

RESEARCH ARTICLE

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Detection of Epithelial-Mesenchymal Transition Markers in High Grade Bladder Cancer and Special Variants of Urothelial Carcinoma

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Abstract

Transitional cell carcinoma is considered the most predominant type of bladder cancer. Bladder cancer can also be found as squamous cell carcinoma that accounts for 5% of the total bladder cancer due to its etiology. The biomarkers associated with grade, prognosis, and stage of the disease are not well proved and known however, many studies have pointed to the association between SNAIL/SLUG and Twist2 to the overall survival in patients with bladder cancer. These biomarkers were found to have a crucial role in inhibiting cadherin mediators specifically E-cadherin which are found normally in high level to integrate cell adhesion and normal function of the bladder. This research aims to detect SNAIL/SLUG and Twist2 biomarkers in specimens of patients with bladder cancer and to detect their impact on E-cadherin, a tumor suppressor mediator responsible for improving survival and prevent metastasis. **Materials and Methods:** Using 150 archival tissue blocks from human bladder cancer cases to detect expression of SNAIL/SLUG and Twist2 in relation to loss of E-cadherin by immunohistochemical method. **Results:** Our results have revealed that in squamous cell carcinoma 40 specimens showed marked Twist 2 expression, and 30 specimens showed marked snail/slug biomarkers expression while poorly differentiated cancer cases showed marked expression of Twist 2 in 60 specimens and marked expression of Snail/slug marked expression in 50 specimens. Both were associated with E-cadherin loss. Among the 100 specimens with transitional cell carcinoma, 70 specimens showed divergent differentiation with 7 subtypes each showed different medium to high expression of Snail/Slug and Twist 2 biomarkers with the loss of E-cadherin. E-cadherin was strongly associated with the inverse increase in SNAIL/SLUG and Twist2 biomarkers in urothelial carcinoma. **Conclusion:** Detection of SNAIL/SLUG and Twist 2 biomarkers in urothelial cancer is an important predictor for the loss of E-cadherin, a cornerstone in urinary bladder cell adhesion and its loss in urothelial carcinoma may contribute to cancer invasion and poor prognosis.

Keywords: Urothelial bladder cancer- epithelial- mesenchymal transition- SNAIL/SLUG and Twist 2- Ecadherin

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Introduction

Bladder cancer is the most predominant cancer worldwide involving the urinary system (Bellmunt, 2021; Daneshmand, 2021). Urothelial cancer (UC) or previously known as Transitional Cell Carcinoma (TCC) accounts for 90 percent of all bladder cancers. Nevertheless, urothelial cancers can also exist in the renal pelvis, ureter, or urethra (Bellmunt, 2021). Squamous cells represent 5% of worldwide bladder cancer owing to schistosomiasis (Saginala et al., 2020). However, chronic inflammation, recurrent urinary tract infections, and prior exposure to cyclophosphamide chemotherapy are among other risk factors for Squamous Cell Carcinomas (SCC) (Chalansi et al., 2009). While the remaining 5% includes adenocarcinoma, sarcoma, and metastases to the bladder (Saginala et al., 2020). In some developing countries, such as the Middle East and North Africa,

urothelial and non-urothelial types are both exist mostly due to schistosomiasis infection (Daneshmand, 2021). The highest mortality is detected in Egypt at 6.6/100,000 owing to inhabitant schistosome parasite (Saginala et al., 2020; Daneshmand, 2021). [Globally, 549,393 representing 3% of new bladder cancer (BC) cases and 199,922 new deaths or 2.1 % have been recorded in 2018 alone (Bray et al., 2018). According to Global Cancer Observatory (Globocan) recent report, bladder cancer is considered among the highest 10 cancer types worldwide (Bladder Cancer Fact Sheet, 2020) in addition, Egypt has recorded bladder cancer as the fourth most common type of cancers with 134, 632 new incident cases occupying the highest rates among other countries such as Europe and North America as well as Syria and Israel (Richters, 2020) with Age Standardized Rate (ASR) of 13.2 (Bladder Cancer Fact Sheet, 2020; Richters, 2020).

Urothelial cancer tend to differentiate into different

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histological features (Chalasanani et al., 2009) 60% of the urothelial carcinoma exhibits squamous differentiation while 10% could differentiate into foci of glandular differentiation. Squamous differentiation is diagnosed through the existence of intracellular keratin, intercellular bridges, or keratin pearls. Urothelial carcinoma has a poorly differentiated appearance where it cannot be isolated from other types of urothelial cancers consequently, suspected urothelial differentiation should be adopted where squamous and/or glandular differentiation in a poorly differentiated neoplasm exist at a metastatic site of other tumors, i.e. lung and pancreatic tumors (Amin, 2009). Sarcomatous carcinoma is a rare histological variant of urothelial carcinoma with incidence rate between 0.2% to 4.3%. This type has the worst overall survival when compared to patients with high-grade urothelial cancer.

