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Immunohistochemical expression of GATA3, CK5/6 and snail-1 in intrinsic subtypes of bladder carcinoma

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ABSTRACT

Background: GATA3 binding protein has been described as a diagnostic immunomarker for urothelial carcinoma with high sensitivity and specificity. The purpose of this study was the evaluation of immunohistochemical based molecular classification of bladder cancer employing GATA3 and CK5/6, the relation between different subtypes and the available prognostic parameters of bladder cancer, correlation of the immunohistochemical expression of Snail-1 with various types of bladder carcinoma. Expression of GATA3, CK5/6 and Snail-1 immunohistochemistry was assessed in 80 formalin fixed embedded in paraffin blocks from selected cases of UBC (Trans Urethral Resection of bladder tumour 62 and Radical Cystectomy 18 specimens).

Results: GATA3 showed positive nuclear expression in 75% of urothelial carcinoma cases, while all the studied cases of squamous cell carcinoma, adenocarcinoma and the neuroendocrine carcinoma small cell type were GATA3 negative. There was a significant relation between CK5/6 expression and high grade tumours as 100% of high scores were high grade with presence of muscle invasion. Moreover, by using CK5/6 and GATA3 cases were classified into three intrinsic molecular subtypes. Additionally, 62.5% of urothelial carcinoma cases showed positive nuclear and/or cytoplasmic Snail-1 expression, while most of studied cases of non-urothelial carcinoma (87.5%) were Snail-1 positive.

Conclusions: GATA3 is considered as a specific marker for excluding non-urothelial tumours in primary urinary bladder carcinoma. GATA3 and CK5/6 could classify urothelial carcinoma cases into intrinsic molecular subtypes. Since, Snail-1 and CK5/6 expression was correlated significantly to high grade and muscle invasive tumours, we speculated that Snail-1 and CK5/6 considered as independent poor prognostic markers.

Key words: immunohistochemistry, GATA3, CK5/6, snail-1, urinary bladder carcinoma (UBC)

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INTRODUCTION

Urinary bladder carcinoma is one of the most common carcinomas all over the world. According to the recent cancer statistics 2020, it is the tenth most common cancer in men and the twelfth most common cancer in women in the America [1]. Bladder cancer is the fourth most common carcinoma in Egypt, accounting about 30% of all malignancies and accounting for the majority of urinary system malignancy [2].

Despite advances in intravesical treatment and surgical procedures, about 30% of non-muscle invasive bladder carcinoma cases progress to muscle invasion as well as up to 70% of cases have a high recurrence rate within the first year of diagnosis, therefore an appropriate prediction of progress is critical in the treatment of bladder cancer as the selection of biomarkers that can classify the high risk subgroups can improve the challenge [3].

Comprehensive messenger Ribonucleic Acid (mRNA) expression levels in the bladder carcinoma are recently used to classify urothelial carcinoma into different molecular subtypes. Luminal and basal intrinsic subtypes are the primary molecular subtypes, which resemble to the previously reported molecular subtypes of breast carcinoma [4]. Although additional research has expanded the molecular classification of urothelial carcinoma into 6 types, the basal and luminal subtypes continue to be the essential subtypes [5]. A meta-analysis study reported that immunohistochemical expression of GATA3 and Cytokeratin (CK) 5/6 can identify basal and luminal subtypes with accuracy more than 90% [4].

GATA3 protein binding is one of a family of tumour suppressor genes and it is essential for the growth, differentiation, proliferation, and apoptosis .It trans locates from the cytoplasm into the nucleus by importin-a to control gene expression. GATA3 affinity to importin-a regulation is mediated by Mitogen Activated Protein Kinase (MAPK), which enhances nuclear transport [6]. GATA3 is an immunomarker that is high expressed in breast and bladder cancers [7]. It is an accurate indicator of bladder cancer with only urothelial differentiation [8].

Cytokeratin's belong to water insoluble intermediate filaments family, which present in the cytoplasm of epithelial cells and epithelial tumours, more than 25 subtypes of cytokeratin's are recognized. Moreover, cytokeratin's are divided into three categories: low-, high-, and pan keratin cocktail (AE1 and AE3), which retains a wide variety of cytokeratin's [9]. CK5/6 is a reliable basal cell marker. Basal CK is detected in stratified epithelium basal cell layers and basal-like ductal epithelial cells of the breast [10]. CK5/6 usually present in squamous cell

carcinoma in bladder that shows pure squamous differentiation with the absence of any urothelial component [11].

