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Stem Cells Expression in Bladder Carcinoma

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Abstract: Increasing evidence has proposed that tumors contain tumor-initiating cells or cancer stem cells (CSC) that are responsible for their progression and relapse. Aldehyde dehydrogenase 1 A1 (ALDH1A1) has recently been identified as a marker for cancer stem cells in some human malignancies. This study focused on assessment of immunohistochemical expression of ALDH1A1 in cancer bladder and its correlation with other clinicopathological features to rule out its utility as a prognostic marker and thus offer the patients the best theraputic and follow up modalities. This study included 60 cases of urinary bladder cancer in Egyptian patients. Immunohistochemical reactions were carried out by using ALDH1A1 monoclonal antibody. A total score of ALDH1A1 expression is obtained by multiplying the score of staining intensity and mean percentage of stained cells to obtain ranging from 0% up to 300%. We used a cutoff value of 10% to determine whether a tumor had low or high ALDH1A1 expression. Statistical analysis was conducted to correlate ALDH1A1 expression with other clinic-pathological features. High ALDH1A1 expression (>10%) was found in 55 cases (91.7%).There was highly significant correlation between ALDH1A1 expression and histologic types of bladder cancer where the highest expression was among adenocarcinoma followed transitional cell carcinoma and then squamous cell carcinoma. A significant correlation was noticed between ALDH1A1 expression and tumor grade among patients of high score; between grade III on hand and grades I &II on the other hand. A significant correlation was noticed between ALDH1A1 expression and tumor stage (T) among patients of high score. There was a significant correlation between vascular invasion and ALDH1A1 expression among patients of high score. Non-significant correlations were noticed between ALDH1A1expression and age, sex, tumor shape, site, size, both nodal and distant metastasis I conclusion, ALDH1A1 may be considered a useful marker for categorizing bladder cancer patients with poor prognosis who might benefit from adjuvant and effective treatments. In addition, could greatly enrich our understanding of the tumor biology of bladder.

Key words: Bladder cancer · Cancer stem cells · ALDH1A1 · Prognosis

INTRODUCTION

Cancer of the urinary bladder accounts for about 3.2% of all cancers worldwide. It ranks ninth in worldwide cancer incidence and the 13th most numerous cause of death from cancer. An estimated 386,300 new cases and 150,200 deaths from bladder cancer occurred in 2008 worldwide. The majority of bladder cancer occurs in males (rates in males are three to four times those in females) and there is a 14-fold variation in incidence internationally [1]. In Egypt, the Cancer Pathology Registry of National Cancer Institute, Cairo University showed that during the years 2003-2004, bladder cancer was the commonest among urinary system malignancies (90.71%) and the third among all cancers (12.22%) [2]. Sixty percent of bladder carcinomas are low grade and noninvasive cancers. Twenty-five percent of newly diagnosed bladder tumors are high-grade/muscle invasive lesions. The patients with low grade and non muscle-invasive carcinomas are routinely treated by endoscopic resection. After endoscopic resection, the majority of the patients develop cancer recurrences. The patients with high histologic grade and muscle-invasive tumors receive more aggressive therapies including cystectomy and/or radiation/chemotherapy; at least half of the cases eventually progress with local and distant metastases. Periodic cystoscopy and urine cytology are currently used to monitor the patients for cancer recurrence or progression [3]. However, these clinical means are associated with high cost, substantial patient discomfort,

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variable and poor accuracy. Therefore, novel and clinically applicable prognostic markers are urgently needed to identify patients at high risk for poor prognosis. To date, numerous potential markers have been identified by a variety of molecular biology and genetic studies [4]. However, the role of these molecular markers in clinical diagnosis and therapeutic decision making still remains uncertain. New diagnostic modalities have to be developed [3]. Accumulating evidence has proposed that tumors contain tumor-initiating cells or cancer stem cells (CSC) that are responsible for its progression and relapse. Although being present in a small population in tumor, CSCs can undergo self-renewal, recapitulate the phenotype of the cancer from which they were derived, proliferate and drive continued expansion of malignant cells [5]. Bladder carcinoma growth and metastasis might also be promoted by CSCs that are responsible for its aggressiveness [6]. Therefore, analysis of molecular aberrations that are associated with CSCs would deepen our understanding of the tumor biology of bladder carcinoma. Most importantly, these molecular changes could be developed as a new diagnostic system for monitoring the progression of bladder tumor and offer the best opportunity to prevent its recurrence and probably cure the challenging malignancy [7]. Human cytosolic aldehyde dehydrogenase 1 (ALDH1) plays a role in the biosynthesis of retinoic acid. Altered metabolism of retinal to retinoic acid is likely to play a major role in stem cell biology. ALDH1A1 is a major member of the ALDH1 family. Activation of ALDH1A1 has been found in stem cells populations in multiple myeloma and acute myeloid leukemia [8], breast cancer [9] and lung cancer [10]. ALDH1A1+ bladder cancer cells were endowed with extensive tumorgenicity and self-renewal potential, being able to generate tumors that resembled the histopathological characteristics and heterogeneity of the parental tumor cells and thus had the properties of CSCs or stem-like cancer cells. Furthermore, ALDH1A1 could function as a prognostic factor for predicting outcome in patients with malignancy. Nevertheless, a longitudinal clinicopathological study to validate its prognostic value for improving treatment efficiencies of bladder cancer will be needed [3].

