ORIGINAL ARTICLE

CONTENTS

Immunohistochemical evaluation of SOX-10 in patients with urinary bladder cancer

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ABSTRACT

Aim: To evaluate the expression levels of SOX-10 in tissues of bladder tumor and to prove the correlation between SOX-10 expression and clinicopathological characteristics of bladder tumors, including patient age, sex, tumor grade, and muscle invasion.

Materials and Methods: Forty formalin fixed paraffin embedded FFPE tissue blocks gathered by transurethral resection of bladder tumor are collected from teaching hospitals at Al-Najaf governorate. Those blocks were stained by hematoxylin and eosin. The histopathological characteristics were examined and immunostained by SOX10.

Results: An assessment of the expression of SOX-10 in urothelial carcinoma showed that the SOX-10 test was positive in five samples of urothelial carcinoma, while the SOX-10 test was negative in thirty-five samples. The correlation of SOX-10 with other variables (age, sex, and grade) was statistically non-significant.

Conclusions: SOX-10 is an immunohistochemical sensitive marker for the diagnosis of urothelial carcinoma. SOX-10 expression appeared to be non-significant relation with age, gender and histopathological parameters and its expression has been showed to be lost or decreased in substantial proportion in relation to urothelial carcinoma. This variation or down regulation of SOX-10 is correlated with higher tumor grade and stage.

KEY WORDS: urothelial carcinoma, SOX-10, immunohistochemistry

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INTRODUCTION

Bladder carcinoma (BC) is the most frequently malignant tumor of urological field in world [1]. Transitional cell carcinoma (TCC) is the commonest kind and reports about 90% of cancers bladder [2]. Despite aggravating factors like smoking and obesity was determined for long time, the underlying causes of bladder cancer formation and development still unknown [3, 4]. Although an advancement of techniques of surgical and chemotherapy in the treatment of bladder cancer, the end results of therapy (for invasive and superficial bladder cancer) still substandard. Proof demonstrates that the formation bladder cancer is multistep mechanism which involves initiation, promotion and progression [5]. In spite of carcinoma of urothelium (UC) may be affect many areas of the urinary tract, more frequently occurs in the urinary bladder. Bladder cancer is sometimes determined in older age patients (more than 50) [6]. Hematuria (blood in urine) is the commonest symptom [7]. Nearly ninety percent of cancers of bladder are carcinomas of urothelium originated from the tissue of urothelium [8]. Urothelial carcinomas (non–invasive) occupy the most of primary bladder tumors and are divided depend on their structure as flat and papillary lesions [9]. On the other hand, seventy-five percent of carcinomas (invasive) are non-muscle invasive of bladder cancers and twenty-five percent of carcinomas are muscle invasive of bladder cancers [10]. Superficial type of bladder cancers may found in the mucosal layer, invaded to the lamina propria layer, or carcinoma in situ (noninvasive). The muscularis propria was recorded like a progressed cancer [11-13]. During the time of treatment of urothelial carcinoma, the tumor grade and stage are very important step. Meanwhile low grade tumors progression is uncommon, on other hand, progression and recurrence of high grade tumors are more frequent [14]. Bladder cancers grading and staging must be properly determined for the type of treatment to be used. It is critical for deciding the follow-up of the disease [15]. SOX-10 sex determining region is a type of SOX family, that is a group of translation factors coding a high-mobility group (HMG) domain that are joined to the factor of determination of testis [15]. Firstly, SOX-10 is known depend on its important

function in crest cells differentiation of neuron [16, 17]. Mutations in SOX-10 gene of human are joined with the appearance of neurocristo pathies of Warrensburg Shah Syndrome and patients show hypopigmentation, cochlear neurosensory deafness and enteric aganglionosis [18, 19]. Newly, SOX-10 was proved to be included in the genesis and development of many cancers [20, 21]. However, in digestive cancers SOX10 act a tumor suppressor and work against the signaling pathway of WNT/β-catenin [22]. We are well aware that SOX-10 is more appeared in bladder cancer tissues and cells, and its appearance is connected to the clinical and pathological features of bladder cancer patients. Besides, SOX-10 was played an important role in the bladder cancer cells growth, migration and invasion that connects with tumor genesis, infiltration and metastasis of bladder cancer [23]. SOX-10 is joined with salivary gland tumors, breast, nasopharyngeal, ovarian and prostate cancers growth and progression. SOX-10 high expression represents an oncogene for stimulating the signal pathway of WNT/β-catenin in hepatocellular carcinoma, and represents a tumor suppressor for suppressing the signal pathway of WNT/β-catenin in cancers digestive system [24-26].