The Epithelial Mesenchymal Transition (EMT) is a procedure implicated in the invasion and migration of tumour cells. Epithelial cells differentiate through complex processes into highly invasive, motile mesenchymal-like cells that promote tumour metastasis. EMT is characterized by absence of E-cadherin expression and increase expression of many transcriptional suppressor of E-cadherin expression as Zeb, Slug, Twist, and Snail [12]. Snail is a family of zinc finger transcription factors that induces EMT by downregulating epithelial markers and upregulating mesenchymal markers. Thus, Snail could be linked to cancer development, metastasis, and treatment failure [13].

The aim of this work was to study the expression of GATA3 and CK5/6 to classify bladder carcinoma into luminal and basal subtypes, evaluation of the relation between luminal and basal subtypes and the available prognostic parameters of bladder cancer. Moreover, we aimed to study the correlation between Snail-1 expression and different subtypes of bladder carcinoma.

MATERIALS AND METHODS

Study design

This retrospective study was carried out on 80 formalin fixed embedded in paraffin blocks from selected cases of Urinary Bladder Carcinoma (UBC) specimens (62 Trans Urethral Resection of bladder tumour and 18 Radical Cystectomy specimens) from January 2018 to December 2021. The study was done after approval from the Research Ethics Committee (approval code number: 33820/5/20).

Data collection and histopathological evaluation

Clinical data were obtained from patient reports. Hematoxyline and Eosin (H and E) staining sections were reviewed for diagnosis and for evaluation of various histopathological features which include the pathologic grade, depth of invasion, lymph vascular invasion.

The UBC cases were microscopically classified according to the World Health Organization (WHO) system into: Urothelial Carcinoma Cases High Grade (UCHG), Urothelial Carcinoma Low Grade (UCLG) tumours, squamous cell carcinoma, adenocarcinoma cases and neuroendocrine carcinoma [14]. Staging of the studied cases was done according to American Joint Committee on Cancer (AJCC) Tumour Node Metastasis (TNM) Staging of Urinary Bladder cancers (8^{th} edition) [15].

Immunohistochemical staining

Bladder cancer sections (5 um) were deparaffinised then hydrated, and immersed in 3% hydrogen peroxide for 20 minutes for blocking endogenous peroxidase activity at room temperature then washed by Phosphate Buffer Solution (PBS) for few minutes and left for drying.

GATA3 a mouse monoclonal antibody (Cat. No. Sc-268, 1:100 dilution Santa Cruz Biotechnology, Inc), CK5/6 a mouse monoclonal antibody (Cat. No. Sc-53262, 1:100 dilution Santa Cruz Biotechnology, Inc) and Snail-1 a rabbit polyclonal antibody (Cat. No. A5243, 1: 50 dilution AB clonal Biotechnology) were placed on each slide for 30 minutes. Two to three drops of the secondary biotinylated antibody were placed on the slides for 45 minutes then the slides were dried. The slides were incubated for 5-10 minutes in Diamino-Benzedine (DAB) chromogen then washed in tap water, counter stain with Mayer's hematoxylin for one minute was done, and then washed in tap water, and sections were dehydrated in ascending grades of alcohol, cleared in xylene, and then covered by using Canada balsam [16]. Negative controls were acquired by skipping the step of primary antibody incubation.

Interpretation of GATA3, CK5/6 and Snail-1 positivity: Using the semi quantitative score (immunoreactive score), that incorporates both: the percentage of immune reactive cells and the intensity of staining. The percentage of immunoreactive cells: 0% (0), 1%-49% (+1), 50%-70% (+2), >70% (+3). The intensity of staining was scored as the following: negative (0), weak staining (+1), medium staining (+2), strong staining (+3). An IHC result was assigned by using semi quantitative scoring system.

Immunoreactive score was calculated by multiplying the percentage of immunoreactive cells by the intensity of staining: (0): Negative (0), (1-3): Weakly positive (+1), (4-6): Moderately positive (+2), >6: Strongly positive (+3). For statistical purpose, Snail-1 immunohistochemical scores (0 and +1) were considered negative and immunohistochemical scores of (+2 and +3) were considered positive [17-19].