Epithelial to Mesenchymal Transition (EMT) is a reversible vital process occurs during embryonic development and wound healing. Cells exhibit EMT process are characterized by decreased proliferation which make it more resistant to antiproliferative therapy (Schulte et al., 2012; Summers et al., 2019). High grade invasive tumors exhibit structural and functional defects in the p53 and retinoblastoma protein pathways which start in situ or as de novo invasive tumors with high metastatic rates and poor prognosis (Schulte et al., 2012; Summers et al., 2019). EMT play a vital role in cancer progression and metastasis has been proved in many in vitro studies. EMT activation enables the transition of neoplastic cells from in situ to distal sites (Singh et al., 2017).

E-cadherin suppressors such as Snail, Slug, Twist, and Zeb1 all regulate stromal fibroblast and cancer invasion in urothelial bladder cancer. The de novo expression of N-cadherin along with suppression of E-cadherin are thought to play a role in cancer invasion, poor prognosis, and control of clinical parameter as grading and staging (Schulte et al., 2012). Recently, Twist is a suppressor of E-cadherin gene, was proved to be involved in tumor progression and its upregulation has been associated with metastasis (Yu et al., 2010). This study aims to detect the expression of Twist 2, Snail, and Slug in high grade transitional cell carcinoma and squamous cell carcinoma and special variants of transitional cell carcinoma in correlation with E-cadherin.

Materials and Methods

One hundred and fifty archival tissue blocks from human bladder cancer were obtained from the pathology department at Theodor Bilharz Research Institute. Tumor specimens were obtained by means of cystoscopy (transurethral resection biopsies and cystectomy). Only biopsies containing muscle tissue were included, so that muscle invasion by the tumor could be assessed; biopsies were taken from apparent growths. All specimens were from adult cases with bladder cancer with different grades and stages without bilharzial cystitis. The study protocol was approved by the institutional committee for the protection of human participants and conformed to the guidelines of the 1975 Declaration of Helsinki.

Histopathological study

Tissues were fixed in 10% buffered formalin, paraffin embedded, and processed routinely. Hematoxylin and eosin (HandE) stains were used to evaluate all bladder lesions and to assess carcinoma grade and stage (Eble, 2002). 100 specimens with high grade urothelial carcinoma and 50 specimens with squamous cell carcinoma were detected. Urothelial carcinoma samples were classified into seven main groups according to the results of histopathological examination where different subtypes of TCC variants were assessed i.e. Squamoid differentiation, glandular differentiation, Sarcomatoid differentiation, small cell differentiation, large cells, clear cell, and microcystic cell differentiation each had 10 samples.

Immunohistochemistry for E cadherin, Twist 2 and Snail/Slug were performed on sections prepared from the paraffin blocks with a commercially available anti-human E cadherin, Twist 2 and Snail/Slug (monoclonal antibody; Dako, Glostrup, Denmark, at the optimal working dilution of 1:100). Briefly, sections of 4 mm thickness were prepared and AQ8 placed on positively charged slides (Superfrost plus; Menzel-Glaser, Germany), and the slides were stained on an automated platform: the Dako Autostainer Link 48 (Dako). Heat-induced antigen retrieval was used for AQ10 30min at 97°C in the high-pH EnVision FLEX Target Retrieval Solution and the primary antibody was used at a dilution of 1 in 100.

Interpretation of immunostaining

Immunohistochemistry scoring of Twist 2

Five microscopic fields were randomly selected, and the percentage of stained cells (cytoplasmic or nuclear staining) was evaluated (Seyedmajidi et al., 2014).

The entire section was examined to find the area with maximum positivity and stained nuclei for Twist 2, positively stained cytoplasm and nuclei were scored using the 40 X objective in 20 fields. Immunohistochemistry evaluation was done by two independent observers. For evaluation of Twist 2 expression, each slide was scored according to percentage of positively stained cytoplasm cytoplasmic and nuclear staining. The following ranges were used: negative score where no stained cells, mild score when 1 to 15% positive staining occurred, moderate score when 15-40% staining occurred, marked expression score was considered when 40% or above in cytoplasmic and nuclear expression occurred (Wishahi et al., 2018).

For interpretation of Immunohistochemical staining of E-cadherin, membranous expression was graded based on the percent of positive cells and classified as normal when > 90% of cells have positive membranous expression and when 89% or less of tumor cells showed cytoplasmic or nuclear staining the tumor cells were considered weak to lost (Moussa et al., 2019).