AIM

To evaluate the expression levels of SOX-10 in tissues of bladder tumor and to prove the correlation between SOX-10 expression and clinic-pathological characteristics of bladder tumors, including patient age, sex, tumor grade, and muscle invasion.

MATERIALS AND METHODS

The cross-sectional study of 40 samples of Iraqi patients with urothelial carcinoma was performed at faculty of medicine of University of Kufa (middle Euphrates unit for cancer research). Formalin fixed paraffin embedded tissue blocks (FFPE) gathered by transurethral resection of bladder tumor are collected from teaching hospitals at Al-Najaf governorate, and the diagnosis of urothelial carcinoma performed by histopathologist. Those blocks were stained by hematoxylin and eosin (Fig.1). The processing of immunohistochemistry (IHC) was done using positive charge coated slides. IHC staining protocol by labeled streptavidin biotin (LSAB) method was performed after de-paraffinization, heat induced antigen retrieval for 20 minutes, then blocking with peroxidase enzyme for 5 minutes, followed by primary antibody SOX-10 (Bio SB, clone: EP268). After incubation with primary antibody, the secondary antibody was added for 30 minutes followed by horse reddish peroxidase (HRP) for 30 minutes, and chromogen for 15 minutes. Each step washed twice with buffered solution. Finally, counter staining and mounting. The reactivity of SOX-10 was considered as positive when there was nuclear staining.

SCORING

Depending on the intensity of staining and the percentage of positivity, immunohistochemical evaluation of slides stained with SOX-10 was scored. An intensity score was assessed as 0 (no stain), score 1 (light stain), score 2 (moderate stain) score 3 (strong stain) divided by score percentage, both scores will be recorded apart between the groups.

STATISTICAL ANALYSIS

Statistical methods employed to assess the correlation between SOX-10 expression and clinicopathological features of urothelial carcinoma, like age, sex, histological grade, and muscle invasion was chi-square test. SPSS version 26 was used for the calculation. P-value equal to 0.05 or less was regarded as statistically significant.

RESULTS

Samples of urothelial carcinoma in this study were forty FFPE tissue blocks. The age categories of patients were over 67 (50%) and less than 67 (50%). The microscopic study of these samples was made by using SOX-10 tumor marker test. SOX-10 test was positive in five samples of urothelial carcinoma (Fig.2), while SOX-10 test was negative in thirty-five samples of urothelial carcinoma (Fig.3-5). There was male predominance 77.5%; details of subject and their numbers were shown in the following (Table 1, Table 2).

Table 1 shows that the age category was less than 67 by about 50% and more than 67 by about 50%. The majority of sex group was at the male categories by about 77.5% more than females 22.5%.

Table 2 shows that most cases were positive for SOX-10 among patients suffering from urothelial carcinoma. Regarding to age, sex, grade, muscle invasion categories, SOX-10 was positive in age less than 67 by about 15%% (p value=0.9), positive in male by about 16.1% more than female (p value=0.6), positive in low grade by about 18.2% more than in high grade by about 5.6% (p value=0.4), and positive in the absence of muscle invasion by about 14.8% (p value=0.9).

DISCUSSION

Our study was completed at University of Kufa, faculty of medicine (Iraq, middle Euphrates unit for cancer research) to prove the relation between SOX-10 and urothelial carcinoma. In the present study, there was a high negativity of SOX-10 at age categories less than 67 years about 90 %, with

