RESEARCH ARTICLE

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The Immunohistochemical Expression of SOX-10 in Urothelial Carcinoma and the Non Neoplastic Urothelium; and a Correlation with the Tumor Features

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Abstract

Objective: Evaluation of SOX-10 expression in malignant urothelial cells, comparing it with the phenotypically non neoplastic urothelium, and correlating it with the various clinicopathological variables, with a focus on the invasive pattern. **Methods:** Eighty paraffin blocks of urothelial carcinoma were stained by H&E. Histopathological features were evaluated and then immunostained with SOX-10 to evaluate its expression. **Results:** The evaluation of SOX-10 expression in urothelial carcinoma, revealed a high grade of SOX-10 expression in the malignant urothelium (43\80 cases; 53.7%), while the adjacent the non neoplastic urothelium expressed high SOX-10 in (12\42 case; 28.6%). Correlation of SOX-10 score with the various variables revealed a statistically significant correlation with the gross shape (P value=0.002), the tumor grade ((P value=0.009), the muscle invasion by the tumor ((P value=0.004), the tumor T stage, (P-value <0.001), N stage (P value=0.003), associated Schistoma hematobium infestation (P-value =0.016), and the presence of vascular tumor emboli (P-value =0.009). It was statistically insignificant with the gender, the anatomical site, and the perineural tumor invasion. Correlating the mean of SOX-10 score with some tumor features revealed a statistically significant correlation with the muscle invasion by the tumor, Tumor grade, T stage, and non neoplastic urothelium; P-value <0.001 each and N stage P value=0.006. **Conclusion:** SOX-10 is overexpressed in urothelial carcinoma and it was also detected in a significant part of the surrounding non neoplastic urothelium, which may contribute to understanding its role in multistep urothelial carcinogenesis as transcription or tumor-promoting factor, thus it could be used in future trials for specific targeted therapy.

Keywords: Urothelial carcinoma- normal urothelium- SOX-10

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Introduction

Cancer urinary bladder is a distressingly incident malignancy that contributes by 3% to the newly developing cancer pool. It ranks the tenth in the incidence in the global cancer registry 2018. Its incidence shows a male gender preponderance; constituting the sixth incident and the ninth fatal male malignancy (Bray et al., 2018; Ferlay et al., 2018).

The etiopathogenesis can be largely traced to environmental and occupational factors. Occupational exposure to industrial chemicals like amines, polycyclic aromatic hydrocarbons, and heavy metals is incriminated (Cumberbatch et al., 2015).

But the long-standing tobacco smoking is still the most risky, which mediates cellular conversion through a pro-inflammatory effect (Mushtaq et al., 2019).

Chronic Inflammation is an established risk factor in bladder cancer. It produces a variety of chemokines, and cytokines, that facilitate tumor cell proliferation, and angiogenesis. Also, it elaborates mutagenic reactive oxygen species and induces changes in the tumor micro-environment enhancing the tumor spread (Pikarsky et al., 2004; Michaud, 2007). In Egypt; it is notably associated with chronic Schistosoma haematobium infestation (Felix et al., 2008).

The conversion of the normal urothelium to a neoplastic one occurs through a complex and overlapping series of molecular events; known as epithelial plasticity. These changes are reversible at early stages (Horst et al., 2012). This process is contributed and even initiated by epithelial-mesenchymal transition (EMT) transcription factors, namely; ZEB, SNAIL, and TWIST (Nieto and Cano, 2012).

Mesenchymal stem cells in the bladder can differentiate into multiple cell lines including endothelial cells, and fibroblasts that also promote autocrine growth (Tachibana et al., 1995).

The molecular-based cancer diagnosis for the purpose of targeted therapy is uprising, but urothelial cancer is not

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yet enlisted (Netto and Cheng, 2012; Xylinas et al., 2014).

The urothelial carcinogenesis is divided molecularly into two divergent pathways with some overlap resulting in 2 different morphological and prognostic subtypes, which are papillary non-muscle invasive and the muscle-invasive forms (Bakkar et al., 2003).