This study focused on assessment of immunohistochemical expression of ALDH1A1 in cancer bladder and its correlation with other clinicopathological features to rule out its utility as a prognostic marker and thus offer the patients the best theraputic and follow up modalities.

MATERIALS AND METHODS

Study Design: Retrospective cross-sectional study. This study included 60 paraffin blocks of urinary bladder cancer from radical cystectomy specimens in Egyptian patients, collected from the Pathology Department, Kasr El-Einy Hospital, Cairo University, during the time period from January 2012 to December 2013. Patients' data were obtained from the clinical and medical reports: age at diagnosis; sex; site, shape and size of the tumor; extent of tumor invasion; presence or absence of metastases and tumor stage. Sections of 4 μm thickness were cut by microtome from the paraffin blocks. Serial sections from each tumor block were stained with hematoxylin and eosin for histopathological assessment and a section was mounted on charged slide for immunohistochemical staining by monoclonal antibody against ALDH1A1antigen.

Histopathological Evaluation: Hematoxylin and Eosin stained slides were evaluated for the histological type of the tumor according to World Health Organization (WHO) histological classification of tumors of the urinary tract; 2004 and for tumor grade based on AJCC/UICC TNM, 7th edition; 2010. Other architectural features were assessed as differentiation of TCC cases (squamous, glandular…etc) as well as extent of invasion, presence of vascular and/or nerve invasion, lymph node metastases and associated schistosomal infestation.

ALDH1A1 Immunostaining: Immunohistochemical reactions were carried out by using ALDH1A1 monoclonal antibody (Rabbit IgG) (EP1933Y clone), 1.0 ml concentrated, diluted at 1:200-400, manufactured by BIOCARE MEDICAL. Paraffin sections were deparaffinized and then rehydrated. Slides were immersed in 90 ml methanol + 10 ml H_2O_2 for 30 minutes for blocking endogenous peroxidase. Antigen retrieval was done by boiling the section in 1mM EDTA, pH 8.0, for 10-20 min followed by cooling at room temperature for 20 minutes. Excess buffer was removed by blotting paper and slides were covered by blocking reagent and incubated for 30 minutes in humid chamber to suppress nonspecific binding of immunoglobulin.

The slides were then covered by the primary monoclonal antibody ALDH1A1 and incubated in humid chamber at room temperature overnight. Secondary antibody (DAKO) was put and incubated in humid chamber for 45 minutes. Application of peroxidase and

incubation in humid chamber for 45 minutes was conducted, followed by application of DAB (3-3\ diaminobenzidinetetrahyrochloride) substrate (DAKO) and incubation for 3 minutes (or until the color of the control become brown). Counter staining was done by Mayer's hematoxylin. The sections were washed in between the successive steps by distilled water and by phosphate buffer saline (PBS) (pH 7.2). A section of breast cancer known to be positive for ADH1A1 was used as positive control.

ALDH1A1 Immunostaining Interpretation: The positively stained cells were characterized by the presence of brownish cytoplasmic coloration. The staining intensity was scored according to the following scale: no visible staining, 0; faint staining, 1; moderate staining, 2; and strong staining, 3. The total number of cells with positive staining for the antibodies was quantized in 20 fields on each tissue section. Percentage of cells with positive staining was graded from: 0% up to 100%. Then multiplying the score of staining intensity and mean percentage of stained cells to obtain a total score ranging from 0% up to 300%. To assess the clinical significance of ALDH1A1 expression in bladder cancer patients, we used a cutoff value to determine whether a tumor had low or high ALDH1A1 expression, which was a tumor specimen with >10% overall score defined as one with high ALDH1A1 expression [3].

