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Biochemical study of E-Cadherin and SATB1 in Urothelial Bladder Carcinoma of Egyptian Patients

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Abstract

Bladder cancer was considered the third most common tumor in Egypt according to the National Cancer Institute (NCI) registry. However, Transitional cell carcinoma of the urinary bladder represents about 90% of all bladder cancer cases. **Method;** The aim of the study was to evaluate the expression levels (mRNA and protein) of E-cadherin and special AT-rich sequence binding protein-1 (SATB1) as biomarkers for transitional cell carcinoma (TCC) using immunohistochemistry and quantitative reverse transcription PCR (RT-qPCR) techniques of the Egyptian urinary bladder patients compared to the noncancerous bladder tissues. The study included 48 TCC tissues (41 males and 7 females) of different grades and stages and 12 noncancerous bladder tissues (7 males and 5 females). **Results;** A statistically significant association regarding smoking and gender were found between the bladder transitional cell carcinoma (BTCC) group and the control group. However, there was no statistically significant difference on comparing the presence of schistosomiasis in both studied groups. The expression of E-cadherin was significantly down expressed and SATB1 was significantly over expressed in TCC tissues compared to the noncancerous bladder tissues at both mRNA and protein levels. Decreased expression of E-cadherin and over expression of SATB1 were statistically associated with tumor stage, tumor grade, and tumor size. On the other hand, no statistically significant difference was detected between the expressions of the two studied genes and either gender or age. A significant association was found between the protein expressions of E-cadherin and SATB1. Pearson correlation test showed significantly negative correlation between mRNA expressions of E-cadherin and SATB1. **Conclusion;** E-cadherin and SATB1 expressions may be considered as good biochemical markers for the diagnosis and prognosis of transitional cell carcinoma in urinary bladder of Egyptian patients.

Keywords: SATB1, E-cadherin, Transitional cell carcinoma, Immunohistochemistry, and RT-qPCR

Introduction:

Bladder cancer is considered the 10th most common tumor worldwide with an expected 549,000 new cases and 200,000 deaths annually. Males have higher risk factor than females for bladder cancer with an incidence and mortality rates of 9.6 and 3.2 per 100,000 in men. So, it is regard that becomes the six common cancer and ninth leading death. The most incidence rates for bladder cancer in males and females are in Southern Europe, Western Europe, Northern America and in Lebanon among females. There were many risk factors for bladder cancer such as chemicals, contamination of water, cigarette smoking [1]. In United State, fifty percent of increasing rates of bladder cancer causes by smoking in males and females [2, 3].

In Egypt, the bladder cancer was the third common type of cancer (6.9%) after breast cancer and hepatocellular carcinoma. In males, it is prevalent than females as in Europe and USA.4 Recent data display that it was a significant decrease of the incidence in squamous cell carcinomas (SCCs) in bladder cancer patients due to better surveillance of schistosomiasis [5]. In the Africa and Middle East, the farthest of bladder cancer tumors are squamous cell carcinomas

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(SCCs) those cases by the infection of Schistosoma or smoking [6, 7]

E-cadherin gene (CDH1) mutation plays an important role in the molecular evaluation of some cancers. Also, E-cadherin related to cell adhesion in bladder cancer [8]. Decrease of E-cadherin- mediated cell adhesion related to the loss of epithelial cell morphology as well as the causes of metastatic status by the neoplastic cells. It is situated on chromosome 16q22 [9]. It is 120KD transmembrane glycoprotein [10]. The special AT-rich sequence binding protein-1 (SATB1) is a protein that plays an important role in the cancer progression and metastasis. This is by its ability to change the transcriptional profile of hundred genes. It is a chromatin organizer and transcriptional regulator worldwide. The base-unpairing regions (BURs), (The AT-rich sequences), is present in every 40000 DNA base pairs. SATB1 bind to BURs forms docking site that important to bind more chromatin transcription factors as well as to modify enzymes [11].

Methods:

The study included sixty formalin-fixed paraffin-embedded tissue samples (FFPET) which were collected from the Pathology Department of the National Cancer Institute (NCI), Cairo University and the National Institute of Urology and Nephrology, Egypt, during the period between January 2013 to December 2015. All tissue samples were collected from adult Egyptian patients, their ages ranged between 41 to 79 years, and the use of human tissue sample in the current study was conducted following the Helsinki Declaration. The collected samples were divided into two groups:

1- Transitional cell carcinoma of the bladder (BTCC group).

This group included 48 tissues samples (41 males and 7 females) from patients were pathologically diagnosed as primary transitional cell carcinoma of the bladder according to the World Health Organization (WHO) histological classification criteria of tumors of the urinary tract (2004) [12]. The grading of tumors was done according to the AJCC/UICC TNM, 7th edition (2010) [13].

2- Control group.

This group consisted of 12 tissues samples from patients who had noncancerous bladder lesions (7 males and 5 females), Samples from normal bladder tissues obtained from the adjacent tumor as far away as possible from the tumor (tumors less than 2 cm in diameter).