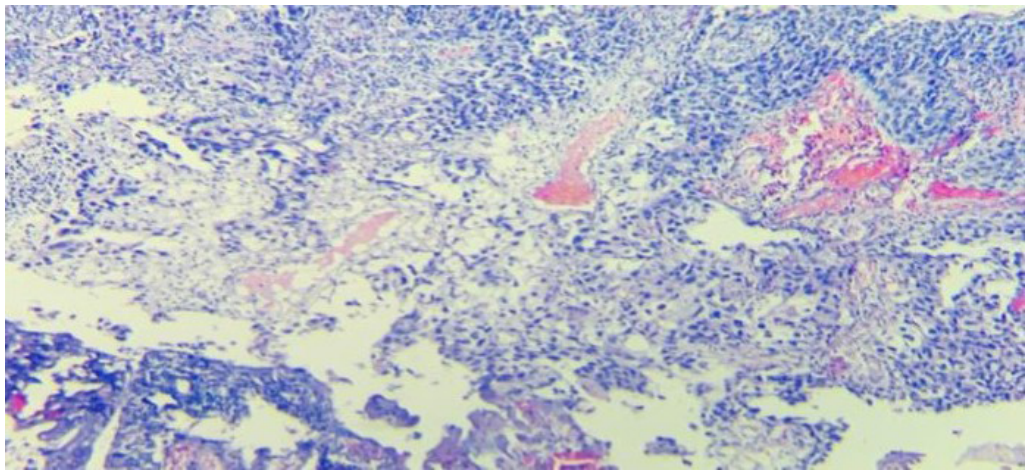

Fig. 1. Transurethral resection, high grade urothelial carcinoma, H&E. Magnification power X100.

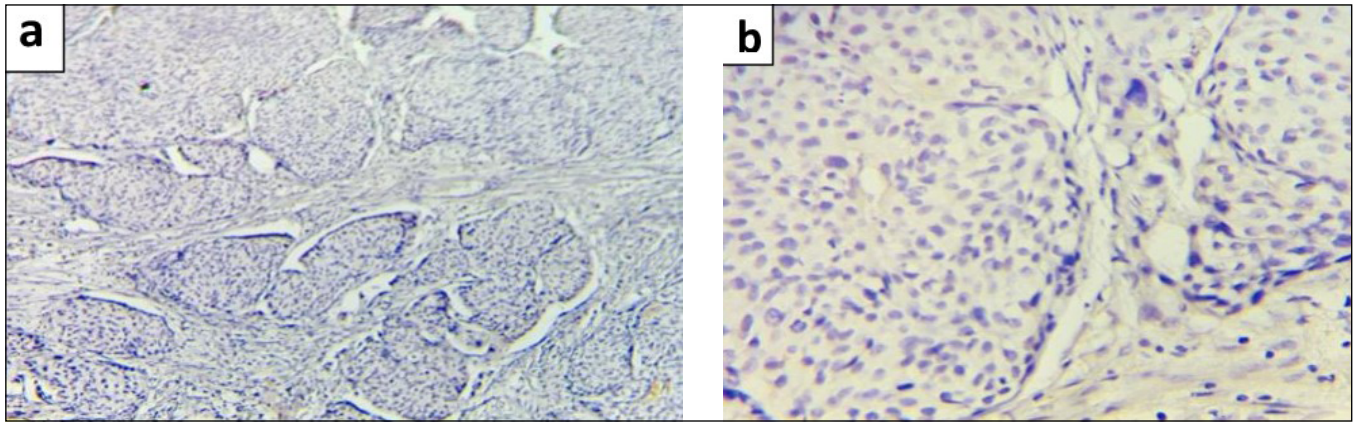

Fig. 2. Transurethral resection, low grade urothelial carcinoma, immunohistochemical positive for SOX-10. Magnification power (a) X100, (b) X400.

