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Original article

GATA3 and CK5/6 Immunohistochemical Expression in Urothelial Carcinoma: Diagnostic, Biological and Prognostic Significance

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ABSTRACT

Background: Urothelial carcinomas [UCs] can be diagnostically challenging, particularly transurethral bladder tumors resection biopsies with limited material. Immunohistochemistry is a valuable instrument for diagnosis when morphology alone is inadequate. GATA-binding protein 3 [GATA3] and cytokeratin 5/6 [CK 5/6] contributes to the growth of UC. However, their correlative expression in UC and prognostic value has not been sufficiently investigated.

The aim of the work: The current study aimed to evaluate the utility of GATA3 expression in UC and correlate that with CK 5/6 expression to verify different subtypes of UC and assess their prognostic significance.

Methodology: Here, we immunohistochemically stained GATA3 and CK 5/6 in 90 UCs samples by transurethral bladder tumor resection in a retrospective study, between May 2018 and February 2020. All were histopathologically evaluated and immunohistochemically stained for GATA3 and CK 5/6, and then correlated them with the clinicopathological parameters to investigate their clinical significance.

Results: GATA3 expression was seen in 76 patients [84.44 %]. There was significant correlation between GATA3 expression with the tumor histological grade and degree muscle invasion. There was a weak or even negative expression in high-grade, invasive than the low-grade, non-invasive tumor [P= <0.001]. CK5/6 was positive and focally positive in 27.78% with a significant correlation of CK5/6 expression with tumor grade and muscularis propria invasion. On the other side, tumors with diffuse GATA3 expression had low CK5/6 expression.

Conclusion: GATA3 and CK 5/6 should be used as sensitive and specific markers for UC. They can also be effectively used in the prediction of probable grade and tumor invasion in biopsied material with poor morphological characters; and thus, help in the future appropriate treatment.

Keywords: Urothelial Carcinoma; Transurethral bladder tumor resection; Muscle-invasive bladder cancers; GATA-3, CK 5/6; Immunohistochemical

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* Main subject and any subcategories have been classified according to the research topic.

INTRODUCTION

Acute abdomen represents about 9% of childhood. The lining epithelium of the renal collecting tubules, calyces, renal pelvis, the ureter, the bladder and urethra arising from the same foetal origin, and the term "urothelium" is used to define this epithelium. Approximately all cases of urothelial carcinoma [UC] are urinary bladder carcinomas [UBCs]. Upper tract urothelial carcinomas [UTUC] represent 5–10% of all urothelial malignancies [1]. In Middle East countries, non-urothelial tumors are more frequent due to the high prevalence of schistosomiasis. For example, in Egypt, urinary bladder cancer was the most frequent cancer in urinary tract. Genetic factors might have a role in the initiation and advancement of urothelial carcinomas [2].

For instance, Choi et al. [3] and Damrauer et al. [4] defined subtypes of bladder UC, as the molecular subtypes of the breast cancer [5]. Subsequent studies classified the bladder UC into 5 or 6 sub-categories. However, the specific criteria of basal and luminal sub-categories still used for most of cases [6, 7].

Choi et al. [3] reported consistency between the results of immunohistochemical [IHC] staining and expression of mRNA in basal (cytokeratin [CK] 5/6-positive) and luminal (CK20-positive) sub-categories. Another meta-analysis suggested that the IHC study of GATA3 and CK5/6 may adequately recognize these two subtypes with > 90% accuracy [11].

Illustration of both grade and muscle invasion is of great influence on the management and overall outcome. Clinical staging is determined by transurethral resection [TURB] of all visible lesions followed by histopathological examination for the evaluation of the depth of the tumor invasion and subsequently the proper staging, and grading. Although, histopathology plays a crucial role in the prediction and prognosis of the bladder cancer. In addition, it could direct therapy in those patients. However, it is a subjective issue and thus has had poor specificity [8].

The presence of a specific prognostic biomarker is crucial. It could be used to follow up the disease advancement, irrespective of treatment intervention. Two types of such biomarkers were defined; one to predict recurrence and the other to track disease progression. A predictive biomarker can be used to give information about the effective treatment, and could be used as a targeted therapy [9].

Many immunohistochemical markers have been studied; GATA3 is a transcription factor beneficial in the diagnosis of UC [10]. It is also used as a biomarker for luminal bladder cancer [BC] [6,11]. Subsequently, GATA3 has been used for prediction of breast cancer development and progression [4,12].

It is being recognized as a urothelial-associated immunohistochemical marker. There have been few studies done to study expression in different grades, stage, and histological variants or urothelial carcinoma with variable results [13,14].