Exclusion criteria:

1) Patients had no medical records or absent pathology material; 2) muscularis propria free biopsies or children patients; 3) patients who had any tumor other than urinary TCC (such as squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, or metastatic tumors); and 4) patients who had received preoperative treatment (such as chemotherapy or radiotherapy).

Immunohistochemistry:

The protein expressions of E-cadherin and SATB1 were assessed by imunnohistochemistry in 4 μm sections of FFPE according to standard protocol as following: deparaffinization, followed by rehydration, and finally antigen retrieval were performed in one step using the EnVision™ FLEX Target Retrieval Solution (Dako), at low pH (6.0) for 53 minutes at room temperature in PT Link (Dako PT100). This was followed by incubating slides with FLEX peroxidase block (Dako) for 5 min, and then washed for 5 minutes by EnVision™ FLEX Wash Buffer (Dako). The primary antibody for E-cadherin was diluted 1:100 (clone M3612, Dako) and SATB1 was diluted 1:100 (EPR3951, GeneTex), and then were applied to slides for 60 minutes at room temperature using the Dako Autostainer Link 48 (Dako, Carpinteria, CA), this were followed by incubation with the EnVision™ FLEX HRP visualization reagent (Dako) for 30 minutes at room temperature, and then washed for 5 minutes by EnVision™ FLEX Wash Buffer (Dako). Immunoreactivity for the E-cadherin and SATB1 were done using the 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen (Dako) as a substrate for 10 minutes at room temperature followed by DAB enhancer for 5 minutes at room temperature and Mayer's haematoxylin (Dako) as a counterstain [14]. The staining intensities of E-cadherin in the tumor cells were evaluated compared to the normal urothelium as a positive (normal control). The T lymphocytes in the colorectal wall served as a positive control for SATB1. Normal goat serum was substituted for the primary antibody as a negative for E-cadherin and SATB1.

Evaluation of immunohistochemical staining and scoring:

The samples sections were scored by the examination of more than ten visual fields per slide. One hundred cells were counted in each fields using the 400× magnification lenses from different areas of the tested section using the immunoreactive score, IRS = SI (staining intensity) × PP (percentage of positive cells) [15]. As for E-cadherin expression, the immunoreactive score based on the intensity and percentage of the stained membranous cells and scored as normal or decreased E-cadherin expression (according to IRS values: IRS≥7 or IRS<7, respectively). The intensity of stained membranous cells was classified as follows: 0 (no staining); + (weak staining); ++ (moderate staining); +++ (strong

staining, identical to that of normal bladder epithelium). The percentage of expressed E-cadherin as follows: 0-9% (score 0); 10-24% (score 1); 25-49% (score 2); 50-74% (score 3); and ≥ 75% (score 4) [16].

As for SATB1 expression, the immunoreactive score based on the intensity and percentage of the nuclear staining of tumor cells and scored as negative or positive SATB1 expression (according to IRS values: IRS≤1 or IRS>1, respectively). The intensity of SATB1 immunoexpression was scored as follows: 0 (no staining); + (weak staining); ++ (moderate staining); +++ (strongly staining). The percentage of stained tumor cells was assigned as follows: <10% (score 1); 10-50% (score 2); and >50% (score 3) [17].

Total RNA extraction and quantitative RT-qPCR analysis:

The total RNA was extracted from paraffin-embedded tissue samples using the Total RNA isolation kit (GeneDireX, Las Vegas City, NV, USA) according to the instructions of the manufacture. The RNA integrity and quantity were checked by the NanoDropTM ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The RNA samples with the OD260/OD280 value between 1.8 and 2.2 only were accepted. The RT-qPCR was performed using the HERA SYBR® Green RT-qPCR Kit (HERA SYBR® Green, Willowfort, Uk) and in the (ABI) 7500 Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.).

A total reaction volume of 20 µl in each PCR tube, containing 1 µl of RT Enzyme Mix (20X), 10 µl of HERA SYBR® Green Master Mix (2x), 1.0 µl of each pair of primers (5 µM), 100-200 ng (variable volume) of the target RNA, and complete the definite volume with free RNase H_2O. The RT-qPCR protocol was as follows: in the first stage (reverse transcription) each PCR tube was incubated at 50 °C for 15 minutes, and then the temperature was increased (HERA enzyme activation) to 95 °C for 5 minutes. This was followed by 40 cycles of amplification as follows: all PCR tubes were incubated (denaturation) at 95 °C for 30 seconds, then temperature was decreased (annealing/extension) to 60 °C for 30 seconds. The HERA SYBR® Green was intercalated with the amplicon to give a fluorescent signal that was detected by using the ABI7500 system. Primers were designed using NCBI Primer-BLAST. The synthesis of oligonucleotides was carried out by Eurofins Genomics as follows in Table (1).