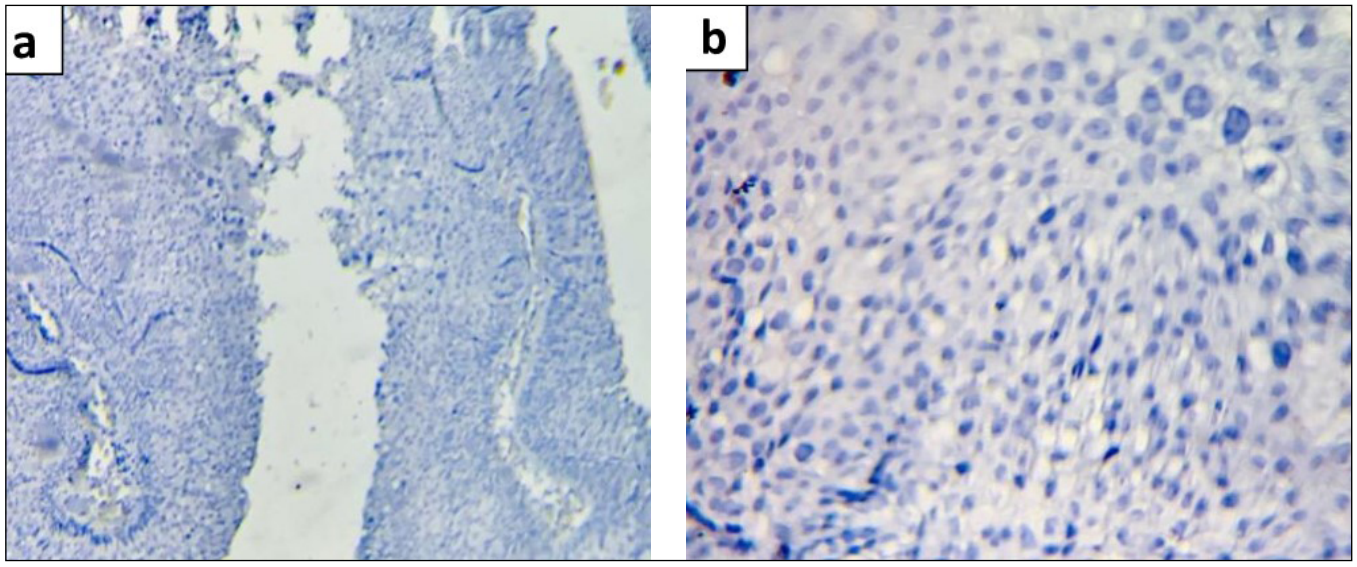

Fig. 3. Transurethral resection, high grade invasive urothelial carcinoma, immunohistochemical negative for SOX-10. Magnification power (a) X100, (b) X400.

male was less than female – 83.9 % to 100% and positive in low grade urothelial tumor 4/22 cases, 18.2% in comparison with high grade expression 1/22 case, 5.6% with no significant difference, which comparable with study made by *Selcen I et al.* [24], who proved low expression of SOX-10 (19 out of 50; 38%) of bladder tumor. According to grade and muscle invasion, there was no statistically significant relation of SOX-10 expression with grade of urothelial tumor (p-value equal to 0.4), muscle invasion (p-value equal to 0.9), and those results disagree with other study performed by

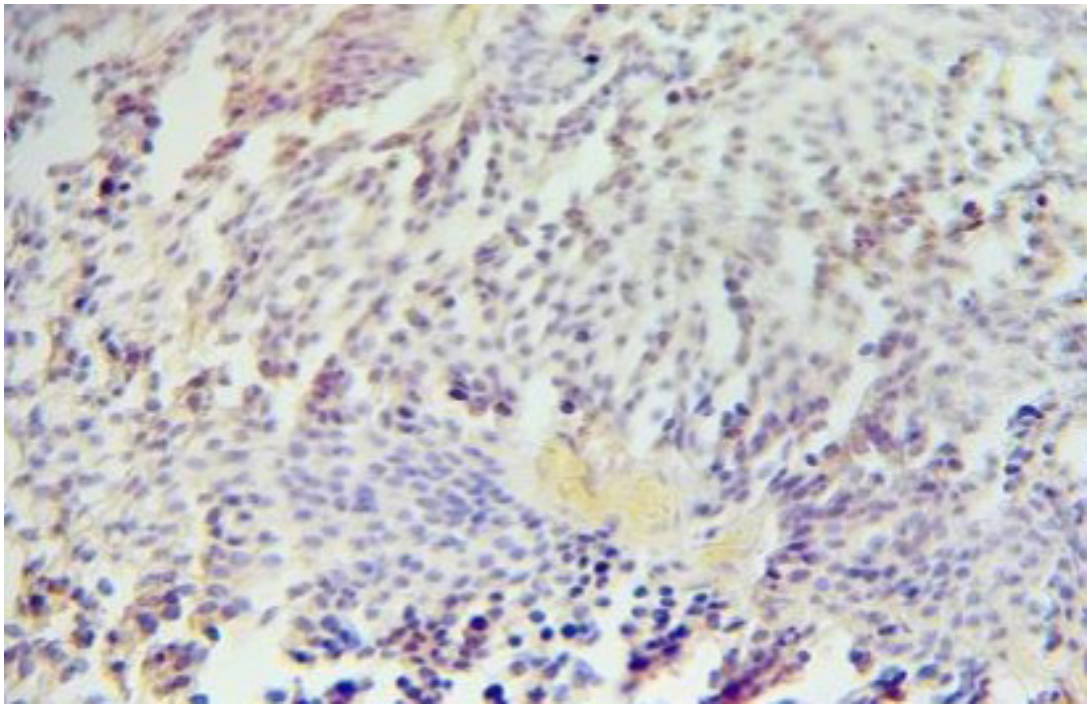

Fig. 4. Transurethral resection, low grade urothelial carcinoma, immunohistochemical negative for SOX-10. Magnification power (a) X100, (b) X400.