Statistical analysis

The collected data was statistically analysed using Statistical Package for the Social Sciences (SPSS) software statistical computer version 20. Data were expressed in terms of frequencies (number of cases) and percentages for categorical variables and range, mean ± Standard Deviation (SD) for continuous variables. Chi-square test was used for categorical variables, to compare different groups. Fishers Exact or Monte Carlo correction was used to correct chi-square when more than 20% of the cells have expected count less than 5. The level of significance was selected at p-value <0.05.

RESULTS

Clinicopathologic characteristics of urinary bladder carcinoma cases

The eighty studied cases were divided into 64 cases of urothelial carcinoma (80%) and non-urothelial carcinoma (20%), including 10 cases of SCC (12.5%), 5 cases of adenocarcinoma (6.3%) and 1 case of neuroendocrine small cell carcinoma (1.3%). The clinicopathological features of the studied cases of urinary bladder carcinoma are summarized in Table 1. The age of the studied cases ranged between 47 and 86 years, with a mean age 64.69. Male to female ratio was 9:1. Urothelial carcinoma cases were categorized into low grade 14 cases and high grade 50 cases out of 64 cases urothelial carcinomas. All the studied cases of Squamous cell carcinoma, adenocarcinoma and the neuroendocrine carcinoma small cell type were of high histological grade. Most of the studied cases showed muscle invasion (T2a) at least 56 cases (70%). Vascular invasion was detected in 13 cases (16.2%).

Tab. 1. Clinicopathological features of studied cases of urinary bladder carcinoma

Clinicopathological features	(80) Total	%
Age (Mean ± SD.)	64.36 ± 8.26	
Gender		
Male	72	90
Female	8	10
Histopathological type		
Urothelial	64	80
Non urothelial	16	20
SCC	10	12.5
Adenocarcinoma	5	6.25
Neuroendocrine carcinoma	1	1.25
Grade		
High grade	66	82.5
Low grade	14	17.5
Degree of muscle invasion		
Non muscle invasion	24	30
Muscle invasion	56	70
Vascular invasion		
Absent	67	83.8
Present	13	16.2

Tab. 2. Distribution of the studied cases according to GATA3, CK 5/6 expression and Snail-1 expression (n=80)

Total			Histologic type				
			Urothelial carcinoma		Non urothelial carcinoma		
			Non-invasive	Invasive	Squamous cell carcinoma	Adenocarcinoma	Neuroendocrine carcinoma
GATA3							
Negative		32(40%)	0	16(29.6%)	10(100%)	5(100%)	1(100.0%)
Positive	**+2**	21(26.2%)	0	21(38.9%)	0	0	0
	+3	27(33.8%)	10(100%)	17(31.5%)	0	0	0
CK 5/6							
Negative		24 (30%)	6 (7.5%)	12 (15%)	0	5 (6.25%)	1 (1.25%)
Positive	**+1**	23(28.75%)	2 (2.5%)	20 (25%)	1 (1.25%)	0	0
	+2	23(28.75%)	1 (1.25%)	19(23.75%)	3 (3.75%)	0	0
	+3	10 (12.5%)	1 (1.25%)	3 (3.75%)	6 (7.5%)	0	0
Snail-1							
Negative	**0**	7 (8.75%)	4 (5%)	3 (3.75%)	0	0	0
	+1	19 (23.75%)	5 (6.25%)	12 (15%)	1 (1.25%)	1 (1.25%)	0
Positive	**+2**	26 (32.5%)	2 (2.5%)	16 (20%)	4 (5%)	4 (5%)	0
	+3	28 (35%)	3 (3.75%)	19 (23.75%)	5 (6.25%)	0	1 (1.25%)