Table (1): The primer sequences

Primer	Sequence
SATB1	forward, 5'-GTGGAAGCCTTGGGAATCC-3'
	reverse, 5'-CTGACAGCTCTTCTTCTAGTT-3'
E-cadherin	forward, 5′-CTGGACGCTCGGCCTGAAGT-3′
	reverse, 5′-GGGTCAGTATCAGCCGCTTT-3′
B-actin	forward, 5′-TTAGTTGCGTTACACCCTTTC-3′
	reverse, 5′-ACCTTCACCGTTCCAGTTT-3′

The quantity of the each target gene was calculated by subtracting the Ct for the reference from the Ct for the target gene (ΔCt sample), the same calculation was performed for the control gene (ΔCt control), and the ΔΔCt was subsequently calculated as follows: (ΔΔCt = ΔCt sample - ΔCt control), finally the result was expressed as 2-ΔΔCt [18].

Statistical analysis:

The data were analyzed using the SPSS® software (Version 23, SPSS Inc., Chicago, IL, USA). Quantitative data were demonstrated as mean±standard deviation, while qualitative data were expressed as frequencies and percentages. Student's t-test was used for quantitative independent variables, one-way ANOVA test was used to compare all pairwise differences, and Chi-square test was performed for categorical variables. Furthermore, Correlation studies were performed by Pearson and Spearman, P-value equal or less than the 0.05 level of significance was considered statistically significant.

Results:

Table (2) shows the general characteristics of the tested BTCC and control groups. The age of the BTCC patients group ranged from 41 to 78 with a mean ±SD were (61.68±9.11) years whereas the age of the control group ranged from 42 to 75 years with a mean ±SD (57.08±11.0) years. No statistically significant difference was detected upon comparing the mean values of the two studied groups regarding the age (t=1.5, p=0.139). Moreover, the majority of BTCC patients were 85.4% males and the remaining (14.6%) were females. The ratio of males to females equal 5.8:1.

Schistosomiasis confirmed by the detection of bilharzial ova in bladder tissues of patients. In the present study found that 2/12(16.7%) of the control cases were positive with bilharzial ova versus 20/48 (41.7%) of the BTCC patients and the difference was statistically not significant (χ2=2.584, p=0.108). As for history of smoking, 3/12(25.0%) were smokers in control patients and 30/48 (62.5%) were smokers in the BTCC patients. There was difference significant

between the smokers and bladder cancer (χ2=5.45, p=0.020).

The protein and mRNA expression levels of E-cadherin. The E-cadherin expression was significantly lower in the tumor tissues compared to the non-tumorous tissues at the mRNA level as for the protein level (t=4.909, p=0.000 and χ2=13.125, p=0.0001; respectively) as shown in Table (2) and Figure (3).

The relationship between the clinicopathological features of the patients and the E-cadherin expression was illustrated in Table (3) and (4). Decreased mRNA expression of E-cadherin was significantly associated with tumor stage (F=11.03, p=0.000), and tumor grade (F=11.06, p=0.001). Similarly, abnormal protein expression of E-cadherin was significantly correlated to tumor stage (χ2=10.667, p=0.031, and tumor grade (χ2=12.583, p=0.002).

Aberrant E-cadherin expression was also associated significantly with tumor size at the mRNA level (t=3.695, p=0.001) and the protein level (χ2=14.885, p=0.000). However, no significant correlation was found between E-cadherin expression at the mRNA and protein levels and either the age or the gender of the BTCC patients.

As for Table (2) and Figure (3) shows that, the mRNA expression of SATB1 was higher in the BTCC tissues compared to the non-tumor tissue samples and the difference was highly statistically significant (t=5.648, p=0.000). Similar results were found by immunohistochemistry staining analysis (protein expression) for detecting SATB1 which was mainly expressed in the nucleus of the bladder tumor tissues compared to completely negative expression in non-tumor tissues (χ2=19.615, p=0.000).

As for Table (3) and (4), increased mRNA expression of SATB1 was significantly associated with tumor stage (F=5.42, p=0.001) and tumor grade (F=7.38, p=0.002). Similarly, increased protein expression of SATB1 showed a highly significant correlation with tumor stage (χ2=11.694, p=0.020) and tumor grade (χ2=8.721, p=0.013). Figure (2) illustrates the protein expression of SATB1. The same finding was also reported with mRNA expression. Increased mRNA expression was statistically associated with tumor size at both mRNA level (t=3.165, p=0.003) and protein level (χ2=8.957, p=0.004). On the other hand, no significant correlation was detected between SATB1 expression (at mRNA and protein levels) and either the age or sex of the BTCC patients.

The relation between the protein expression level of E-cadherin and SATB1 together in BTTC group is illustrated in Table (3) and (5). A statistically significant difference was found between decreased expression of E-cadherin and increased expression of SATB1 (χ2=7.203, p=0.007). Pearson correlation test showed a statistically negative correlation between the mRNA expressions of E-cadherin level and SATB1 level (r=-0.442, P=0.002).