The papillary non-invasive carcinoma arises from normal urothelium as a continuum of proliferative events; starting with hyperplasia, then papillary urothelial neoplasia; Non-muscle-invasive bladder cancer (NMI-BC), with a minor contribution (10–15%) to the pool of high-grade non-invasive and invasive urothelial cancer. Despite its less muscle-invasive potential, it has a distressing recurring course (Crawford, 2008; Cheng et al., 2010). The primary genetic alterations affect the Tyrosine kinase receptor, Fibroblast growth factor receptor-3 (FGFR-3), Harvey rat sarcoma viral oncogene homolog (H-RAS), and Phosphatidyl inositol-4,5-bisphosphate 3-Kinase, catalytic subunit alpha (PI3KCA) which promote cell proliferation (Millis et al., 2015).

While most invasive carcinoma arises through cumulative genetic mutation of tumor suppressor genes including p53, p16, and Rb (Wu, 2005; Mitra et al., 2006; Knowles and Hurst, 2015) that establishes a sequence of dysplasia to flat carcinoma in situ (CIS) then high-grade non-invasive to muscle-invasive urothelial carcinoma (MI-BC) (Mitra and Cote, 2009; Al Hussain and Akhtar, 2013).

Both groups share common genetic alterations, with the early loss of heterozygosity (LOH) on chromosome 9, which represents the vehicle for the genetic instability that facilitates the accumulation of genetic defects. Also, mutations in PI3K, Tuberous sclerosis 1(TSC1), Protein patched homolog 1 (PTCH), Cyclin- dependant kinase inhibitor2A (CDKN2A), and deleted in breast cancer 1(DBC1) were encountered (Kawauchi et al., 2009).

Sex determining region Y-BOX10 (SOX-10) is a transcription factor that belongs to the SOX family (Kiefer, 2007), it has a role in embryonic development (Haldin et al., 2010; Stolt et al., 2010) and an essential one in the differentiation of neural crest cells (Watanabe et al., 2013). SOX-10 has been implicated in the genesis and development of various cancers (Kwon et al., 2016; Panaccione et al., 2016).

SOX-10 has two extremes of aberrant expression (low and high) in different malignancies, so it can perform dual sets of opposing actions. The outcome action is determined by the transformed neoplastic cell histogenesis. It may act as a tumor oncogene associated with an increased expression as in gliomas (Ferletta et al., 2007) and melanoma (Shakhova et al., 2012). In hepatocellular carcinoma; it accomplishes its action by activating the Wnt/B-catenin/TCF4 cascade (Zhou et al., 2014).

A tumor-suppressing activity with inhibited Wnt/B-catenin signaling pathway, the epithelial-mesenchymal transition (EMT), and stemness of tumor cells, was detected in digestive cancers (Tong et al., 2014). However, the expression and the role of SOX10 in the bladder cancer genesis and progression of tumors are still controversial.

Aim of the study: Evaluation of SOX-10 expression in the malignant urothelium, correlating the degree of expression with the invasive pattern of the tumor and other pathological variables for future trials of targeted therapy. Also detection of its expression in the phenotypically normal urothelium to investigate its role in the process of tumorigenesis.

Materials and Methods

Study sample and data collection: A retrospective cross sectional study was conducted on 80 tumor paraffin blocks for urothelial carcinoma of the urinary bladder, that were diagnosed in Cairo university hospital, during the period from 2019-2021. Permission for utilizing archived medical records and the release of the preserved samples were gained from the ethical committee of the scientific research. The relevant clinical and pathological data were retrieved from the records and tabulated.

Histopathological evaluation: Examination of H&E stained sections was blindly performed by 2 independent pathologists.

1- The tumor's histopathological features were assessed according to the WHO 2016 classification for the tumors of the urinary system and male genital organs. They were classified into non-invasive papillary and invasive urothelial carcinoma and graded using a two-tier grading system into a low and high grade (Humphrey et al., 2016).

Tumors were staged according to the AJCC cancer staging manual (Amin et al., 2017). The presence of associated Shistosomal infestation was reported.

2- All the previous data were tabulated and correlated with the detected immunohistochemical findings.

Immunohistochemical procedures: A section of formalin-fixed, paraffin-embedded tumor tissue on a positively charged side was immunohistochemically stained by Ventana autostainer, following the manufacturer protocol using anti-human SOX-10 monoclonal antibody (clone EP268, Rabbit Ig G) pre-diluted at (1:100), manufactured by BIO SB, USA. The reaction was carried out using the Avidin-Biotin immunoperoxidase system.