Well differentiated keratinized squamous element may be recognized as part of UC and sometimes non-keratinizing squamous differentiation closely looks-like urothelial differentiation. On the other side, World Health Organization [WHO]/International Society of Urologic Pathologists [ISUP] doesn't endorse the routine usage of IHC indicators to recognize squamous differentiation in UC. On the other side, indicators of squamous differentiation [e.g., p63, p40, CK5/6] could be positive in UC [15].

The predictive significance of CK5/6 expression in the absence of apparent squamous differentiation is still indistinct [16].

THE AIM OF THE WORK

This study aimed to recognize the role of GATA-3 expression in UC and to assess the frequency of CK 5/6 expression in UC without apparent squamous differentiation and its prognostic significance in Egyptians. We correlate their immunohistochemical expression with clinical and histopathological parameters that may have diagnostic, therapeutic, as well as prognostic utility along with site specificity. To the best of what we know, no such study and correlation have been done in Egyptian population so far.

PATIENTS AND METHODS

This study included 90 patients of primary urothelial carcinoma. The cases were collected from the archived patients' files in the histopathology and urology

departments of Al-Azhar University Hospitals during the period May 2018– February 2020. Since this study was a retrospective study, no written or verbal consent could be obtained. Otherwise, an administrative consent was obtained and the study protocol was approved by the local research and ethics committee.

The inclusion criteria were patients with properly recorded clinical, radiological and operative data in their files, who had primary urothelial carcinoma. On the opposite side, exclusion criteria were: patients with insufficient data in their files, and cases of non-urothelial histology or those of urothelial carcinoma showing squamous differentiation.

The collected data included 1] full detailed history, focusing on urinary tract symptoms and hematuria. 2] Clinical examination including the local urologic Bimanual Digital Rectal Examination [DRE]. 3] Biochemical and hematologic profiles including Full Blood Count, Kidney Function Tests and Urinalysis. 4] The tumor characteristics [site, size, multiplicity and extent of local invasion] were assessed with baseline CT Urography, prior to any intervention. 5] A bladder diagram with all macroscopic features of the tumor [site, size, number and appearance] and abnormal-looking urothelium recorded on, prior to TURBT, with cystoscopy under white light. 6] Systematic monopolar TURBT under classical white light using the 26-Fr. Karl Storz resectoscope set with complete resection of all visible/suspicious lesions and their underlying muscularis propria. 7] A report of the findings at the time of surgery with a precise description of the specimen; and 8] Histopathological Evaluation.

For the histopathological examination, 5-mm-thick sections were prepared from each tissue paraffin block and stained with hematoxylin and eosin. All cases were evaluated by two independent pathologists (Mohamed Yousef Ali and Marwa A. El Kholy) and reported as per the WHO/ISUP Classification 2016.

For immunohistochemical staining, two sections were used for each patient on positively charged slides and submitted to immunohistochemical staining by streptavidin–biotin alkaline phosphate procedures. GATA3 immunohistochemical staining was done on 3-aminopropyl-triethoxysilane-coated slides.

Staining and evaluation using specific Rabbit monoclonal antibody to GATA3 [Clone no. EPR16651, 1:300 dilution, abcam] were done. Human neuro-blastoma tissue served as a positive control. For the negative control, primary antibodies were omitted while performing immunohistochemical staining. Both negative and positive controls were included in each batch of IHC staining. CK5/6 immunohistochemical was done by FLEX Monoclonal Mouse Anti-Human Cytokeratin 5/6, clone D5 / 16 B4 [Lot No. 20042129] by DAKO envision manoeuvre as described by manufacturer. All patients were evaluated by two independent pathologists (Mohamed Yousef Ali and Marwa A. El Kholy).

Interpretation of immunohistochemical staining

Immunohistochemical staining evaluation, the slides were examined at 400× magnification. Only nuclear staining by GATA3 was considered positive. The percentage of tumor cells labelled by GATA3 was scored as follows: Score 0: No tumor cells stained, Score 1: 1–10%, Score 2: 11–50%, Score 3: 51–80%, Score 4: 81–100%. The staining intensity of tumor cells labelled by GATA3 was scored as follows: Staining Scores, 0 for no tumor cells stained. Staining Score 1 for weak. Staining Score 2 for moderate. Staining Score 3 for strong. Finally, immunoreactivity score for GATA3 expression was calculated by multiplying the number representing the percentage of immune-reactive cells by the number representing staining intensity and cases were categorized into four groups shown in Agarwal et al. [8] [Table 1]. All cases were evaluated by two independent pathologists (Mohamed Yousef Ali and Marwa A. El Kholy).