Table (2): The correlation between the relevant clinicopathological characteristics of the patients compared to the control and tested BTTC groups

Item		Control group	BTTC group	P-value	Sig.
Age (years) Range		57.08±11.0 (42-75)	61.68±9.11 (41-78)	0.139	NS
Gender	**Male (%)**	7 (58.3%)	41 (85.4%)	0.036	S
	Female (%)	5 (41.7%)	7 (14.6%)		
Smoking	**Yes (%)**	3 (25.0%)	30 (62.5%)	0.020	S
	No (%)	9 (75.0%)	18 (37.5%)		
Schistosomiasis	**Yes (%)**	2 (16.7%)	20 (41.7%)	0.108	NS
	No (%)	10 (83.3%)	28 (58.3%)		
IHC expression of E-cadherin	**Normal (%)**	12 (100%)	20 (41.7%)	0.000	HS
	Decrease (%)	0 (0.0%)	28 (58.3%)		
IHC expression of SATB1	**Negative (%)**	12 (100.0%)	14 (29.2%)	0.000	HS
	Positive (%)	0.0 (0.0%)	34 (70.8%)		
E-cadherin mRNA expression Range (min – max)		1.06±0.38 (0.56-1.78)	0.52±0.33 (0.08-1.55)	0.000	HS
SATB1 mRNA expression Range (min – max)		1.12±0.57 (0.55-2.08)	7.97±4.16 (1.23-19.02)	0.000	HS

Table (3): The relation between the relevant clinicopathological features of the patients and protein expressions using immunohistochemistry staining of E-cadherin and SATB1 in the BTTC group

Item		E-cadherin expression			SATB1 expression		
		Decreased NO. (%)	Normal NO. (%)	P-value	Negative NO. (%)	Positive NO. (%)	P-value
Age	≥60	18(64.3%)	10 (35.7%)	0.322	9 (32.1%)	19(67.9%)	0.750
	<60	10 (50.0%)	10 (50.0%)		5 (25.0%)	15 (75.0%)	
Sex	Male	24 (58.5%)	17 (41.5%)	0.945	12 (29.3%)	29 (70.7%)	0.970
	Female	4 (57.1%)	3 (42.9%)		2 (28.6%)	5 (71.4%)	
Tumor size	≥3 cm	20 (87%)	3 (13%)	0.000	2 (8.7%)	21(91.3%)	0.004
	<3 cm	8 (32%)	17 (68%)		12(48.0%)	13(52.0%)	
Tumor stage	Ta	3 (30.0%)	7 (70.0%)	0.031	6 (60.0%)	4 (40.0%)	0.020
	T1	2 (28.6%)	5 (41.7%)		4 (57.1%)	3 (42.9%)	
	T2	7 (63.6%)	4 (36.4%)		2 (18.2%)	9 (81.2%)	
	T3	12 (75.0%)	4 (25.0%)		2 (12.5%)	14 (87.5%)	
	T4	4 (100.0%)	0 (25%)		0 (0.0%)	4 (100.0%)	
Tumor grade	G1	1 (12.5%)	7 (87.5%)	0.002	5 (62.5%)	3 (37.5%)	0.013
	G2	7 (46.7%)	8 (53.3%)		6 (40.0%)	9 (60.0%)	
	G3	20 (80.0%)	5 (20.0%)		3 (12.0%)	22 (88.0%)	
SATB1 expression	Negative	4 (28.6%)	10 (71.4%)	0.007			
	Positive	24 (70.6%)	10 (29.4%)				

Table (4): The relation between the relevant clinicopathological features of the patients and mRNA expressions of E-cadherin and SATB1 in the BTTC group

Item		E-cadherin mRNA expression		SATB1 mRNA expression	
		Mean±SD	P-value	Mean±SD	P-value
Age	≥60	0.50±0.33	0.733	8.01±4.33	0.940
	<60	0.54±0.34		7.91±4.02	
Sex	Male	0.49±0.33	0.259	8.18±4.39	0.390
	Female	0.65±0.33		6.70±2.22	
Tumor size	≥3 cm	0.35±0.22	0.001	9.78±4.36	0.003
	<3 cm	0.67±0.34		6.30±3.22	
Tumor stage	Ta	0.76±0.31	0.000	4.92±3.17	0.001
	T1	0.72±0.32		5.81±1.92	
	T2	0.66±0.27		8.05±4.01	
	T3	0.25±0.14		9.43±3.92	
	T4	0.21±0.09		13.3±3.41	
Tumor grade	G1	0.84±0.32	0.000	4.53±3.04	0.002
	G2	0.62±0.23		6.74±3.39	
	G3	0.35±0.28		9.80±4.01	

Table (5): The correlation between mRNA expressions of SATB1and E-cadherin in the BTTC group

Parameters	SATB1 expression		
	r-value	**P-value**	**Sig.**
E-cadherin expression	-0.442	0.002	**HS**