In each staining session, skin tissue was used as a positive control, and as a negative control, a tumor tissue section was processed in the same setting but the primary antibody was not added, and instead, PBS was used.

Immunohistochemical evaluation

Sox-10 immunoreactivity: Positive Sox-10 reaction was identified by brownish nuclear staining within the urothelial cells that were evaluated by 2 independent pathologists with blind clinical data.

*The immunoreactivity was assessed according to the immunoreactivity score intensity (IRS), which was the result of the multiplication of both the percentage and the intensity of staining.

* The percentage of positive cell was calculated per x200 PF and it was defined as follows; (0; No stained cells), (1; 1–25%), (2; 26–50%), and (3; 51–100%).

*Tissue staining intensity was graded as follows: (0; No staining), (1; light brown), (2; moderate brown), and (3; strong brown).

*The immunoreactivity score was dichotomized into (low=1–3) and (high =4–9) scores of expression (Han et

al., 2014).

* Correlating scores of Sox-10 expression with the clinicopathological parameters was done.

* Detection of SOX-10 expression in phenotypically normal urothelium and determination of its score.

Statistical methods

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 24. Comparisons between two groups with respect to normally distributed numeric variables were done using the t-test. Non-normally distributed numeric variables were compared by the Mann-Whitney test. For categorical variables, differences were analyzed with 2 (Chi-square) tests and Fisher's exact test when appropriate. All P values are two-sided. P values< 0.05 were considered significant.

Table 1. SOX-10 Expression in Urothelial Cells

SOX-10 score	Malignant urothelium	Non neoplastic urothelium (42\80) (52.5%))	P-value
High	43 (53.7%)	12 (28.6%)	0.007s ignificant
Low	37 (46.25%)	30 (71.4%)	
Total	80 (100%)	42 (100%)	

Table 2. The Correlation between the Age and SOX-10 Expression

Age	Sox score	Number	Mean	Std. Deviation	P-value
	High	43	57.05	10.845	0.588
	Low	37	58.32	10.022	

Results

Clinico-Pathological features: The current study included 80 cases of vesical urothelial carcinoma. Their age ranged between 25 and 82 years, with a mean value of 58 and a median value of 60 ± 10 standard deviations. They were composed of (67 males, 83. 8%), and (13 females, 16.3 %). The specimens were obtained through cystoscopic biopsy in (27 cases; 33.8 %) & radical cystectomy in (53 cases; 66.2 %). The morphological features of the tumor revealed the following; the predominant site in descending order was at the posterior wall (36 cases; 45%), the dome (26 cases; 32.5%), the lateral wall (9 cases; 11.3 %), the whole wall (8 cases; 7.5%), the anterior wall (2 cases; 2.5%), and at the neck (1 case; 1.3%). The gross shape was polypoid (30 cases; 37.5%), fungating (13 cases; 16.3%), ulcerative (19 cases; 23.8%), and infiltrative(18 cases; 22.5%). The tumor was papillary non-invasive in (15 cases; 18.75%) and invasive in (65 cases; 81.4 %). The tumor histological grade was low in (27 cases; 33.8%) and high in (53 cases; 66.3%). Lymphovascular invasion was detected in (22 cases; 27.5%) and perineural invasion was noted in (8 cases; 10%). Shistosomal hematobium cystitis was associated in (21 cases; 26.3%). Classifying the tumor according to TNM stage revealed that (15 cases; 18.8%) were Ta stage, (11 cases; 13.8%) were T1, (17 cases; 21.3%) were T2, (25 cases; 31.3%) were T3, and (12 cases; 15%) were T4. As regards the lymph node invasion; (8 cases; 10%) were N1, and (12 cases; 15%) were N2.

Immunohistochemical features

Evaluation of SOX-10 expression in the urothelium revealed that malignant urothelium showed a low SOX-10 score in (37\80 cases; 46.25%) (Figure 1) and high SOX-10 score in (43\80 cases; 53.7%) (Figures 2, 3 and 4) and. The normal urothelium adjacent to the urothelial carcinoma showed SOX-10 expression in (42\80 cases; 52.5%); (30\42 cases; 71.4 %) displayed low expression (Figure 5) and (12\42 cases; 28.6%) displayed high expression. The correlation was statistically significant. P Value=0.007 (Table 1).