Immunohistochemical staining of Snail/slug was defined as detectable immunoreaction in perinuclear and/or cytoplasm

Regarding the extent of the positivity, classification was done as mild where less than 25% of the tumor cells had expressed Snail/Slug; moderate where < 25% to 50% of the tumor cells had expressed Snail/Slug; and marked

where <50% up to 100% of the tumor cells had expressed Snail/Slug.

Statistical analysis

Data available for statistical evaluation was performed with Statistical Package for the Social Sciences SPSS (version 20, IBM, Chicago, IL, USA) for Windows software. Data are presented as mean \pm SEM. Probability of $P < 0.05$ indicated a significant difference and $P < 0.01$ indicated highly significant difference.

Results

Twist 2 expression and mild Twist 2 expression ($p < 0.05$); ** Significant difference between squamous cell carcinoma with marked Twist 2 expression and mild and moderate Twist 2 expression ($p < 0.05$); # Significant difference between squamous cell carcinoma marked Snail/slug expression with mild and moderate Snail/slug expression ($p < 0.05$); #### Significant difference between squamous cell carcinoma lost E cadherin expression with normal E cadherin expression ($p < 0.05$).

In Table 2, 70 variants were detected in transitional cell carcinoma. Clear cell TCC showed a significant increase in mean value of Twist marker with insignificant increase in its expression in 90% of the samples. In addition, Snail

and Slug biomarkers showed a significant increase in their expression in Clear cell with marked insignificant expression in 60% of the samples and medium expression in the remaining samples. E-cadherin showed 70% loss in those cells with a significant increase in the mean value associated with clear cell TCC subtype.

Glandular cell variant was significantly associated with high expression of Snail and slug biomarkers with insignificant marked expression (80%). E-cadherin was insignificantly lost in 80% of those cells. Microcystic variant showed significant expression in both snail/slug and Twist biomarkers with 60% staining of those cells with mean value of 65 ± 5.4 and 77.5 ± 2.7 ; respectively ($P < 0.05$). E-cadherin was lost in 90% of the cells with significant increase in the mean value (65.83 ± 2.04 , $P < 0.05$).

Large cell variant also exhibited a significant increase in snail/slug as well as Twist biomarkers with marked staining of 100% of the cells with an associated increase in the E-cadherin loss in 90% of the cell variants ($P < 0.05$). Sarcomatoid variant showed high expression in the means of Snail/Slug as well as Twist with 100% marked expression of Snail/Slug as moderate staining in those cells. E-cadherin was found lost in 80% of those cells. In Small cell variant, Twist and Slug/Snail biomarkers both showed a significant mild expression in 20% of the