Table [1]: Grouping of GATA3 along with interpretation

Group	The Immunoreactivity score	Interpretation
I	0-1	Negative
II	2-4	Weakly positive
III	5-8	Moderately positive
IV	9-12	Strongly positive

For the quantification of CK 5/6, at least one thousand cells were counted in 10 HPFs [high power fields] [40×]. Intermediate to strong staining of the cytoplasm and membranes in > 10% of tumor cells was considered positive. Weak to intermediate staining in

less than 10% was focal positive, while no staining was negative [16]

Statistical analysis: Data were analysed using Statistical Package for Social Science software computer program version 26 [SPSS, Inc., Chicago, IL, USA]. Qualitative data were presented in frequency [number-percent]. Fischer's exact tests were used to compare the qualitative data for table [2x2] and Monte-Calrol for table with more than [2x2]. P value less than 0.05 was considered statistically significant

RESULTS

The study included 90 cases of urinary bladder urothelial carcinomas, with an age range between [53-74] with a mean age 63.5 years. There was a male predominance with 69 cases were males and 21 females. Demographic and clinico-pathological details were available for all cases [Table 2]. GATA3 expression was seen in 76 cases [84.44 %] of urothelial carcinoma. GATA3 expression was analysed per immunoreactivity score. GATA3 staining was positive when nuclear staining seen within malignant cells and no cytoplasmic staining were noted. GATA3 expressions are categorized as groups in [Table 3]. Of 61 cases presented by hematuria 54 cases [88.52%] gave positive GATA3 expression that gave significant statistical value [P= 0.01]. Regarding the size of the tumor GATA3 expression was positive in the tumor < 3cm. There was no significant association between GATA3 expression and age, gender, site and size of lesion. On correlation of histological grade and stage with GATA3 expression, there were highly significant statistically correlations with histological grade [P= <0.001*] and muscle invasion [P = <0.001*] in which, of 74 cases of low-grade urothelial carcinomas 62 case [83.78%] and only 5 cases of 16 [31.25%] high grade showed moderate to strong expression while 9 and 3 cases of low grade exhibited weak and negative respectively while 11 cases [68.75%] of high grade gave negative expression. As regard tumor stage, of 12, 53 and 25 cases of non-invasive tumors, lamina propria invasive and muscle invasive carcinomas 12[100%],47[88.67%] and 8[32%] cases respectively had moderate-to-strong expression [Table 4] [figure 2].

CK5/6 was positive and focally positive in 25 cases [27.78%] of 90 cases of urothelial carcinomas [Table 5]. CK5/6 expression exhibited a significant association with patient age with higher expression in ≥ 60 years [P=0.01]. On considering gender, signs and symptoms, site, size, tumor grade and muscularis propria invasion there was a highly significant statistical expression of CK5/6 with higher expression in male gender, with hematuria, lateral wall tumor, and the size less than 3 cm. With 13 of 16 cases [81.25%] of high grade gave positive and focally positive CK5/6 expression, when compared to low grade urothelial carcinomas 62 of 74 cases [83.78%] were negative for Ck5/6. Regarding tumor stage all cases, 46 of 53 [86.79%] and 7 cases of 25 [28 %] of non-invasive papillary carcinomas, lamina propria and muscularis propria invasive carcinomas respectively were negative [figure 3].

Table [2]: Patient demography details and clinico-pathologic characteristics at diagnosis

	Variable	No. of patients	Percentage [%]
The age group [years]	< 60	28	31.11
	≥ 60	62	68.89
Gender distributions	Male	69	76.67
	Female	21	23.33
Signs and symptoms	Hematuria	61	67.78
	Urinary outflow obstruction	20	22.22
	Pain	9	10
The site of lesions	Lateral wall	67	74.44
	Neck bladder	17	18.89
	Others	6	6.67
The tumor size	< 3 cm	63	70
	≥ 3 cm	27	30
The tumor grade	Low grade	74	82.22
	High grade	16	17.78
Pathological stage	Ta [non-invasive papillary carcinoma]	12	13.33
	T1[invade lamina propria]	53	58.89
	T2[invade muscularis propria]	25	27.78

Table [3]: Groups of GATA3 expression

Group	GATA3 expression	No. of patients	Percentage
Group I	Negative	14	15.56
Group II	Weak	9	10
Group III	Moderate	25	27.78
Group VI	Strong	42	46.66