The correlation between SOX-10 score and the age was inversely proportionate; as lesser mean age (57.05) was detected with a higher SOX-10 score, and vice versa. The correlation was statistically insignificant. P value=0.588

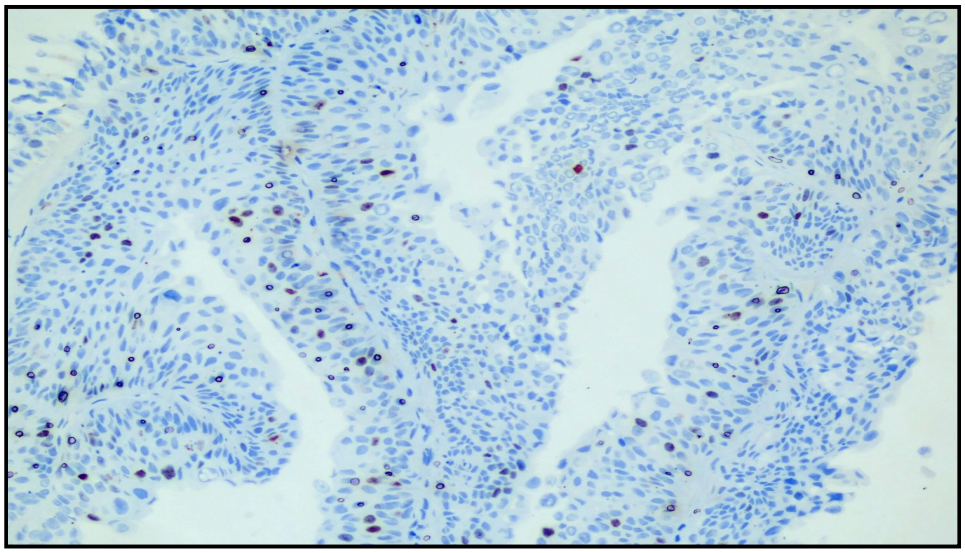

Figure 1. Non Muscle Invasive Low Grade Papillary Urothelial Carcinoma Showing Less than 25% of the Urothelial Cells Stained Light Brown with SOX-10 Immunostaining (low SOX-10 score). (Magnification power 200x).

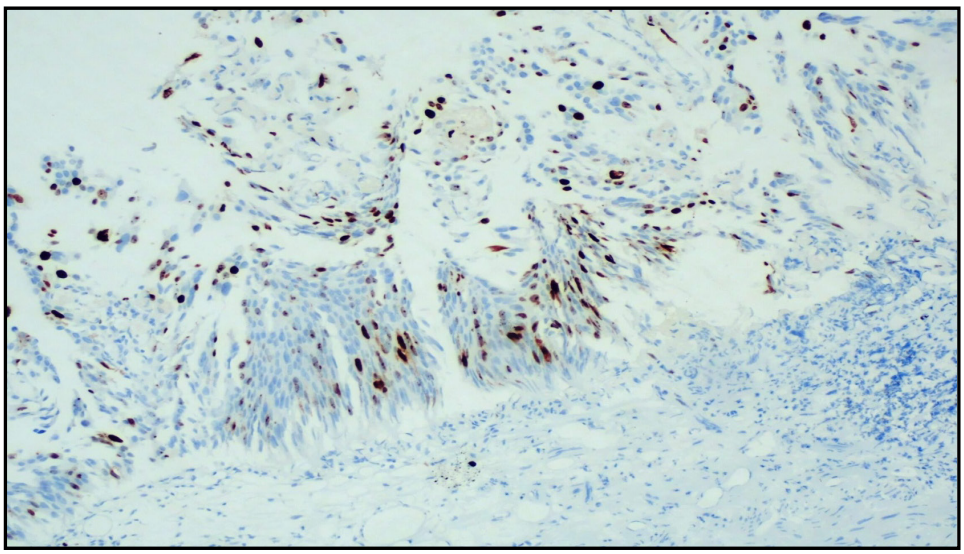

Figure 2. Non-Muscle Invasive Low Grade Papillary Urothelial Carcinoma Showing more than 25% of the Urothelial Cells with Strong SOX-10 Immunostaining, (high SOX-10 score). (Magnification power 200x).