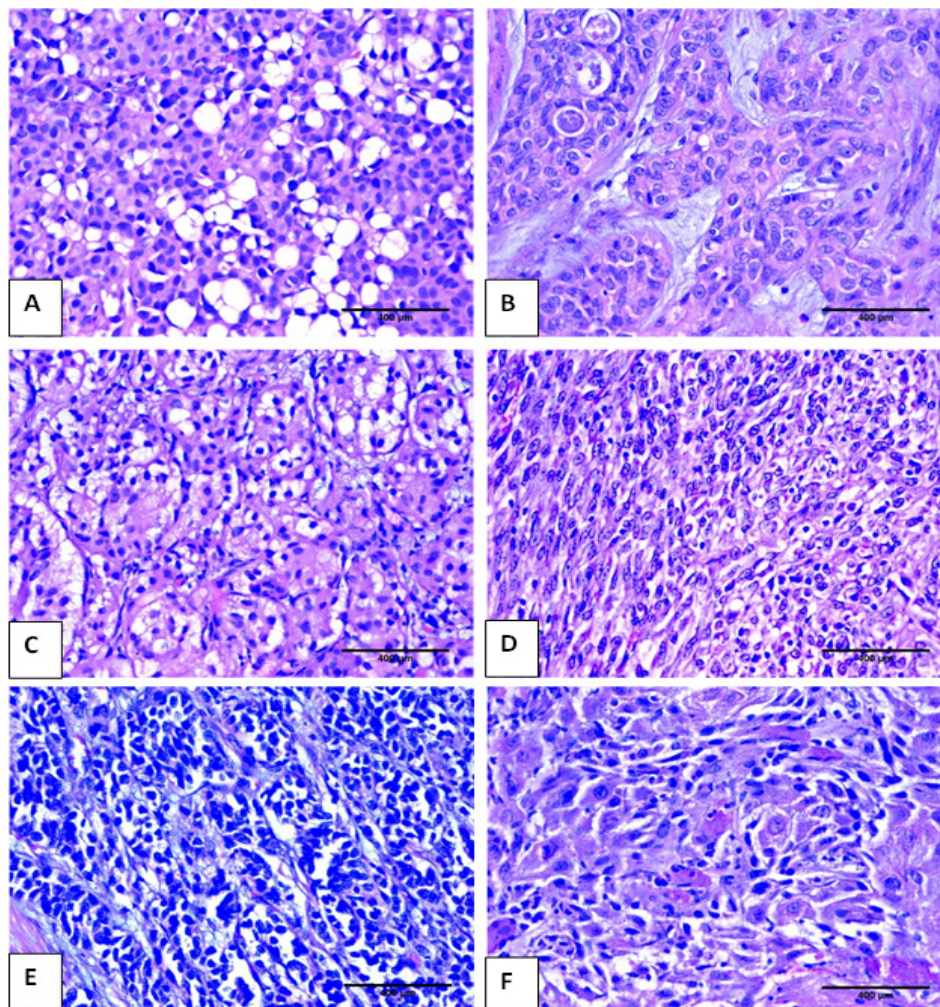


Figure 1. Histopathological Features of Different Subtypes of TCC. A, clear cell; B, Glandular variant; C, Microcystic variant; D, Sarcomatoid variant; E, Small cell variant; F, Squamoid variant. (HandE, $\times 400$).

Table 1. Types of TCC and Expression of Twist, Snail/slug in Presence of E- cadherin

Types	Twist 2 score			Snail/Slug score			E cadherin	
	Mild	Moderate	Marked	Mild	Moderate	Marked	Lost	Normal
Poorly differentiated (High grade) urothelial carcinoma. (100)	10 (10%)	30 (30%)	60 (60%)*	20 (20%)	30 (30%)	50 (50%)	60 (60%)	40 (40%)
Squamous cell carcinoma (50)	5 (10%)	5 (10%)	40 (80%)**	5 (10%)	15 (30%)	30 (60%) #	40 (80%) ###	10 (20%)

*, Significant difference between high grade urothelial carcinoma with marked

slides, moderate expression in 20%, and 60% staining of the cells in those variants. E-cadherin was found in only half of those cells. Finally, Squamoid cell variant showed a significant marked expression in both Twist and slug/Snail biomarkers in 100% of the cells with 60% loss of E-cadherin in those cells. In Figures 1-4, expression of snail/slug in association with loss of E-cadherin exhibits.

Discussion

Many studies have mentioned the association between mesenchymal biomarkers in bladder cancer and poor prognosis (Schulte et al., 2012; Summers et al., 2019; Singh et al., 2017; Yu et al., 2010; Eble, 2009; Seyedmajidi et al., 2014; Ganesan et al., 2016; Kosaka, 2002). Snail/slug is zinc-finger transcriptional inhibitor

contain cysteine/histidine moiety that is identical to E-box responsible for helix-loop- helix transcription (Nieto, 2002). Evidence has suggested a remarkable role of Snail not only in EMT transition, nonetheless in cell death or survival as well as asymmetric cell division (Kosaka et al., 2010; Nieto, 2002). Whereas, Twist 2 belongs to twist family by HLH transcription factor 2 which encoded to inhibit osteoblast maturation and maintain cells in a pre-osteoblast phenotype through osteoblast development (Li et al., 2019). Detection of these biomarkers have been associated with inhibition of E-cadherin with increased metastasis and delayed prognosis in many cancer types as breast, pharyngeal and bladder cancer (Bryan et al., 2008).

As mentioned before, SCC in urinary bladder cancer is mostly common in developing countries by 59% while comprises only around 2.7% of the total carcinomas in