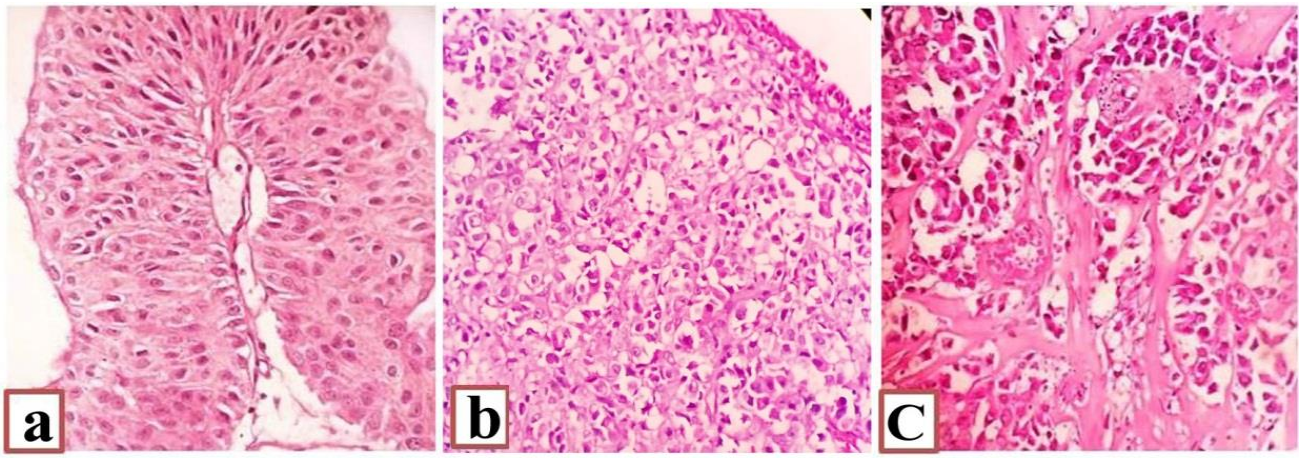

Figure 1: [a] Superficial papillary urothelial carcinoma; low grade PTa – h&E X200, **[b]** Superfascial urothelial carcinoma infiltrating lamina propria; High grade T1 – h&EX200, **[c]** Invasive urothelial carcinoma infiltrating the muscle layer; High grade T2 – h&EX200.

Table [4]: Comparison of GATA3 expression groups with clinico-pathologic features of the study population

Groups		Group I [n=14]		Group II [n=9]		Group III [n=25]		Group VI [n=42]		P- value
		No.	%	No.	%	No	%	No.	%	
The age group [years]	< 60	5	35.71	4	44.44	10	40	9	21.43	0.25
	≥ 60	9	64.28	5	55.56	15	60	33	78.57	
Gender distributions	Male	9	64.28	7	77.78	19	76	34	80.95	0.62
	Female	5	35.71	2	22.22	6	24	8	19.05	
Signs and symptoms	Hematuria	7	50	4	44.44	15	60	35	83.33	0.01*
	Urinary outflow obstruction	6	42.86	2	22.22	7	28	5	11.90	
	Pain	1	7.14	3	33.33	3	12	2	4.76	
				P1=0.32		P1=0.64 P2=0.58		P1=0.02* P2=0.02* P3=0.15		
Site of lesions	Lateral wall	8	57.14	6	66.67	18	72	35	83.33	
	Neck bladder	3	21.43	3	33.33	6	24	5	11.90	0.17
	Others	3	21.43	0	0.00	1	4	2	4.76	
Tumor size	< 3 cm	5	35.71	5	55.56	15	60	38	90.48	0.67
	≥ 3 cm	9	64.28	4	44.44	10	40	4	9.52	
Tumor grade	Low grade [n=74]	3	21.42	9	100.0	20	80.00	42	100.00	<0.001*
	High grade [n=16]	11	78.57	0	0.00	5	20.00	0	0.00	
				P1=<0.001*		P1=0.001* P2=0.29		P1=<0.001* P2=1.00 P3=0.006*		
Pathological stage	Ta [non-invasive] [n=12]	0	0.00	0	0.00	5	20.00	7	16.67	
	T1[invade lamina propria] [n=53]	2	14.29	4	44.44	15	60.00	32	76.19	<0.001*
	T2[invade muscularis propria] [n=25]	12	85.71	5	55.56	5	20.00	3	7.14	
				P1=0.16		P1=<0.001* P2=0.11		P1=<0.001* P2=<0.001* P3=0.22		

Data expressed either as frequency [No-%], P: Probability *: significance <0.05, Test used: Monte-Carlo for more than [2x2] table & fisher exact for table [2x2] for data expressed as frequency [No-%], P1: significance relative to Group I, P2: significance relative to Group II, P3: significance relative to Group III.