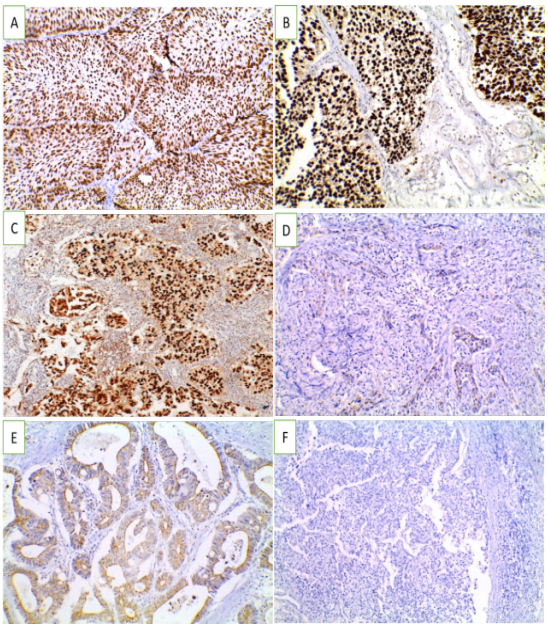

Fig. 1. GATA3 immunoexpression in studied UBC cases (X 200) **(A).** Non-invasive low grade urothelial carcinoma showing positive nuclear expression (+3) **(B).** Non-invasive high grade urothelial carcinoma showing positive nuclear expression (+3) **(C).** Invasive urothelial carcinoma showing positive nuclear expression (+3) **(D).** Squamous cell carcinoma showing negative expression of GATA3 **(E).** Adenocarcinoma showing negative nuclear expression of GATA3 **(F).** Neuroendocrine carcinoma showing negative expression of GATA3

GATA3, CK5/6 and snail-1 immunohistochemical results

Forty-eight cases showed positive nuclear GATA3 expression (60%) (21 cases showed moderate positivity +2 and 27 cases were strong GATA3 positive +3).The remaining 32 cases were GATA3 negative (Figure 1). Fifty-six c ases s howed p ositive C K5/6 expression (70%) (23 cases showed CK5/6 positivity +1, 23 cases showed positivity +2 and 10 cases were positive +3), while 24 cases were CK5/6 negative (30%) (Figure 2). Out of our 80 cases, 54cases showed Snail-1 expression (67.5%) distributed as 26 cases showed Snail-1 score +2 and 28 cases were Snail-1score +3), while 26 cases(32.5%) were negative distributed as 7 cases of Snail-1showed no stain and 19 cases showed Snail-1 score +1. (Figure 3), Table 2.

By using CK5/6 and GATA3 immunomarkers in the current study, three intrinsic molecular subtypes of urothelial carcinomas were stratified as luminal (CK5/6-ve/GATA3+ve) composed of 18 cases (16 cases of pure urothelial carcinoma and 2 cases with divergent differentiation), basal (CK5/6+ve/GATA3-ve) composed of 16 cases (15 cases of pure urothelial carcinoma and one case with divergent differentiation) and mixed (CK5/6+ve/GATA3+ve) composed of 30 cases (16 cases of pure urothelial carcinoma and 14 cases with divergent differentiation). These three molecular classifications was significantly correlated with the histologic types, grade, degree of muscle invasion P-value was ≤ 0.05 as demonstrated in Table 3.

Snail-1 expression is insignificantly correlated with histologic type, vascular invasion and GATA3 expression (p-value was >0.05). On the other hand, it was significantly correlated with the tumour grade, degree of muscle invasion and CK5/6 expression (p-value was <0.05) of studied urinary bladder carcinoma cases as in Table 4.

DISCUSSION

Urinary bladder carcinoma is an international health problem constituted an essential cause of morbidity and mortality. It is the