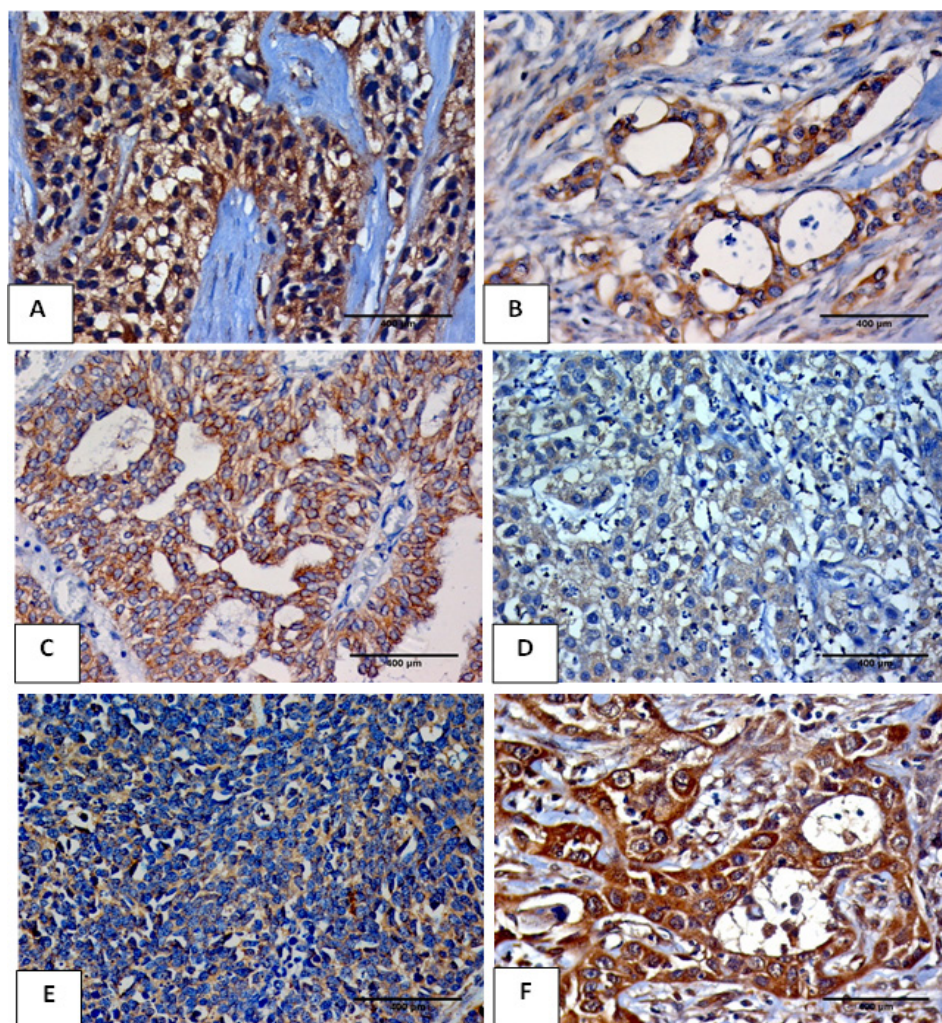


Figure 2. Histopathological Features of Twist Monoclonal Antibody in Different Subtypes of TCC. A, Clear cell; B, Glandular variant; C, Microcystic variant; D, Sarcomatoid variant; E, Small cell variant; F, Squamoid variant. (IHC, Twist, DAB ×400).

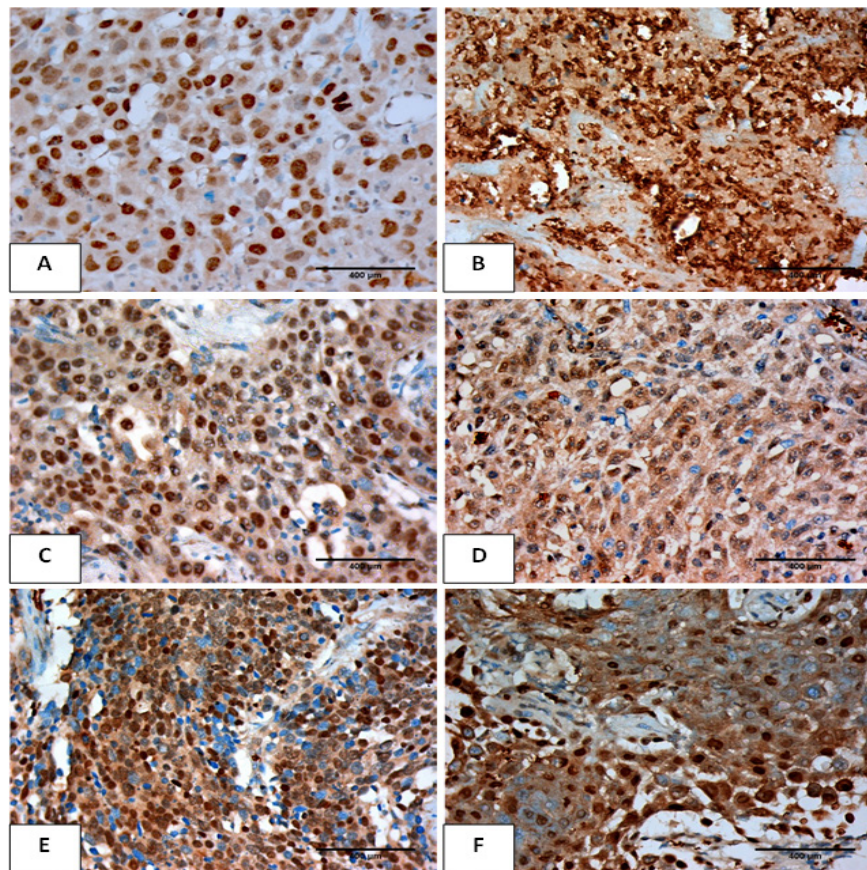


Figure 3. Histopathological Features of Snail/Slug Monoclonal Antibody in Different Subtypes of TCC. A, Clear cell; B, Glandular variant; C, Microcystic variant; D, Sarcomatoid variant; E, Small cell variant; F, Squamoid variant. (IHC, Snail/Slug, DAB $\times 400$).

