumour grade and stage), Shariat SF et al., showed a positive correlation between loss of E-cadherin expression and the severity of bladder carcinoma in their studies [6, 29-33].

In the present study, a statistically significant relationship was found between increasing GATA3 score and increasing grade and stage of the tumour. The study results corroborated the findings of previous studies by Agarwal H et al., Leivo MZ et al., (p-value=0.03 for tumour stage), Liu H et al., Frederick ED et al., and Naik M et al., (p-value 0.002535 for stage) [4,34-37].

For p63 analysis in present study, a statistically significant relationship was noted between high- and low-grade tumour stages (p=0.0092) and p63 values. The study results were similar to the available literature [5,38-44].

Limitation(s)

The duration of the study was not adequate for proper follow-up, and survival analysis could not be conducted.

CONCLUSION(S)

The GATA3, p63, and E-cadherin can be used as prognostic markers in UCs, as there was a statistically significant relationship between the loss of E-cadherin and p63, along with an increase in GATA3 expression with increasing tumour grade and severity. No significant relationship was found between Her2Neu expression and tumour grade or tumour stage.

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