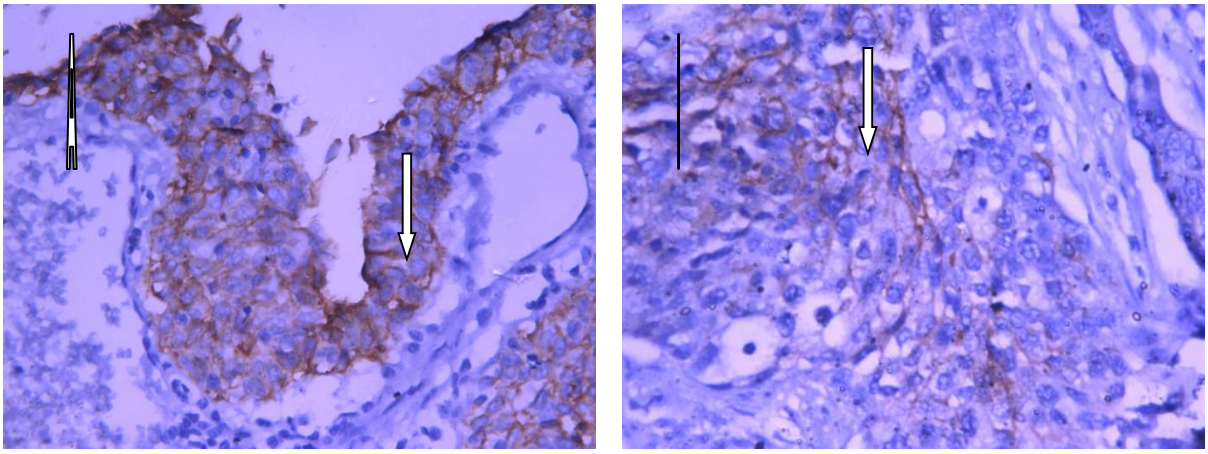

Figure (1): Immunohistochemistry staining of E-cadherin, (A) non-tumor tissue (cystitis) showing positive (normal) cell membrane expression, (B) high grade urothelial carcinoma showing decreased cell membrane expression (brown color) (IHC ×400).

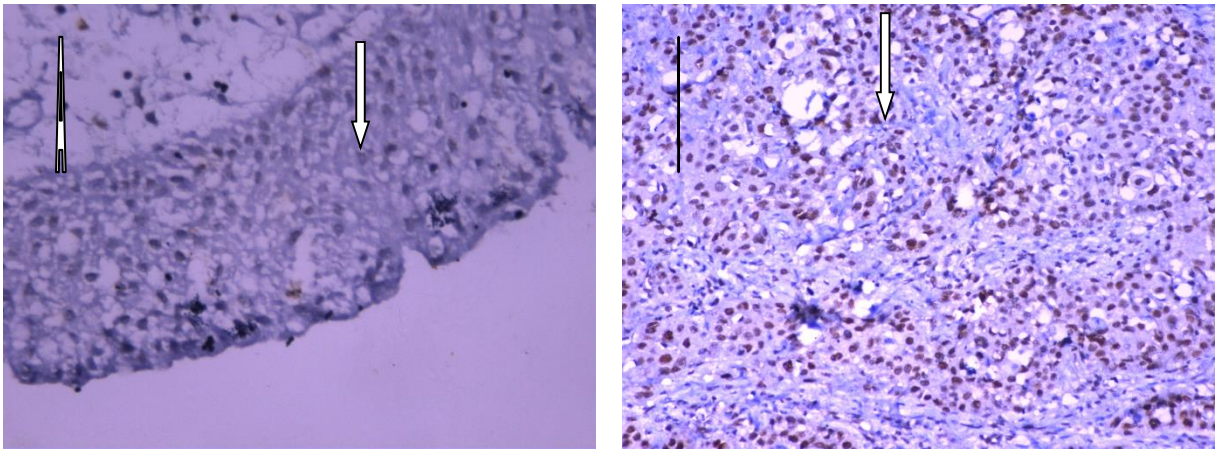

Figure (2): Immunohistochemistry staining of SATB1, (A) non-tumor tissue (cystitis) showing negative nuclear expression(IHC ×400), (B) high grade urothelial carcinoma showing strong nuclear expression (brown color) (IHC ×200).