(Table 2).

The correlation between SOX-10 score and other clinicopathological parameters as illustrated in (Table 3) and summarized as follows; SOX-10 score was insignificantly correlated with the gender; as high score was detected in (34 males; 50.7%), and low score was detected in (33 ones; 49.3%). The female gender showed a high score in (9 cases; 69.2%) and low in (4 cases; 30.8%). P-value=0.221.

Comparing SOX-10 score with the anatomical site revealed a predominance of high SOX-10 score at the dome 65%, equal incidence at the anterior and the posterior walls, the whole wall 50%, less at the lateral wall 44.4%, and absence at the neck. The relation was statistically insignificant. P-value = 0.672.

Correlating SOX-10 score with the tumor gross morphology revealed that high SOX-10 score was detected in (12 fungating cases; 92.3%), (12 ulcerative cases; 63.2%), (10 infiltrative cases; 55.6%) and in (9 polypoid cases; 30%). The correlation was statistically significant. P value=0.002.

SOX-10 score showed a statistically significant correlation with the tumor grade as High SOX-10 score was detected in (34 high-grade cases; 64.2%), and in (9 low-grade cases; 33.3%), while low SOX-10 score was detected in (19 high-grade cases; 53.8 %), and in (18 low-grade cases; 66.7%). P-value =0.009.

SOX-10 was significantly correlated with the invasive pattern of the tumor; as papillary non-invasive tumor displayed high SOX-10 score in (3\15 cases; 20%) and low SOX-10 score in (12\15 cases; 80%), while invasive carcinoma showed a high SOX-10 score in (40\65 cases; 61.5%) and low SOX-10 score in (25\65 cases; 38.5%). P-value= 0.004.

Tumors associated with vascular tumor emboli displayed a high SOX-10 score (17\22 cases; 77.3%) and

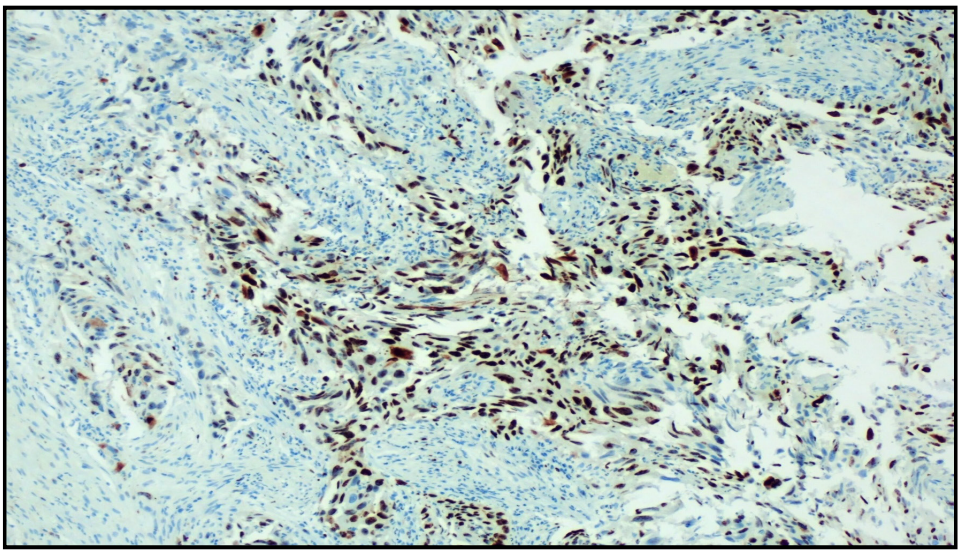

Figure 3. High Grade Papillary Urothelial Carcinoma Infiltrating the Lamina Propria Showing more than 50% of the Invasive Malignant Urothelial Cells with Strong SOX-10 immunostaining (high SOX-10 score). (Magnification power 200x).