Statistical Analysis: Data was statistically analyzed by Statistical Package of Social Science Software program, version 21 (SPSS). Data was summarized using mean and standard deviation for quantitative variables and frequency and percentage for qualitative ones. Comparison between groups was performed using independent sample t-test or one way ANOVA with post hoc Tukey's test for quantitative variables and Chi square or Fisher's exact test for qualitative ones. Pearson or Spearman correlation coefficients were calculated to signify the association between different quantitative or ordinal variables, respectively. P values less than 0.05 were considered statistically significant and less than 0.01 were considered highly significant.

RESULTS

The age of cases ranged from 38 to 80 years with a mean of 60.6±7.9, 65% of cases were between 60 and 80 years and 35% of cases were between 38 and 59 years. Forty seven cases (78%) were males and 13 cases (22%) were females. The size of tumors ranged between 1.5cm and 11cm with a mean of 5.2± 2.3cm; the tumor sizes of 35 cases (58.3%) were equal or greater than 5cm and 25 cases (41.7%) were less than 5cm in their greater dimensions. The tumors were either ulcerative (30 cases; 50%), or fungating (19 cases; 31.7%), or infiltrative (11cases; 18.3%). The tumors were located within the urinary bladder as follows; posterior wall (26 cases; 43.3%), anterior wall (14 cases; 23.3%), lateral wall (12 cases; 20.0%), dome (4 cases; 6.7%), the entire bladder (4 cases; 6.7%).As regarding tumor type; 39 cases were transitional cell carcinoma TCC (65%), 18 were squamous cell carcinoma SCC (30%) and 3 were adenocarcinoma (5%). According to histological divergent differentiation of TCC cases (39 cases): 19 cases were conventional urothelial (48.7%) and 14 cases showed squamous differentiation (35.9%), 3 cases showed glandular differentiation (7.7%) and 3cases showed mixed squamous and glandular differentiation (7.7%). Regards tumor grading; 2 cases (3.3%) were grade I, 21 cases (35%) were grade II and 37 cases (61.7%) were grade III. Tumors were classified according to primary tumor stage (T) into; T1: 1(1.7%); T2: 10(16.7%); T3: 36 (60%) and T4: 13 (21.7%) and according to lymph node metastasis (N) into; N0:39 (65%); N1: 10 (16.7%); and N2:11 (18.3%) and according to distant metastasis (M); M0:58 (96.7%) and M1:2 (3.3%). Vascular emboli were seen in 33 cases (55%). 18 cases (30%) showed evidence of nerve involvement. 25 cases (41.7%) showed schistosomal infestation.

Total ALDH1A1 expression score ranged from 0.6 % to 300% with a mean of 174.4%±111.1%. 5 cases (8.3%) showed low score (<10%) and 55 cases (91.7%) showed high score (>10%). We observed that normal adipose tissue and nerve bundles (Fig. 1e) within tumor specimens showed strong positive ALDH1A1 expression There was non-significant correlation between age, sex of patients and ALDH1A1 expression score. ALDH1A1 expression score was not significantly related to tumor size. As regard tumor shape: Ulcerative tumors showed highest level of ALDH1A1expression (mean 211.3%±87.5SD), followed by infiltrative (mean 190.1%±110.4 SD) then fungating forms (mean 158.0%±115.9 SD), among cases with high score (>10). However, ALDH1A1 score was not significantly related to tumor shape. As regard tumor site: tumors involving the whole walls of urinary bladder showed the highest level of ALDH1A1 score (mean 264.4%± 57.54 SD) followed by posterior wall (mean 191.7%±109.1 SD), anterior wall (mean 181.6%± 104.4 SD),

Table 1: Correlations between total ALDH1A1 expression and various clinic-pathological parameters included in the study

	Total ALDH1A1 score				
Variable	Low (≤ 10) (n=5)		High (> 10) (n=55)		P value
Age (years) *mean ± SD*	60.8 ± 9.0		60.6 ± 7.9		0.95* NS
Age grouping n, %					
< 60 years	1	20.0%	20	36.4%	0.6@NS
≥ 60 years	4	80.0%	35	63.6%	
Sex					
Male	5	100.0%	42	76.4%	0.6@ NS
Female	0	0.0%	13	23.6%	
Tumor size; mean ± SD	5.3 ± 0.4 cm		5.2 ± 2.4 cm		0.8* NS
Tumor size					
< 5 cm	0	0.0%	25	45.5%	0.07@NS
≥ 5 cm	5	100.0%	30	54.5%	
Tumor type					
TCC	4	80.0%	35	63.6%	0.7∞ NS
SCC	1	20.0%	17	30.9%	
Adenocarcinoma	0	0.0%	3	5.5%	
Tumor grade					
Grade I	0	0.0%	2	3.6%	0.7∞ NS
Grade II	1	20.0%	20	36.4%	
Grade III	4	80.0%	33	60.0%	
T staging					
T1	0	0.0%	1	1.8%	1.0∞ NS
T2	1	20.0%	9	16.4%	
T3	3	60.0%	33	60.0%	
T4	1	20.0%	12	21.8%	
N staging					
N0	3	60.0%	36	65.5%	0.3∞ NS
N1	0	0.0%	10	18.2%	
N2	2	40.0%	9	16.4%	
Distant metastasis					
Present	0	0.0%	2	3.6%	1.0@NS
Absent	5	100.0%	53	96.4%	
Vascular invasion					
Present	3	60.0%	30	54.5%	1.0@NS
Absent	2	40.0%	25	45.5%	
Nerve invasion					
Present	3	60.0%	15	27.3%	0.03@NS
Absent	2	40.0%	40	72.7%	