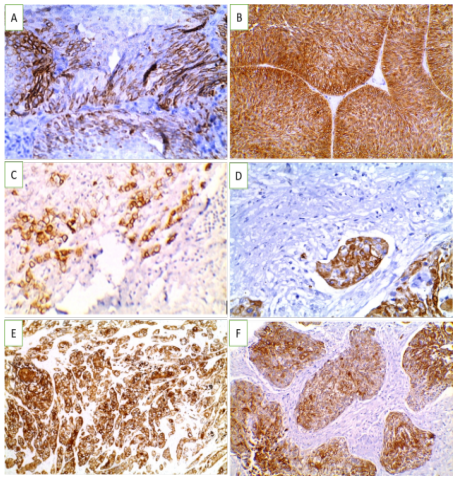

Fig. 2. CK5/6 immunoexpression in studied UBC cases (A-D X 400) **(A).** Non-invasive high grade urothelial carcinoma showing positive cytoplasmic and membranous expression (+2) **(B).** Non-invasive high grade urothelial carcinoma showing positive cytoplasmic and membranous expression (+3) **(C).** Invasive high grade urothelial carcinoma showing positive cytoplasmic and membranous expression (+1) **(D).** Invasive urothelial carcinoma with vascular embolus showing positive cytoplasmic and membranous expression (+3).(E-FX200) **(E).** Invasive high grade urothelial carcinoma with squamous differentiation showing positive cytoplasmic and membranous expression (+3) **(F).** Squamous cell carcinoma showing positive cytoplasmic and membranous expression (+3)

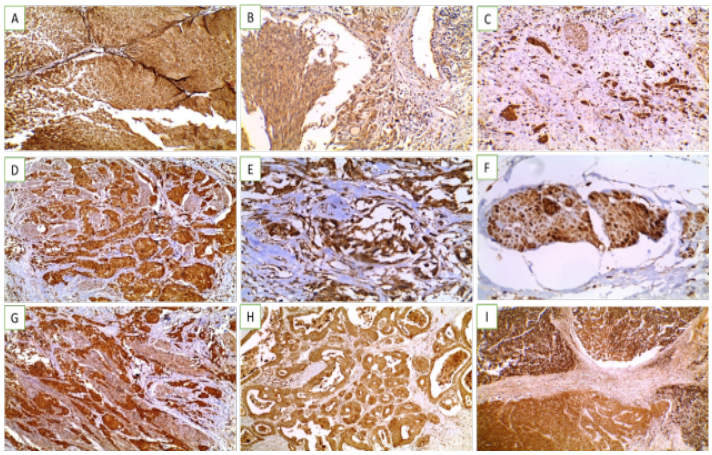

Fig. 3. Snail-1 immunoexpression in studied UBC cases(A-D X 200) **(A).** Non-invasive high grade urothelial carcinoma showing positive cytoplasmic and nuclear expression (+3) **(B).** Invasive laminal low grade urothelial carcinoma showing mainly cytoplasmic expression (+2) **(C).** Invasive high grade urothelial carcinoma showing positive cytoplasmic and nuclear expression (+3) **(D).** Invasive high grade urothelial carcinoma infiltrate muscularis propria showing positive cytoplasmic and nuclear expression (+3) .(E-FX400) **(E).** Lipoid cell variant showing positive cytoplasmic and nuclear expression (+3) **(F).** Invasive urothelial carcinoma with vascular emboli showing cytoplasmic and nuclear expression (+3) (G-I X200) **(G).** Squamous cell carcinoma showing positive cytoplasmic and nuclear expression (+3) **(H).** Adenocarcinoma showing positive expression of (+2) **(I).** Neuroendocrine carcinoma showing positive nuclear expression (+3).

Tab. 3. Relation between molecular subtypes, histologic types, grade and muscle invasion in urothelial carcinoma cases (n= 64).

		CK5/6 / GATA3			X^2	^{MC}p
Histologic type		Basal ±) (n= 16)	Luminal (∓) (n= 18)	Mixed (+/+) (n= 30)		
Pure urothelial		15 (93.7%)	16 (88.9%)	16 (53.3%)	11.212	0.004
With differentiations		1 (6.3%)	2 (11.1%)	14 (46.7%)	-	-
Grade	Low grade	0	9 (50%)	5 (16.7%)	12.633	0.001
	High grade	16 (100%)	9 (50%)	25 (83.3%)		
Degree of muscle invasion	Non muscle invasive	0	10 (55.6%)	12 (40%)	12.381	0.002
	Muscle invasive (pT2 at least)	16 (100%)	8 (44.4%)	18 (60%)		

Tab. 4. Relation between Snail-1 with different clinicopathologic parameters, CK 5/6 and GATA3 expression (n=80).