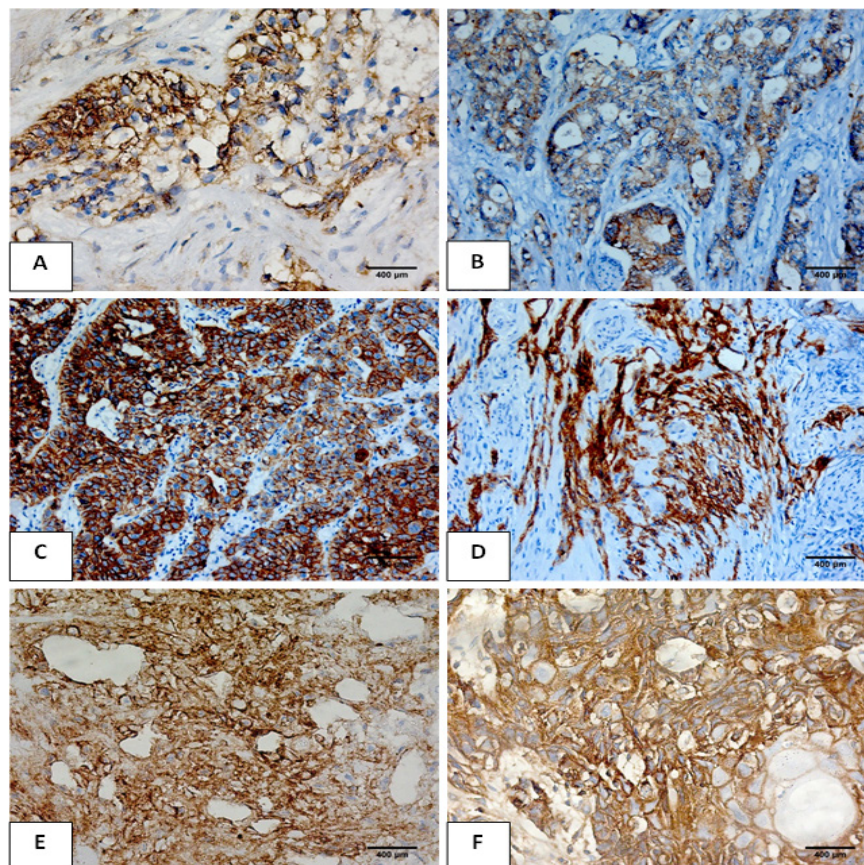


Figure 4. Histopathological Features of E cadherin Monoclonal Antibody in Different Subtypes of TCC. A, Clear cell; B, Glandular variant; C, Microcystic variant; D, Sarcomatoid variant; E, Small cell variant; F, Squamoid variant. (IHC, E cadherin, DAB $\times 400$).