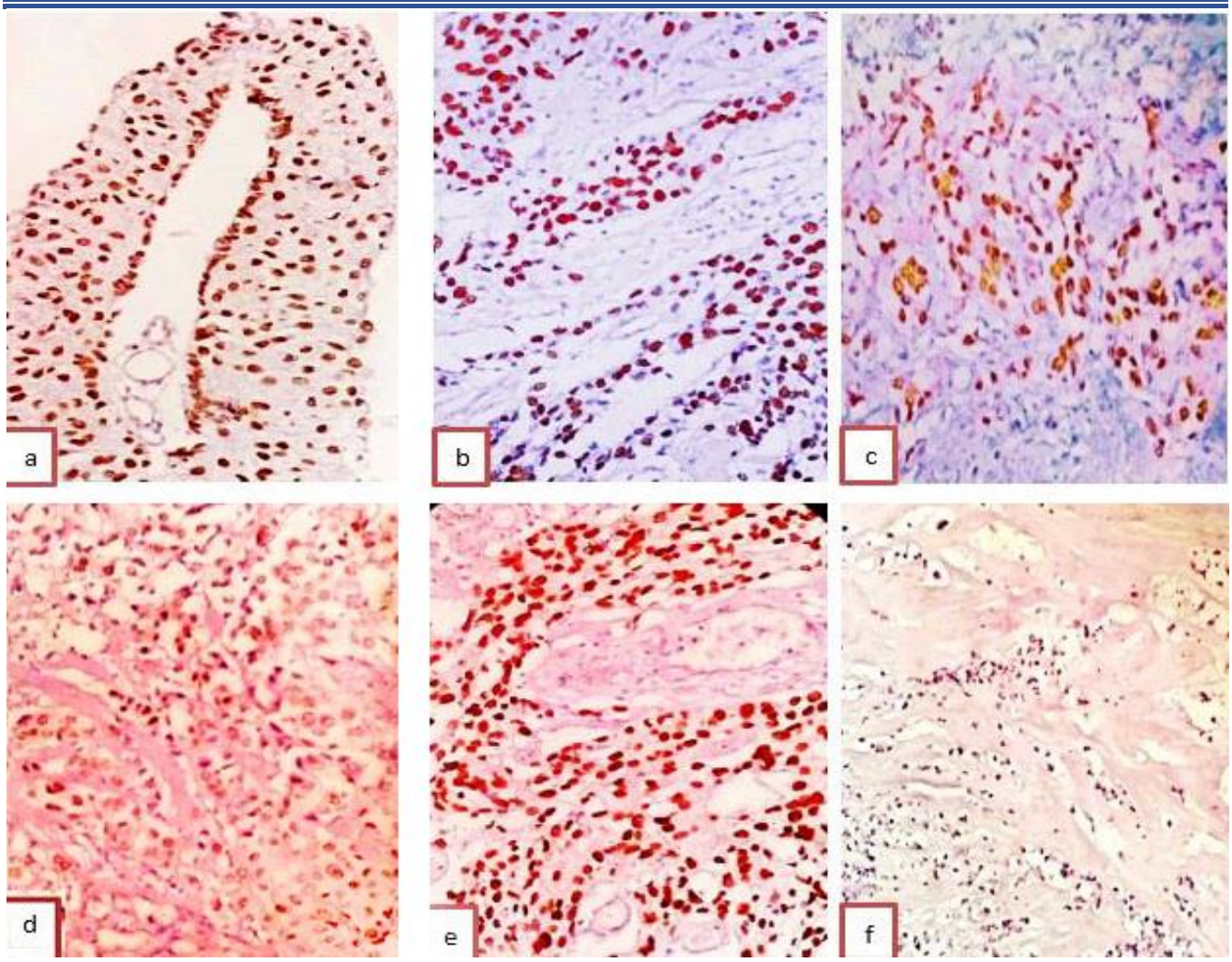

Figure 2: [a] Superficial papillary urothelial carcinoma ; low grade PTa – Strong positive for GATA – 3 [nuclear] X200 .[b] Superficial urothelial carcinoma infiltrating lamina propria ; High grade T1 Strong positive for GATA – 3 [nuclear] – X200, [c] Superficial papillary urothelial carcinoma infiltrating lamina propria ; High grade T1 moderate positivity for GATA – 3 [nuclear] –X200,[d] Superficial urothelial carcinoma infiltrating lamina propria ; High grade T1 weak for GATA – 3 – X200, [e] Invasive urothelial carcinoma infiltrating muscle layer; High grade T2-Strong positive for GATA – 3 [nuclear] – X200, [f] Invasive urothelial carcinoma infiltrating muscle layer ; High grade T2-Negative for GATA – 3 – X100.