Table 3. Correlation of SOX-10 Score in Urothelial Cells, with the Clinicopathological Parameters

Variables		SOX-10			P-value
		High	Low	Total	
Sex	Male	34 (50.7%)	33 (49.3%)	67	0.221
	Female	9 (69.2%)	4 (30.8%)	13	
Biopsy	Cystoscopic	6 (22.2%)	21 (77.8%)	27	<0.001*
	Radical	37 (69.8%)	16 (30.2%)	53	
Site	anterior	1 (50.0%)	1 (50.0%)	2	0.672
	dome	17 (65.4%)	9 (34.6%)	26	
	lateral	4 (44.4%)	5 (55.6%)	9	
	neck	0 (0.0%)	1 (100.0%)	1	
	posterior	18 (50.0%)	18 (50.0%)	36	
	whole	3 (50.0%)	3 (50.0%)	6	
Shape	Fungating	12 (92.3%)	1 (7.7%)	13	0.002*
	Infiltrating	10 (55.6%)	8 (44.4%)	18	
	Polypoid	9 (30.0%)	21 (70.0%)	30	
	Ulcerative	12 (63.2%)	7 (36.8%)	19	
Grade	High	34 (64.2%)	19 (35.8%)	53	0.009*
	Low	9 (33.3%)	18 (66.7%)	27	
Invasion	Invasive	40 (61.5%)	25 (38.5%)	65	0.004*
	Papillary Non invasive	3 (20.0%)	12 (80.0%)	15	
Vascular emboli	Yes	17 (77.3%)	5 (22.7%)	22	0.009*
	No	26 (44.8%)	32 (5.2%)	58	
Perineural invaion	Yes	6 (75.0%)	2 (25.0%)	8	0.275
	No	37 (51.4%)	35 (48.6%)	72	
Schistosoma hematobium	Yes	16 (76.2%)	5 (23.8%)	21	0.016*
	No	27 (45.8%)	32 (54.2%)	59	
T stage	Ta- T1	4 (15.4%)	22 (84.6%)	26	<0.00001*
	T2-T4	39 (72.2%)	15 (7.3%)	54	
N stage	N0	26 (52.0%)	24 (8.0%)	50	0.001*
	N1- N2	16 (80%)	4 (20%)	20	
	Nx	1 (10.0%)	9 (90.0%)	10	

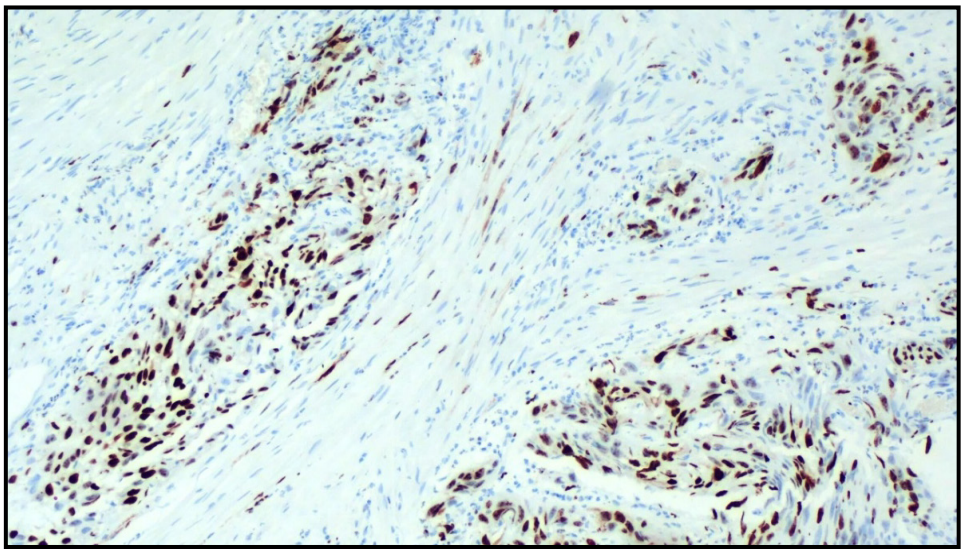

Figure 4. Muscle Invasive Urothelial Carcinoma Showing more than 50% of the Invasive Malignant Urothelial Cells with Strong SOX-10 Immunostaining (high SOX-10 score). (Magnification power 200x).