developed countries (Chalasanani et al., 2009). A study conducted between 1988 to 2003 in bladder cancer to compare SCC with other urothelial carcinomas revealed advanced stage and grade linked to SCC. From 1422 SCC in comparison with 107, 613 other urothelial cancers, SCC was independent predictor for stage III and IV disease unlike other types (Scosyrev et al., 2008). As well, Chen et al study revealed that among the 2,634 patients with metastatic cancer, all patients with SCC had the worst prognosis (P < 0.001) (Chen et al., 2017). This could be attributed to the associated biomarkers that suppress E-cadherin and accelerate cell EMT process and worse cancer prognosis. Chen et al., (2017) study had included a total of 2634 eligible patients with metastatic bladder cancer. The study revealed that 75.2% of the total patients had TCC; adenocarcinoma was found in 3.3%; SCC was in 4.1%; and small cell carcinoma was in 4.3%. In our study, Twist 2 was found significantly higher in 80% (40 specimens) of the SCC while snail and slug biomarkers were significantly high in 60% of the SCC specimens with a corresponding loss in E-cadherin staining (80%, P < 0.05). Our study finding could justify other study results correlating high association between expression of Twist, Snail, Slug biomarkers and bad prognosis. In a study by Jouppila-Mättö et al., (2011) pharyngeal SCC patients were found with highly stained stroma of Twist 2 and were all found highly relapsed (p = 0.04). In addition, all positive staining specimens with Twist 2 and SNAI1 were at least Stage II (p = 0.05) unlike negative immunostaining of Twist and SNAI1 (p = 0.008). Another study result concluded that upregulation of Twist 2 was found correlated with poor prognosis and higher mortality rate in pharynx cancer patients. The study has also revealed a downregulation of E-cadherin in association with the increased expression of Twist 2, Snail and Slug (Gasparotto et al., 2011). Moreover, bad prognosis and poor survival were detected in 87 patients with bladder cancer where high immunohistochemical staining with Twist 2 existed. Twist 2 in SCC had low and medium expression only with a detected down regulation of Twist 2 and correlated good survival and low metastasis (Wishahi et al., 2017). Scosyrev and colleagues' study has concluded that among patients' cancer records from 1988 to 2003, SCC were found more invasive in 85% of the patients with BC unlike UC where only 22% of them were muscle invasive at diagnosis (Scosyrev et al., 2008). Another study by Antunes et al. reported that disease recurrence was detected in 64% of patients with squamous differentiation versus 34% of patients without squamous differentiation as well, higher mortality occurred in 40% of patients with squamous carcinoma versus 16% of patients without squamous differentiation (Antunes et al., 2007). In our study, SCC, although it is a rare disease, it has been associated with marked elevation of Snail, Twist, and Slug biomarkers associated with E-cadherin loss. The results agree with other studies' findings interpreting the crucial role of the presence of these biomarkers with the aggressiveness of SCC. Managing SCC characteristics could help in decreasing resistant to radiotherapy and improving survival. The study findings disclose the importance of the detected biomarkers in prognosis of UC.

Table 2. Descriptive Data of Twist, Snail/slug in Presence of E-cadherin Expression in Subtypes of High Grade TCC Samples

Subtypes of TCC	Twist Twist Score				Snail/Slug Snail/Slug Score				E cadherin E cadherin Score		
	Mean±SD	Mild	Moderate	Marked	Mean±	Mild	Moderate	Marked	Mean±SD	Lost	Normal
Clear cell (10)	75.00±5.47*	0 (0%)	1 (10%)	9 (90%)	80.00±21.90**	0 (0.0%)	4 (40%)	6 (60%)	72.5±2.73@ and@@@	7 (70%)	3 (30%)
Glandular variant (10)	68.33±10.32	0 (0%)	4 (40%)	6 (60%)	77.50±5.4**	0 (0.0%)	2 (20%)	8 (80%)	75.00±35.12	8 (80%)	2 (20%)
Microcystic variant (10)	53.33±5.16##	2 (20%)	2 (20%)	6 (60%)	71.66±13.29*	2 (20%)	2 (20%)	6 (60%)	65.83±2.04*#\$\$@@	9 (90%)	1 (10%)
Large cell variant (10)	77.50±2.78**	0 (0.0%)	0 (0.0%)	10 (100%)	65.00±5.47\$@	0 (0.0%)	0 (0.0%)	10 (100%)	85.005.47* #\$\$	9 (90%)	1 (10%)
Sarcomatoid variant (10)	80.00±10.95**	0 (0%)	4 (40%)	6 (60%)	82.5±10.83**	0 (0.0%)	10 (100%)	0 (0.0%)	74.17±5.4	8 (80%)	2 (20%)
Small cell variant	80.83±12.41**	2 (20%)	2 (20%)	6 (60%)	81.66±11.69**	2 (20%)	2 (20%)	6 (60%)	83.33±9.83\$	5 (50%)	5 (50.0%)
Squamoid variant (10)	62.50±2.73	0 (0.0%)	0 (0.0%)	10 (100%)	55.00±5.47	0 (0.0%)	0 (0.0%)	10 (100%)	75.00 ±5.47	6 (60%)	4 (40%)

TCC, Transitional cell carcinoma. In the (*) symbols above denote statistically significant (P < 0.05); ** in the (**) symbols above denote statistically significant (P < 0.01)