Table [5]: Comparison of CK 5/6 expression with clinicopathological features of the study population

Variables		CK5/6 N. [%]								P- value
		Negative		Positive				Total		
				Focally positive		Positive				
		No.	%	No.	%	No.	%	No.	%	
The age group [years]	< 60	23	82.14	4	14.29	1	3.57	28	31.11	0.01*
	≥ 60	42	67.74	5	8.06	15	24.19	62	68.89	
				P1=0.7		**P1=0.03*** **P2=0.04***		**P1=0.074** **P2=0.18** **P3=0.4**		
Gender distributions	Male	51	73.91	6	8.69	12	17.39	69	76.67	<0.001*
	Female	14	66.67	5	23.81	2	9.52	21	23.33	
				P1=0.13		P1=0.72 P2=0.17		P1=<0.001* P2=0.02* P3=<0.001*		
Signs and symptoms	Hematuria	57	93.44	3	4.92	1	1.64	61	67.78	<0.001*
	Urinary outflow obstruction	4	20	12	60	4	20	20	22.22	
	Pain	4	44.44	1	11.11	4	44.44	9	10	
				P1=<0.001*		P1=<0.001* P2=0.11		P1=<0.001* P2=1.00 P3=<0.001*		

Variables		CK5/6 N. [%]								P- value
		Negative		Positive				Total		
				Focally positive		Positive				
		No.	%	No.	%	No.	%	No.	%	
Site of lesions	Lateral wall Neck bladder Others	54 9 2	80.60 52.94 33.33	9 5 3	13.43 29.41 50.00	4 3 1	5.97 17.65 16.67	67 17 6	74.44 18.89 6.67	<0.001*
				P1=0.02*		P1=0.09 P2=1.00		P1=<0.001* P2=0.01* P3=0.03*		
Tumor size	< 3 cm ≥ 3 cm	61 4	96.83 14.81	2 19	3.17 70.37	0 4	0.00 14.81	63 27	70 30	<0.001*
				P1=<0.001*		P1=<0.001* P2=1.00		P1=<0.001* P2=0.47 P3=1.00		
Tumor grade	Low grade High grade	62 3	83.78 18.75	12 3	16.22 18.75	0 10	0.00 62.5	74 16	82.22 17.78	<0.001*
				P1=0.076		P1=<0.001* P2=<0.001*		P1=<0.001* P2=<0.001* P3=0.3		
Pathological stage	Ta [non-invasive papillary carcinoma] T1[invade lamina propria] T2[invade muscularis propria]	12 46 7	100.0 86.79 28.0	0 1 4	0.00 1.89 16.0	0 6 14	0.00 11.32 56.0	12 53 25	13.33 58.89 27.78	<0.001*
				P1=0.01*		P1=<0.001* P2=1.00		P1=0.87 P2=0.02* P3=<0.001*		

Data expressed either as frequency [No-%], P: Probability *: significance <0.05, Test used: Monte-Carlo for more than [2x2] table & fisher exact for table [2x2] for data expressed as frequency [No-%], P1: significance relative to Group I, P2: significance relative to Group II, P3: significance relative to Group III.