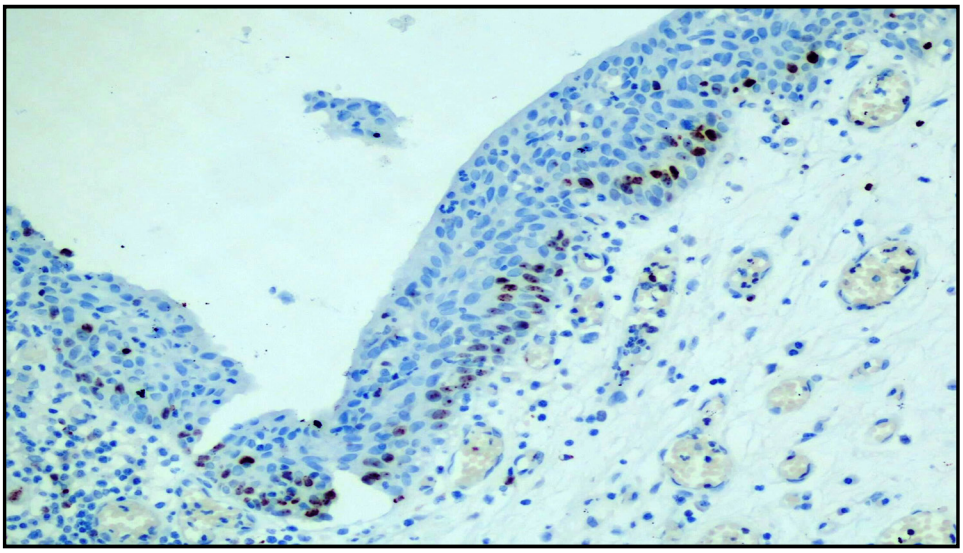

Figure 5. Non Neoplastic Urothelium Showing Strong Brown SOX-10 Immunostaining in Less than 25% of Cells (Low SOX-10 score). (magnification power 200x).

low SOX-10 score (5\22 cases; 22.7%). The correlation was statistically significant. P value= 0.009.

Tumors associated with perineural tumor invasion displayed a high SOX-10 score in (6/8 cases; 75%) and low SOX-10 score (2\8 cases; 25%). The correlation was statistically insignificant. P value= 0.275

Tumor-associated Schistosomal hematobium infestation was statistically significantly correlated with SOX- 10 scores; As it was coupled with a high SOX-10 score in (16\21 case; 76.2%) and with low SOX-10 score in (5\21; 23.8%). (P-value =0.016).

For correlating the tumor T stage with SOX-10 score; the T stage of the tumor was summated into two groups; Non-muscle invasive tumors (T0 and T1) and the muscle-invasive tumors (T2-T4). It revealed that a high SOX-10 score was directly associated with increased depth of invasion as it was detected in (4\26 non-muscle-invasive cases; 15.4%), and (39\54 the muscle-invasive cases; 72.2%), while the reverse was noted regarding low SOX-10 score as it was detected in (22\26 non-muscle-invasive cases; 84.6%), and (15\54 muscle-invasive cases; 27.3%). The correlation was statistically significant. P-value <0.00001.

Correlating the tumor N stage with SOX-10 score showed high SOX-10 score in (16\20 positive cases; 80%) and low SOX-10 score in (4\20 positive cases; 20%). A statistically significant correlation was attained. P-value =0.001.

Discussion

Urothelial carcinoma is prevalent cancer that shares in the global cancer-related mortality by about 150,000 deaths per year. Many risk factors are well established but the tumorigenesis process is complex and still not completely understood (Wyszynski, et al., 2014).

It shows morphologically and genetically diverse features that influence the clinical behavior. The spectrum ranges from multiple recurrences up to mortality, which constitutes a challenge to the onco-therapist despite the progress achieved in the cancer therapy (Torre et al., 2012). Several studies have investigated SOX-10 expression in different tumors but a consensus for a unified role was not approved, instead, a different role linked to different histogenesis was suggested (Shakhova et al., 2012). In hepatocellular carcinoma and nasopharyngeal carcinoma; SOX-10 acted as a tumor promotor but in digestive malignancy a tumor suppressor function was observed (Ohtomo et al., 2013; Zhou et al., 2014; Zhao et al., 2016).

The current study delineated that SOX-10 over-expression was detected in urothelial carcinoma and to a lesser incidence in the adjacent urothelium. It was significantly correlated with the adverse pathological features of the tumor (the invasive pattern, TN stage, high-grade cyto-architectural features, lymphovascular emboli, and perineural invasion) and also with the presence of Shistosoma hematobium infestation suggesting that SOX10 expression might act as tumor transcription or promote factor in urothelial carcinogenesis.