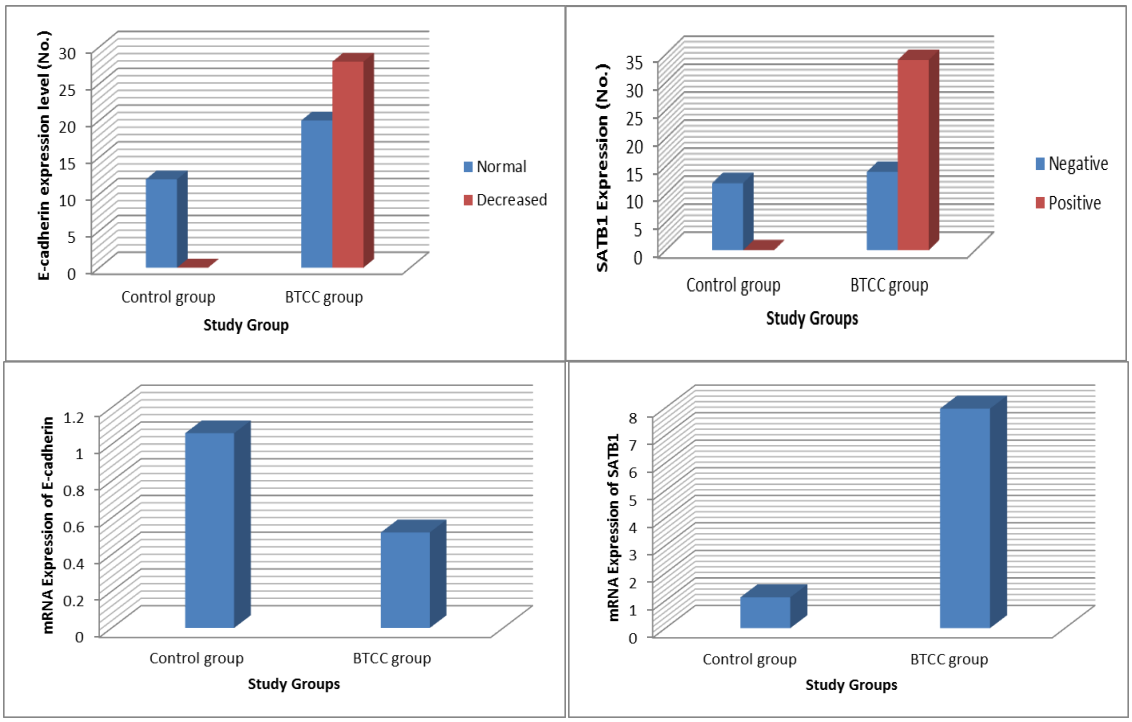

Figure (3): Bar chart shows the protein and mRNA expression levels of E-cadherin and SATB1 in control group and BTCC group.

Discussion:

Bladder cancer is the most common tumor in the urothelial tract. It is the ninth most frequently diagnosed cancer worldwide; bladder cancer has become common globally with an incidence more than 400,000 cases per year [16]. In Egypt, bladder cancer is the common type of cancer. The incidence rate of the bladder cancer in males is more than in females. In ancient time the bladder cancer caused by Schistosoma infection that it is a squamous cell type. Recently, the incidence of transitional cell carcinoma becomes increased and the squamous cell has been declined [19].

The histopathological pattern of bladder cancer has changed among Egyptian patients. Over the last decades, squamous cell carcinoma of bladder (SCC) is declining, while the incidence of bladder transitional cell carcinoma (BTCC) is rising and represents about 90% to 95% of all malignant bladder tumors. The remaining 5% to 10% comprises SCC and adenocarcinoma and other types of epithelial neoplasms [20]. The risk factors including exposure to pesticides, bladder stones, and smoking, these factors (smoking in particular) seem to play more important roles nowadays rather than schistosomiasis in the development of bladder cancer, especially in Upper Egypt [21].

In the present study, smoking was recorded in 25.0% (3/12) of control patients and in 62.5% (30/48) of BTCC patients.There was a high statistically significant association between the BTCC patients and the high risk of bladder cancer among Egyptian smokers. The results of the current study were near to those of Obeauge et al. [22] who reported that after the better control of Schistosomiasis and increased smoking incidence is believed to have contributed to the shift in Egypt towards TCC, which has a stronger smoking association. A mutation or aberration on chromosome 9p21 was common in smokers compared to those in nonsmokers. Benzopyrene diolepoxide (BPDE) is the metabolic product of benzopyrene present in tobacco smoke seems to induce the damage of Chromosome 9p21. Also, smoking may induce the mutations of Tp53 (tumor suppressor protein) [23].

E-cadherin protein is involved in the maintenance and the homeostasis of the normal adult epithelial tissue structure and integrity, where it main function is the construction of adherens junctions. Adhesive contacts between cells provide a physical anchoring system that is necessary to form highly organized tissues [24]. A reduced expression of E-cadherin may be resulting from the loss of heterozygosity (LOH) of the wild-type allele at 16q22.1, inactivation by mutations, high methylation of CDH1 gene promoter in CpG-island or stopping of CDH1 gene expression by specific transcription factors shown in many epithelial tumors [25].

E-cadherin plays a critical role in cancer progression itself, partially through the PI3K/AKT pathway (an intracellular signaling pathway essential in organizing the cell cycle) and MEK-ERK pathway (a group of proteins found in the cell that transmits a

signal from a cell surface receptor to DNA inside the cell nucleus). Integrin performs as a cell-cell adhesion molecule, play a role in increasing migration via its interaction with p-cadherin [26].

In the current study, the expression levels of E-cadherin were reported using immunohistochemical staining and RT-qPCR techniques. However, the expression levels of E-cadherin were revealed to be decreased significantly in the tumorous tissues compared to the non-tumorous tissues at both mRNA and protein levels ($p<0.000$).

In the same context, Hassan [27] who found that the E-cadherin expression in bladder cancer tissues is showed homogeneous memberanous immunoreactivity designated as normal staining in 12 cases (30%), whereas 28 cases (70%) showed abnormal E-cadherin expression. Khorrami et al. [28] estimated that the E-cadherin expression in 180 patients with superficial, unifocal, low grade, and papillary transitional cell carcinoma; the E-cadherin immune expression was absent in 101 (56%) and positive in 79 (44%) patients. On the other hand, Szekely et al. [29] found that E-cadherin expression was retained in 25 out of 40 (62%) cases. Jager et al. [30] reported that E-cadherin expression in bladder tumor did not change significantly from that of non-tumor mucosa ($p=0.146$).