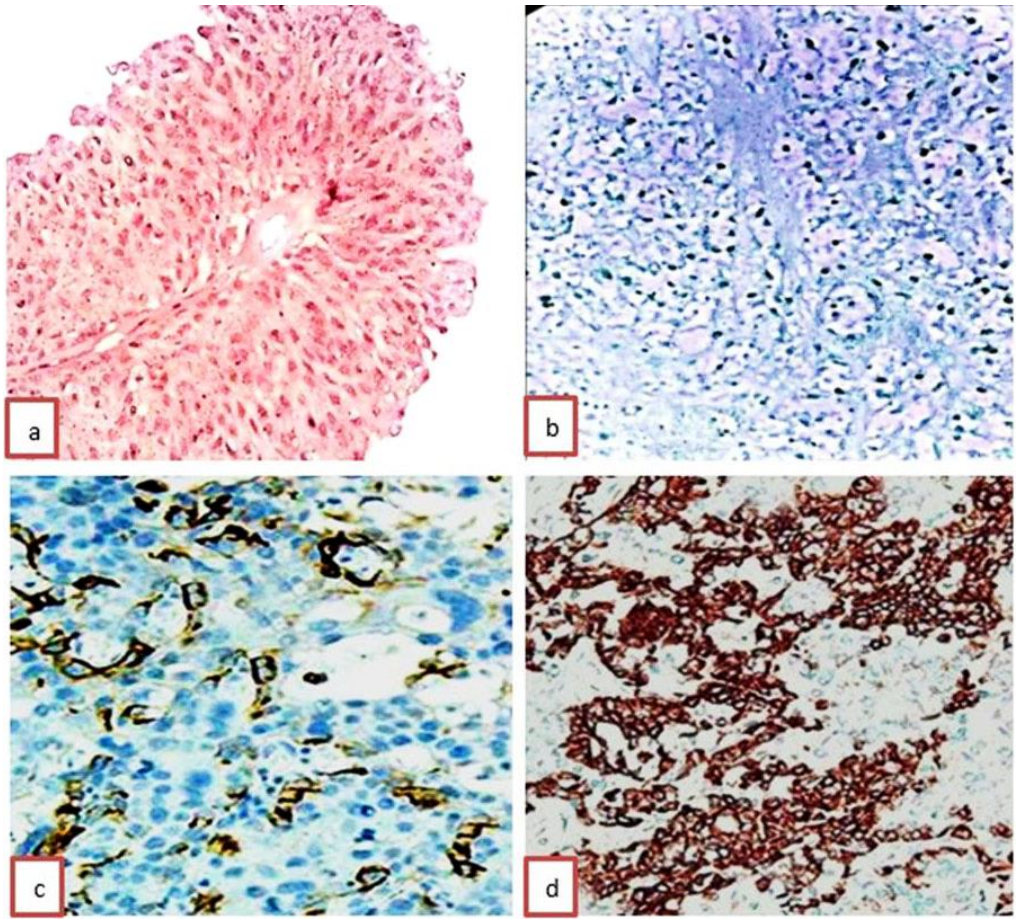

Figure 3: [a] Superficial papillary urothelial carcinoma PTa-Negative for CK5/6, X200, [b] Superficial papillary urothelial carcinoma infiltrating lamina propria; High grade T1 Negative for CK5/6, X100, [c] Superficial urothelial carcinoma infiltrating lamina propria; High grade T1- Moderate for CK5/6 [cytoplasmic and membranous] X400, [d] Invasive urothelial carcinoma infiltrating muscle layer; High grade, T2-Strong positivity for CK 5/6 [cytoplasmic and membranous] X400.

DISCUSSION

Urothelial carcinomas [UCs] are the commonest histologic type of urinary bladder cancer in Egyptians. The identification of high-grade urothelial tumors is crucial for therapy and good prognosis [17]. Most of the urothelial cancers [UC] are superficial at initial diagnosis, and about 70% are characterized by a prolonged course with multiple recurrences after local resection without tumor progression [18]. On the opposite side, a smaller significant percent of patients presents with an aggressive course over a short period. Hence, UBC can be considered as one of overpriced diseases, which requires lifelong surveillance [19, 20].

The surgical modalities and systemic chemo-therapy had recent advances. However, up to 50% of Egyptian patients with invasive urothelial tumors had still suffered from tumor progression, recurrence, and even death [17]. Worldwide, males are more affected by urinary bladder tumors than females [21]. But a previous study had reported worse outcome after radical cystectomy in females than in males. The International Bladder Cancer Nomogram Consortium [IBCNC] created a tool to predict prognosis after surgery. This tool included sex, histological type and grade, as prognostic indicators [23]. Additionally, there is a persistent need to recognize molecular biomarkers to predict the clinical outcomes for BC patients [10]. GATA-3 is a transacting T-cell-specific transcription factor [13]. GATA bind the DNA sequence [A/T] GATA [A/G] in the gene promoters to directly activate or repress the expression of the target genes [24].

In the current study, GATA3 expression was seen in 76 cases [84.44 %] of urinary bladder urothelial carcinoma. which was in line with previous studies done. None of the previous studies had seen any association of GATA3 expression with age, gender, or clinical signs and symptoms, which came in line with our result as we found no significant association between GATA3 expression and age, gender, the site and size of tumor. However, we elucidated that of 61 cases presented by hematuria 54 cases [88.52%] gave positive GATA3 expression [P= 0.01]. Regarding the size of the tumor GATA3 expression was positive in the tumor < 3cm. These results agreed with those of Agarwal et al. [8] who found hematuria as the commonest presenting sign in the study population and its incidence was found to be significantly higher in cases showing GATA3 expression. Mohammed et al. [10] have found that high expression of GATA3 was linked to larger size of the tumor in invasive UC, which is not seen in any other study including ours.