*Independent sample t-test, @Fisher's exact test, ∞ Chi square test

lateral wall (mean 178.8%±105.5 SD) and domal tumors (mean 159.2% ± 88.2 SD), among cases with high score (>10). However, ALDH1A1 score was not significantly related to tumor site (Tables 1 and 2).

There was significant correlation between ALDH1A1 expression and the three histological types of tumors, where the highest expression of ALDH1A1 was among cases of adenocarcinoma (Fig. 1d) followed by TCC (Fig. 1c) then SCC (Fig. 1a,b) which showed the lowest expression ($p < 0.05$); however there was a highly significant difference between TCC and adenocarcinoma cases on one hand and SCC cases on the other ($p < 0.001$). Though highest expression of ALDH1A1 was noticed in cases of TCC with squamous differentiation and lowest expression in mixed differentiation, but was not statically significantly. In SCC cases, we noticed more expression centrally within tumor cell nests, even keratin pearl was positive and among all tumors, tumoral necrotic areas showed evidence of ALDH1A1 expression. Among high ALDH1A1 expression, there was a significant correlation between high tumor grades and higher mean ALDH1A1 expression scores. However, this was concluded between grade III on hand and grades I &II on the other hand, no significant difference was noticed between grade I and II. A significant positive moderate correlation (rho=0.356, among patients of high score) was also observed between

Table 2: Correlations between high ALDH1A1 expression and various clinic-pathological parameters included in the study

Variable	High ALDH1A1 score (n=55); *mean ± SD*	P value
Age	r= 0.023	0.9[@] NS
Age grouping		
< 60 years	195.3 ± 108.3	0.8*NS
≥ 60 years	187.0 ± 100.6	
Sex		
Male	191.4 ± 104.3	0.9*NS
Female	185.5 ± 100.6	
Tumor size	r= 0.121	0.4[@]NS
Tumor size		
< 5 cm	189.8 ± 100.3	1.0*
≥ 5 cm	190.2 ± 106.0	NS
Tumor type		
TCC	223.6 ± 59.3 (B)	<0.001[¤]HS
SCC	103.3 ± 78.7 (A)	
Adenocarcinoma	290.3 ± 9.8 (B)	
Tumor grade		
Grade I	26.1 ± 19.9 (A)	0.001[¤]HS
Grade II	146.4 ± 79.6 (A)	
Grade III	226.4 ± 98.6 (B)	
Tumor grade	rho= 0.485	<0.001[Σ]HS
T staging		
T1	161.9	0.008[$]HS
T2	95.7 ± 62.1(A)	
T3	207.5 ± 102.7(B)	
T4	215.0 ± 95.8 (B)	
T staging	rho= 0.356	0.008[Σ]HS
N staging		
N0	178.6 ± 98.7	0.4[¤]NS
N1	192.8 ± 114.8	
N2	232.6 ± 104.4	
N staging	rho= 0.230	0.091[Σ]NS
Distant metastasis		
Present	269.1 ± 43.7	0.3*NS
Absent	187.0 ± 103.1	
Vascular invasion		
Present	222.1 ± 91.1	0.01*S
Absent	151.6 ± 103.9	
Nerve invasion		
Present	201.9 ± 118.7	0.6*
NS		
Absent	185.6 ± 97.0	

[@]Pearson correlation, r= Pearson correlation coefficient, *independent sample t-test, [¤]ANOVA test, groups having different letter or color label are statistically significantly different at P value of 0.05 (post hoc Tukey's test), [Σ] Spearman correlation, rho= Spearman correlation coefficient

tumor stage (T) and ALDH1A1 expression but no significant difference between T3 and T4. An increase in ALDH1A1 expression was observed with the presence of lymph node metastases, but without statistical significance. As regarding distant metastasis, higher expression of ALDH1A1 was noticed in M1; but without statistical significance. There was a significant relation between vascular invasion and ALDH1A1 expression (p= 0.01) among patients of high score (>10%). As regarding the presence of nerve invasion, there was a significant correlation (p=0.03) with total ALDH1A1 expression; in high scores nerve invasion was more prominent. More expression was noticed with the presence nerve invasion among patients of high score but not statistically significant (p=0.6). Non-significant correlation was found between ALDH1A1 expression and schistosomal association (p=0.3) among patients with high score (>10) (Tables 1 and 2).