In the current study, the decreased of the E-cadherin expression was significantly associated with the increasing of tumor stage for bladder cancer ($P=0.031$). Similarly, the decreased E-cadherin expression was high significant in high grade than in low grade ($P=0.002$). Esmail et al. [31] agree with our results who found that E-cadherin showed positive expression in 65% (13 case) of non-muscle invasive bladder cancer group and in 10% (2 cases) of the muscle invasive bladder cancer group, with high statistical significance ($p<0.001$).

The present results are similar to Xie et al. [32] they declared that; decreased E-cadherin expression in bladder cancer had a significant correlation with pathological T stage and tumor grade. Ismail et al. [33] found that bladder tumor invasion is often correlated with the decreased regulation of E-cadherin expression. There was a significant correlation between the decreased levels of E-cadherin expression and the late tumor grade of bladder cancer.

Also, Otto et al. [34] reported that E-cadherin is considered as a prognostic marker in bladder cancer that is absent in progressive cases. Paliwal et al. [35] found that a reduction of E-cadherin expression was statistically correlated with tumor stage and tumor grade ($P < 0.001$ and $P < 0.01$; respectively). On other hand, Reis et al. [36] detected that there was insignificant association between E-cadherin expression and tumor grade. Mialhe et al. [37] observed that E-cadherin expression was not correlated with the tumor grade, although it was highly correlated with the tumor stage. Zhao et al. [38] found that E-cadherin was not correlated with the grade.

In the present study, mRNA and protein level expressions of E-cadherin showed no significant association with age, gender, or tumor size. Similarly, Jager et al. [30] reported that no significant correlation was observed between E-cadherin mRNA expression and patient age or gender. Szekely et al. [29] found that no significant association between E-cadherin (mRNA or protein) expression and age or gender of the bladder patients.

Throughout carcinomas (cancers of epithelial tissue), E-cadherin expression has been inversely correlated with pathologic stage and grade as well as with patient's prognosis. On contrast, the loss of E-cadherin in carcinomas is promoted invasion and metastasis by decrease of cell to cell interactions [39]. SATB1 protein acts as a 'docking site' for several chromatin modifiers including (ACF, ISWI, and HDAC1) suggesting a molecular mechanism governing cellular plasticity. SATB1 induces histone modifying enzyme (p300) on promoters present in tumor oncogenes and preserves histone activation process (such as histone H3K9/14 acetylation mark highly activated in breast cancer progression). In contrast, SATB1 recruits HDAC1 on promoters of tumor suppressor genes (control of cell proliferation and differentiation), decreases the activation marks and thereby lowering their expression [40].

In this study, the expression levels of SATB1 were detected by performing immunohistochemistry and RT-qPCR analysis. By immunohistochemistry, the protein expression level of SATB1 was localized in the nucleus of the bladder cancer cells compared with completely negative immunoreactivity in non-tumor bladder tissues ($p<0.000$). This agrees with the results of Shaban et al. [41] found that protein expression level of SATB1 showed significant increased expression from complete negativity in normal urothelium to higher in primary bladder carcinoma to highest in lymph node metastases. Wan et al. [42] showed that the over expression of SATB1 at both mRNA and protein levels was statistically significantly in human bladder cancer tissues than that in normal tissues.

Choudhary et al. [43] detected that SATB1 mRNA and protein expression levels were 28-fold and 5-fold higher respectively, in HTB-5 cells (bladder cancer cell line) compared to UROtsa cells (benign transformed human urothelial cell line). Liu et al. [44] observed that the protein expression of SATB1 was significantly increased in urothelial bladder cancer tissues than in normal bladder tissues ($P<0.05$).

In current study, we found that over expression of SATB1 was statistically associated with tumor stage, tumor size, and tumor grade. However,

there was no significant differences between SATB1 over-expression and either gender or age. Likewise, Shaban et al. [41] and Choudhary et al. [43] reported that the levels of SATB1 expression in urothelial carcinoma were significantly associated with poor prognostic factors including advanced pathological T stage and high-grade. Liu et al. [44] found that the expression of SATB1 in the bladder tumor tissues was significantly correlated to the histological stage.