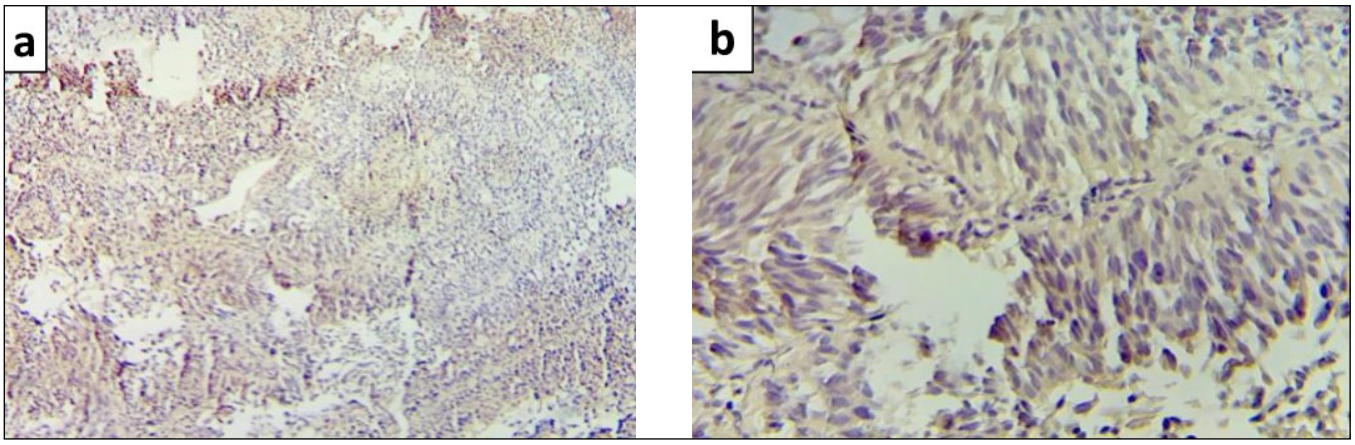

Fig. 5. Transurethral resection, low grade urothelial carcinoma, immunohistochemical negative for SOX-10. Magnification power (a) X100, (b) X400.

Table 1. Clinical and demographic features of studied groups

Variable	Subgroup	No.	%
Age group	less than 67	20	50
	67+	20	50
Sex	Male	31	77.5
	Female	9	22.5
Grade	Low	22	55
	High	18	45
Muscle invasion	Present	13	32.5
	Absent	27	67.5

Yin H et al. [25] who proved a statistic significant relation between SOX-10 appearance and the tumor grade with SOX-10 expression in low grade cases was 33.3% and in high grade cases was 64.2%. Although *Li C et al.*[26] detected that there was no SOX-10 staining in tumor bladder groups, our study showed positive SOX-10 expression in 15% of cases. In addition, our study showed disapproval with that performed by Yin et al [27] who showed over-expression of SOX-10 in high grade cases (40/45) and in low grade cases (27/45) with level of p-value = 0.002.

Table 2. Relationship among studied groups according to immunohistochemistry of SOX-10

		SOX 10		Total	P
		Positive (n=5) No.(%)	Negative (n=35) No.(%)		
Age group	< 67	3(15%)	17(85%)	20(100%)	0.9
	67+	2(10%)	18(90%)	20(100%)	
Sex	Male	5(16.1%)	26(83.9%)	31(100%)	0.6
	Female	0(0%)	9(100%)	9(100%)	
Grade	Low	4(18.2%)18	(81.8%)	22(100%)	0.4
	High	1(5.6%)	17(94.4%)	18(100%)	
Muscle invasion	Present	1(7.7%)	12(92.3%)	13(100%)	0.9
	Absent	4(14.8%)	23(85.2%)	27(100%)	

CONCLUSIONS

SOX-10 is an immunohistochemically sensitive marker for the diagnosis of urothelial carcinoma. SOX-10 expression appears to have a non-significant relationship with age, gender, and histopathological parameters, and its expression has been shown to be lost or decreased in a substantial proportion of cases in relation to urothelial carcinoma. This variation or downregulation of SOX-10 correlates with higher tumor grade and stage. Our study confirmed a reduced SOX-10 expression in urothelial carcinoma. In addition, low SOX-10 levels in bladder cancer can inhibit cells proliferation, migration and invasion while over expression of SOX-10 promotes the progression of bladder cancer. This explained the role of SOX-10 as transcription factor in the nuclei that suppress or activate the target genes.

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The study was approved by medical committee of ethics at University of Kufa, faculty of medicine (MEC-93). Verbal consent was taken from all patients.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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