In our study, SOX10 over-expression was significantly detected in urothelial carcinoma as high SOX10 expression in malignant urothelium was detected in (43/80 case; 53.7%), low expression in (37 cases; 46.25%) compared to high expression (12/42; 28.6%) and low expression (30/42cases; 71.4) in the surrounding non neoplastic tissues. P value=0.007. This result showed approval with that performed by (Yin et al., 2017) who showed high SOX10 expression in bladder cancer tissues (67/90 cases; 74.4%,) compared to that in normal tissues (15/46; 32.6%) (P = 0.000). They suggested that SOX -10 acted as a transcription factor. But it was contradicted with that (Xu et al., 2016), who detected weak SOX10 expression in (19/50 cases; 38%) of bladder cancer tumor tissue compared to that of non-neoplastic and they suggested a tumor suppressor role.

The current study showed a statistically significant correlation between SOX-10 expression and the tumor grade (P-value <0.009). A high SOX-10 score was detected in (34/53 of high-grade cases; 64.2%), and (9/27

of low-grade cases; 33.3%). This result showed approval with that obtained by (Yin et al., 2017) who detected a high SOX-10 score in (40\45 of high-grade cases) and (27\45 of low-grade cases). P value=0.002. But it showed disapproval with (Xu et al., 2016) who detected high SOX-10 expression in (16\28 high-grade cases), and the correlation was insignificant. P value=0.4247.

The current study revealed a statistically significant correlation between SOX-10 expression and the tumor T stage (P-value <0.00001). A high SOX-10 score was detected in (39\54 of the muscle-invasive cases 72.2%); (T2-T4) and (4\26; 15.4% of the non- muscle invasive cases; (Ta-T1). This result showed approval with that obtained by Yin et al., (2017) who detected a high SOX-10 score in (51\61 of the muscle-invasive group) and (16\29 of the non-muscle invasive group). P value=0004. These results showed disapproval with XU et al., (2016) who showed high SOX-10 expression in (21\29 T1-T2 cases) and (10\21 of Ta cases). The correlation was statistically insignificant. P value=0.0746. This discrepancy may be explained by the different T-stage grouping used in their study.

Regarding the presence of the nodal metastasis, our study showed a statistically significant correlation as a high SOX-10 score was detected in (16\20 positive cases; 80%), similar to that of (Yin et al., 2017) who also detected high score in (30\33 positive nodal cases). P value= 0.006, and also concordant with that of (Xu et al., 2016) who detected high SOX-10 expression in (10\23 positive nodal cases). The correlation was statistically significant. P value=0.0128.

The current study revealed a statistically insignificant correlation for each age and gender. P value=0.588 and 0.221 respectively, which shows concordance with both of (Yin et al., 2017) whose P-value was 0.387 and 14.8 and also with those of (Xu et al., 2016) whose P-value was 0.6653 and 0.3413 for each age and gender respectively.

To sum up SOX-10 was found to be over-expressed in urothelial carcinoma and it also expressed significantly in the surrounding non neoplastic urothelium. This expression in the surrounding non neoplastic urothelium was directly conjugated with the score of SOX-10 expression in the malignant urothelium, shedding a light on its potential role in urothelial carcinogenesis as a promoting effect. The over-expression in urothelial carcinoma that was significantly related to the tumor invasive potential could be used as a nucleus to build up the urothelial targeted therapy.

Author Contribution Statement

Manuscript has been read and approved by all the authors:

Category 1:

a) Conception and design: Samar.Amer.

b) Analysis of data: All authors

c) Interpretation of data: All authors

Category 2:

a) Drafting the article: All authors

b) Revising it critically for important intellectual content: All authors

Category 3:

Final approval of the version to be published: All authors.

6. Availability of data: Available upon request, with official permission, according to the institutional rules.

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Scientific approval

The authors confirm that this research work gained approval from Cairo university, scientific research programme.

Ethics approval

The study was approved by the Institutional Medical Ethical Committee (Faculty of Medicine, Cairo University).

Conflict of interests

The authors declare that they do not have any conflicts of interests.

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