SATB1 function is blocked by using siRNA in the 5637 cell lines with relatively high endogenous expression. SATB1 depletion caused an obvious decrease in the proliferation rate of both cell lines where the levels of cyclin D1 (regulator for cell proliferation) and cyclin E (cell cycle progression) were decreased after SATB1 knockdown. SATB1 depletion increased caspase3 cleavage levels (a critical executioner of apoptosis) in bladder cancer cells treated with cisplatin, which was in accordance with increased apoptosis [45]. Baicalein (anticancer drug) strongly decrease the generation and invasion ability of MDA-MB-231 (breast cancer cells) and this may be through the suppression of SATB1 protein expression. These results may suggest that it could be a favorable target for novel anticancer drugs causing a significant decrease in cancer cells proliferation and invasion abilities [46]. SATB1 could increase the expressions of many genes which has the ability to induce tumor growth and metastasis (e.g MMPs, CDK4, Snail1, and Slug are cell cycle progression-promoting gene). Furthermore, it downregulates many tumor suppressor genes (e.g. epithelial markers E-cadherin, cell cycle arrest factors p16INK4A and promoting apoptosis gene FADD) [47].

A statistically significant difference was found between decreased protein expression of E-cadherin and protein over-expression of SATB1 (χ2=7.203, P=0.007). Pearson correlation test showed a statistically negative correlation between the mRNA expressions of SATB1 level and E-cadherin level (r=-0.442, P=0.002).

Data in the present study agrees with Naik et al. [40] who found that the protein expression of SATB1 was negatively correlated with adhesion protein (E-cadherin) responsible for an epithelial phenotype (EMT) of the cell in bladder cancer. Wan et al 42 suggested that there were a statistically correlations between SATB1 expression (mRNA or protein) and EMT markers in bladder cancer samples, which showed that overexpression of SATB1 is inversely correlated with E-cadherin expression. Han et al. [45] revealed that the knockdown of SATB1 reverses the EMT process through downregulation of Snail and SIPI (E-cadherin transcription repressors) and also through upregulation of E-cadherin in highly aggressive cancer cells (MDA -MB-231). Han et al. [48] mentioned that E-cadherin protein expression was decreased by over expression of SATB1, while SATB1 reduction could increase E-cadherin expression and return EMT process to its normal form, resulting in the reconstruction of acinar-like morphology.

Conclusion

The expression levels (mRNA or Protein) of E-cadherin and SATB1 could be used as a marker in the diagnosis and estimate the prognosis of transitional cell carcinoma in urinary bladder of Egyptian patients.

Conflict of Interests

The authors declare that there is no conflict of interest with the manuscript.

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دراسة كيميائية حيوية للإيكادرين وSATB1 في سرطان الخلايا الإنتقالية في المثانة البولية للمرضي المصريين.

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يعتبر سرطان المثانة ثالث أكثر الأورام شيوعا في مصر وفقا لسجل المعهد القومي للأورام. ومع ذلك، يمثل سرطان الخلايا الانتقالية في المثانة البولية حوالي 90% من جميع حالات سرطان المثانة. هدفت الدراسة الحالية الي تقييم مستويات التعبير للايكادرين وSATB1 (مستويات البروتين وmRNA) كمؤشرات حيوية لسرطان الخلايا الانتقالية باستخدام تقنيات التحليل الكيميائي المناعي للأنسجة وتفاعل البلمرة المتسلسل (RT-qPCR) في أنسجة المثانة البولية للمرضى المصريين المصابين بسرطان الخلايا الانتقالية مقارنة مع أنسجة المثانة الغير سرطانية. تضمنت هذه الدراسة 48 عينة من أنسجة المثانة البولية لمرضي سرطان الخلاية الانتقالية (41 ذكور و7 إناث) مختلفين في درجة الورم ومدي انتشاره و12 عينة من أنسجة المثانة البولية الغير سرطانية (7 ذكور و5 إناث). النتائج, وجد ارتباط إحصائي فيما يتعلق بنوع الجنس والتدخين بين مجموعة مرضي أورام المثانة والمجموعة الضابطة، لا توجد علاقة إحصائية لداء البلهارسيا في المجموعتين محل الدراسة. وجد انخفاض ملحوظ في مستوي تعبير الايكادرين وارتفاع ملحوظة في مستوي تعبير الSATB1 في أنسجة المثانة البولية لمرضى سرطان الخلايا الانتقالية بالمقارنة بأنسجة المثانة البولية الغير سرطانية في كل من مستويات (mRNA) والبروتين، وجد أيضا ارتباط إحصائي بين انخفاض مستوي تعبير الايكادرين وارتفاع مستوي تعبيرSATB1 بدرجة وحجم الورم ومدي انتشاره. من ناحية أخري, لا يوجد فروق ذات دلالة احصائية بين مستويات تعبير الايكادرين وSATB1 وكل من نوع الجنس والعمر. وجد ارتباط ذات دلالة إحصائية في مستويات التعبير البروتيني للأنسجة بين الايكادرين وSATB1، أظهر ارتباط بيرسون علاقة إحصائية عكسية جيدة في مستويات التعبير لـ (mRNA) بين الايكادرين وSATB1. الخلاصة، يمكن اعتبار الايكادرين وSATB1 كمؤشرات كيميائية حيوية جيدة في تشخيص سرطان الخلايا الانتقالية في المثانة البولية ومدي انتشاره للمرضي المصريين.

الكلمات الدالة: الايكادرين، SATB1، سرطان الخلايا الانتقالية، التحليل الكيميائي المناعي للأنسجة، تفاعل البلمرة المتسلسل