Histologic type		Snail-1				X^2	^{MC}p
		Negative		Positive			
		No stain (n= 7)	+1 (n= 19)	+2 (n= 26)	+3 (n= 28)		
Urothelial carcinoma		7 (100%)	17 (89.5%)	18 (69.2%)	22 (78.6%)	9.809	0.287
Non urothelial carcinoma		0	2 (10.5%)	8 (30.8%)	6 (21.4%)		
Grade	High	5 (71.4%)	9 (47.4%)	24 (92.3%)	28 (100%)	20.282	<0.001
	Low	2 (28.6%)	10 (58.8%)	2 (11.1%)	0		
Degree of muscle invasion	Non muscle invasive	2(28.6%)	10(52.6%)	4(15.4%)	8(28.6%)	9.996	0.018
	Muscle invasive	5(71.4%)	9(47.4%)	22(84.6%)	20(71.4%)		
Vascular invasion	No	7 (100%)	18 (94.7%)	23 (88.5%)	19 (67.9%)	7.028	0.054
	Yes	0	1 (5.3%)	3 (11.5%)	9 (32.1%)		
CK 5/6							
Negative		4 (57.1 %)	7 (36.9%)	10 (38.5 %)	3 (10.7%)	25.459	0.001
+1		3 (42.9%)	10 (52.6%)	3 (11.5%)	7 (25%)		
+2		0	2(10.5%)	10 (38.5%)	11 (39.3%)		
+3		0	0	3 (11.5%)	7 (25%)		
GATA3							
Negative		2 (28.6%)	6 (31.6%)	13 (50%)	11 (39.3%)	3.722	0.733
+2		2 (28.6%)	4 (21%)	7 (26.9%)	8 (28.6%)		
+3		3 (42.8%)	9 (47.4%)	6 (23.1%)	9 (32.1%)		

10th most common carcinoma in the world and one of the most common carcinoma in men [1].

As urinary bladder carcinoma has markedly various behavioural features, patients with the same disease stage may have different clinical course and outcomes following the same treatment strategy [20]. This together with the high likelihood of recurrence, have made the development of new urinary markers with the hope of appropriate diagnosis, grading, with prediction of the therapeutic response of the tumours [21].

In the current study, we assessed the expression of GATA3, CK5/6 and Snail-1 in various types of urinary bladder carcinoma and the assessment of the role of GATA3 and CK5/6 in the molecular classification of bladder cancer into luminal and basal subtypes.

GATA3 described as a biomarker that is highly expressed in bladder carcinomas, with high sensitivity especially in pure urothelial differentiation [22]. In addition, absence of GATA3 expression was linked with higher grade tumours, and poorer survival outcomes in most previous studies [4].

In the current study, 48 cases out of 64 studied UC cases showed positive nuclear GATA3 expression, while all the studied cases of squamous cell carcinoma, adenocarcinoma and the neuroendocrine carcinoma small cell type were GATA3 negative. Among those UC cases, strong nuclear GATA3 expression (+3) was detected in most of LGUC, while regarding HG infiltrating UC, 75% of cases showed positive nuclear GATA3 expression, while only 25% were GATA3 negative. This finding agreed with Chang et al [23-25]. Who reported that positive staining for GATA3 was detected in 80%, 70% and 80% of their studied UC cases, respectively? These percentages were slightly lower than those obtained by Gulmann et al [26-28]. Who observed 93%, 90% and 88% overall GATA3 positivity in UC cases, respectively. All included cases of SCC in the present study were GATA3 negative. These findings matched with those of Gulmann et al, who found no GATA3 expression in pure SCC of the bladder [26]. In contrast reported that an overall 7% and 5% of their studied cases of well-differentiated SCCs were positive for GATA3, respectively [24, 29]. They concluded that GATA3 can be used to differentiate urothelial carcinoma with squamous differentiation from SCC. All included adenocarcinoma cases in this study were GATA3 negative. This result agreed with Rao et al [8]. Who stated that all their adenocarcinoma cases showed GATA3 negative expression and concluded that GATA3 may be a useful marker in differentiating UC with glandular differentiation from primary adenocarcinoma [30].

The only case of neuroendocrine small cell carcinoma in this study was GATA3 negative. This agreed with Wang et al. who stated that bladder neuroendocrine small cell carcinoma showed negative GATA3 expression. Verduin et al found that only 1 of their 6 cases (17%) of small cell neuroendocrine carcinomas exhibited GATA3 labelling that tumour with mixed components of UC showed more positivity in the non-small cell components [22]. Our results were in contrast with a study made by Mahmood et al. who found that bladder neuroendocrine small cell carcinoma showed positivity for GATA3 [31].