Here, our study has detected an increase in Snail, Slug, and Twist 2 expression in TCC and the loss of E-cadherin. E-cadherin is known to be highly expressed in normal urothelial cell membranes for normal tissue integrity and urine impermeability (Bryan et al., 2008). The inverse exhibition between Snail in nearly 50% of TCC specimens, Twist significant marked expression in 60% of the specimens, with the loss of E-cadherin in 60% of the specimens suggest a crucial role of these biomarkers in prognosis and mortality. Bladder TCCs have been associated with abnormal E-cadherin staining patterns and were strongly correlated with malignancies characterized by high grade, advanced stage, and poor prognosis (Bryan et al., 2008; Lipponen and Eskelinen, 1995; White, 1998). In a study conducted the role of E-cadherin loss and the deterioration of Progression Free Survival (PFS), out of 70 bladder tumors, 40% of the cases had high expression of Twist expression, and 23% of the cases were negative for E-cadherin expression with associated poor PFS (Fondrevelle et al., 2009). The results came in the same line with our study results where E-cadherin loss is associated with the over expression of Twist biomarkers exhibiting the impact of the expression of EMT biomarker on E-cadherin. Another study by Kosaka et al revealed a significantly higher level of Snail in UC ($p < 0.001$) with advanced pathological stage, RFS, and CSS with associated loss of E-cadherin ($P < 0.001$) (Kosaka et al., 2010). In our study results, Twist, Snail/Slug in UC were markedly elevated in most of the TCC variants with a marked loss in E-cadherin suggesting delayed prognosis, metastasis, and poor overall survival in the corresponding patients.

Among TCC specimens, 70 variants were detected. Recently, studies started to detect diverse differentiation in TCC bladder cancers for better prognosis and proper treatment of the disease. In a study conducted on 448 consecutive Transurethral Resection of Bladder Tumor (TURBT) and 295 subsequent cystectomy specimens, 25% of UC had diverse histologic features with high grade and invasive characters (99%). 40% of the TURBT were squamous, 18% were glandular, and 11% had multiple mixed histologic types. The study also compared the mixed histological UC with pure UC to find that it is more invasive ($P < 0.001$). The study has concluded that presence of diverse differentiation of UC at TURBT was an independent predictor of metastasis ($P = 0.007$) without remarkable association with survival prediction (Wasco et al., 2007). These study results agree with another study result that found a significant difference between divergent differentiation with squamous differentiation and pure UC in PFS, Recurrence-Free Survival (RFS), and Cancer-Specific Survival (CSS) (Fujii et al., 2017). Another study revealed the predominance of variant TCC with 51%. Squamous carcinoma represented 39% of the total cases with some divergent or variant morphology followed by 28% with micropapillary, 20% with plasmacytoid, and 17% were poorly differentiated. The study also revealed higher invasion of SCC (Santana et al., 2020). While one of the studies revealed divergent differentiation in 11.5% of the cases only (Fujii et al., 2017), our study results have detected 70% of the

cases with diverse differentiation. The study revealed a predominance in glandular variant followed by squamoid variant (Fujii et al., 2017). In addition, patients with divergent differentiation had a marginally worse CSS compared with pure UC while patients with glandular differentiation and squamoid differentiation both revealed worse RFS and PFS compared with those with pure UC (Fujii et al., 2017). Our study results revealed squamoid variants with marked expression of Twist, Snail, and slug biomarkers (100% expression) with a 60% inhibition in E-cadherin suggesting a bad prognosis in those patients. As well, our study has detected glandular cell variant with marked expression of Twist, Snail/Slug with an inhibition in E-cadherin with a percentage of 60%, 80%, 80%; respectively predicting bad progression with delayed survival according to the mentioned studies above. Microcystic variant is a rare variant that is mostly detected in the bladder and occasionally in the upper urinary tract and its detection should be only concerned to differentiate benign and reactive lesions (Shanks, Shanks and Iczkowski).

Nationally, a study has concluded that in the last few years, TCC has enormously multiplies in Egypt in contrast to SCC which may contribute to the change in sanitation process of the river Nile and improvement of the drainage system in Egypt. The study has concluded that Gharbeya province had 4 times more TCC cases than SCC (Fedewa et al., 2009). Another study performed in Egypt has revealed the same conclusion with a changing in the pattern of bladder cancer in the last 3 decades with predominance in TCC versus SCC with superiority in males than females (Felix et al., 2008).

Using archival specimens had limited the correlation between demographic data regarding patients' disease stage, grade, age, and overall survival with the existence of the associated biomarker and loss of E-cadherin in both TCC or SCC bladder cancers. As well, the divergent differentiation in TCC could not be linked to overall survival and prognosis in bladder cancer.

In conclusion, detection of Snail, Twist and Slug biomarkers in urothelial cancer is an important predictor for the loss of E-cadherin, a cornerstone in urinary bladder cell adhesion and its loss in urothelial carcinoma may contribute to cancer invasion and poor prognosis.

Author Contribution Statement

NOTE!!!

The contributions of all authors must be described in the following manner: The authors confirm contribution to the paper as follows: study conception and design: X. Author, Y. Author; data collection: Y. Author; analysis and interpretation of results: X. Author, Y. Author, Z. Author; draft manuscript preparation: Y. Author, Z. Author. All authors reviewed the results and approved the final version of the manuscript.

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