As we correlate histological grade and stage with GATA3 expression, there were highly significant statistical correlations with the histological grade and muscle invasion in which of 74 cases of low-grade urothelial carcinomas 62 case [83.78%] and only 5 cases of 16 [31.25%] high grade showed moderate to strong expression, while 11 cases [68.75%] of high grade gave negative expression. These findings agreed with that of Miyamoto et al. [24] who first studied the prognostic significance of GATA3 in UCs and found that loss of GATA3 was an indicator of high-grade and/or muscle invasive tumors, whereas a strong expression was a predictor of poor prognosis. Similarly, Agarwal et al. [8] who found a statistically significant correlation [P < 0.001] between histological grade and GATA3 expression, as 100% of low-grade tumors were moderate-to-strongly immunoreactive for GATA3 as compared to 70% of high-grade tumors showing only weak positivity. The rest of the high-grade tumors did not show any reactivity. Almost these results have passed with that made by other investigators except for Hoang et al. [25] and Higgins et al. [26] where there was low number of low-grade tumor UC and high-grade UC, respectively, showed GATA3 expression.

Regarding the tumor stage we observed that of 12, 53 and 25 cases of non-invasive tumors, lamina propria invasive and muscle invasive carcinomas 12 [100%], 47 [88.67%] and 8 [32%] cases respectively had moderate-to-strong expression. These results came in harmony with that of Agarwal et al. [8] who stated that 66% of muscle invasive tumors showed GATA3 positivity [weak positivity] in comparison to 87% of non-muscle invasive tumors [non-invasive and lamina invasive]. It was also seen that all cases of non-invasive UC and majority of lamina invasive UC showed moderate-to-strong intensity of GATA3 expression. Thus, the loss of GATA3 expression was observed in muscle invasive UCs which are in line with the findings of Miyamoto et al. [24].

The diagnosis of bladder squamous cell carcinoma is confined to tumors which showed a pure squamous

differentiation in the absence of any urothelial component. On the other side, advanced UC can show divergent differentiation [including squamous constituent] in about 50% of patients and is associated with poor disease progression [27].

The morphologic characterization of squamous differentiation in UC is based on the existence of intercellular bridges or keratinization. However, non-keratinizing or poorly differentiated squamous components can be closely resembling the UC and therefore can't be readily apparent [16].

In the present analysis, we have found significant relation of CK5/6 expression with tumor grade and invasion of muscularis propria, as 13 of 16 cases [81.25%] of high grade gave positive and focally positive CK5/6 expression. When we compared that with low grade urothelial carcinomas, there were 62 of 74 cases [83.78%] negative for Ck5/6. Regarding the tumor stage all cases, 46 of 53 [86.79%] and 7 cases of 25 [28 %] of non-invasive papillary carcinomas, lamina propria and muscularis propria invasive carcinomas respectively were negative. CK5/6 expression exhibited a significant association with patient age with higher expression in ≥ 60 years [P=0.01]. On considering gender, signs and symptoms, site, size, tumor grade and muscularis propria invasion there was a highly significant statistical expression of CK5/6 with higher expression in male gender, with hematuria, lateral wall tumor, size less than 3 cm. These results came hand in hand with that of Hashmi et al. [16] who noticed a low CK5/6 expression in UC; however, its positivity reflects adverse prognostic features like higher tumors grade and invasion of muscularis propria. Another one done by Gaisa et al.[28] who used IHC indicators of squamous differentiation [e.g., CK5/6 and CK4/14] and found differentiation of squamous cells in a high percentage of UC without morphological indication of squamous differentiation. Langner et al. [27] evaluated the prognostic value of keratin subtyping in urothelial carcinoma and revealed the prognostic impact of different cytokeratin staining in urothelial tumors including CK5/6.

In conclusion, the reduction of GATA3 staining was significantly associated with high grade of the tumor and poor clinical outcome in muscle invasive bladder cancer [MIBC]. The prognostic impact of different cytokeratin staining in UC including CK5/6 suggesting that squamous differentiation may be displayed in a high percentage of UC without apparent morphologic evidence of squamous differentiation. Although, this study isn't the first study from Egypt that confirms GATA3 as a sensitive and specific indicator for UC that could be used effectively to exclude other genitourinary tumors, the correlation of GATA3 expression and CK 5/6 was firstly studied.

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Conflicts of interest

There are no conflicts of interest.

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