Cytokeratin 5/6 is a cytokeratin expressed in a squamous

epithelial cell. It is used generally as a marker of squamous differentiation,that indicates the basal subtype. CK5/6 expression in urothelial carcinoma was associated with poor outcomes in various studies [11]. This study demonstrated CK5/6 expression in 70 % of all studied cases and 71% of the urothelial carcinoma cases. These results agreed with previous studies [32]. In contrast, other studies documented presence of CK5/6 expression in urothelial carcinoma cases which ranged from 19% to 57% only [33].

In the current study, CK5/6 and GATA3 categorized cases of urothelial carcinoma into three intrinsic molecular subtypes. Both markers are used widely in routine investigation and have an inverse expression pattern. Depending on immunoreactivity for GATA3 and CK5/6, cases are classified into 3 groups: 16 cases were GATA3 -ve/ CK5/6 +ve (basal), 18 cases were GATA3 +ve/ CK5/6 -ve (luminal) and 30 cases were GATA3 +ve / CK5/6 +ve. The latter group was accompanied by intermediated prognostic features between basal and luminal types.

There was significant association between stratified int rinsic molecular subtypes, grade and muscle invasion as basal subtype (CK5/6+ve/GATA3-ve) showed high incidence with poor prognostic factors as high grade, presence of muscle invasion. These results agreed with Calvete et al., who explained that basal bladder tumours which express Cancer Stem Cell (CSC) markers characteristic of epithelial mesenchymal transition, as cytokeratins 5, 6, and 14 [34, 35].

Snail-1 shows a wide spectrum of many biological functions. Additionally, the regulation of cell movements, proliferation and survival, immune suppression and generation of stem cell properties. However, there are more processes for Snail-1-regulation to be discovered [36].

Among UC cases, all strong nuclear and cytoplasmic Snail-1 expression (+3) was detected in HGUC. This finding agreed with who stated that the Snail-1 expression was significant associated with a disagreed with the current study in which their study showed Snail-1 reactions superior in low grade and early-stage carcinomas, this controversy maybe related to using different clone type and dilution [37, 38]. All included cases of SCC in the current study showed Snail-1 positivity. Other studies showed somewhat different result regarding that Snail-1 was highly significant in 60% of the SCC cases [39]. Most of HGUC cases showed Snail-1 positivity. Out of 50 the high-grade cases, 22 cases showed positive Snail-1 expression (+3) and 16 cases showed positive Snail-1 expression (score +2) while 12 were negative (as 0 or +1). The correlation between Snail-1 expression and the pathological grade had statistical significance. Th is ag reed wi th the results obtained by Kosaka et al. [37].

Regarding the vascular invasion in bladder carcinoma cases, 12 cases out of 13 cases showed Snail-1 positivity Also indicated that Snail-1 expression was close related to lympho-vascular relation. In contrast to this study found that there are no significant associations were observed between Snail-1 expression and the pathological stage, grade, and vascular invasion [37, 40].

In the current study, regarding correlation between Snail-1, CK5/6 and GATA3, there was significant direct correlation between CK5/6 and Snail-1 expression. However, no significant correlation was found between Snail-1 expression and GATA3. It is recommended that molecular subtyping of bladder carcinoma may be a successful tool in determination patient's response to different types of treatments and their outcomes, IHC based molecular subtyping, with clinical and pathological parameters increase their efficacy which seems to be more applicable than expensive tissue consuming mRNA based tumour profiling, further study of the significance of Snail-1 as a prognostic marker, its involvement in the control of EMT and metastasis and its role in drug resistance is recommended.

CONCLUSION

GATA3 is positive only in urothelial carcinoma cases while all cases of SCC and adenocarcinoma lack expression. So, GATA3 is a specific marker for excluding non-urothelial tumors. There was significant co-relation between Snail-1 and CK5/6 as both are increased in expression with high grade and muscle invasive tumors, considered as independent prognostic markers, may improve the prediction of bad prognosis for patients with bladder carcinoma.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

FUNDING

The authors did not receive support from any organization for the submitted work.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Not applicable.

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