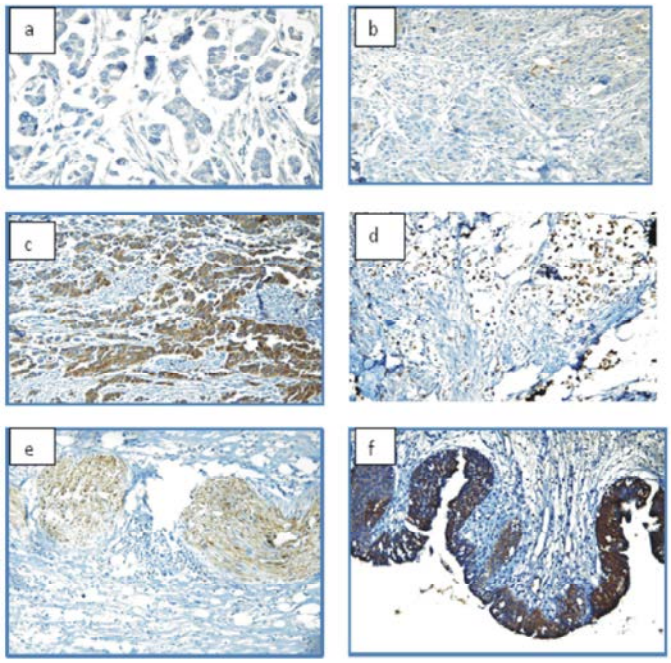

Fig. 1: Positive ALDH1A1 expression in (a) invasive TCC with glandular differentiation, grade II with weak intensity staining 1+. (b) invasive squamous cell carcinoma, grade III showing moderate intensity staining 2+. (c) invasive TCC with focal squamous differentiation grade III with strong intensity 3+, (d) invasive mucoidadenocarcinoma showing strong intensity 3+. (e) Nerve bundles showing positive strong ALDH1A1 expression. (f) Urothelial carcinoma in situ showing positive ALDH1A1 expression with strong intensity 3+

In the current study, the slides of seven cases (6 TCC and 1 adenocarcinoma NOS) were evaluated for ALDH1A1 expression in normal urothelium, dysplasia or carcinoma in situ in close proximity of the invasive cancer. Considering TCC; 3cases were conventional, 1case showed squamous, 1 case showed glandular and 1 case showed mixed differentiation. There was gradual increase in ALDH1A1expression from normal urothelium: 0% (negative) or 50% followed, by dysplasia, then CIS (Fig. 1f) and finally the invasive cancer expressing the highest scores. This was noticed in 5cases. However, there were 2 cases (1adenocarcinoma NOS and 1 TCC with squamous diff.) in which higher ALDH1A1 score was noticed in dysplasia than invasive cancer. In the case of TCC with squamous differentiation, areas of the invasive cancer with squamous differentiation showed lower expression than areas of conventional urothelial cancer.

DISCUSSION

In Egypt, bladder cancer was the commonest among urinary system malignancies (90.71%) and the third among all cancers (12.22%); according to the Cancer Pathology Registry of National Cancer Institute, Cairo University during the years 2003-2004 [2]. The current study was carried out on 60 bladder cancer Egyptian cases randomly selected. The mean age of cases was 60.6 years ranging from 38 to 80 years which was in agreement with other reports from Egypt [11-14]. The majority of cases in this study were between 60 and 80 years which was close to that was stated by Mokhtar *et al.* [2] (61-70 years). In the current study, males were more affected than females with the male to female ratio being 3.6:1. This was near to the results of Salem and Mahfouz [15] where the male: female ratio was 4.2:1 and slightly lower than the results observed by El-Bolkainy *et al.* [11] and Zarzour *et al.* [13], where the male: female ratio was 5:1 and 5.5:1 respectively. The male predominance is probably attributed to the fact that males are more exposed to the established risk factors, including bilharzial infestation, cigarette smoking and occupational exposure to chemical carcinogens [16, 17]. Size influences tumor progression with rates of 9% for tumors less than 5 cm and 35% for tumors more than 5 cm [18]. This was also observed in our current study, as 35 cases (58.3%) were was equal or greater than 5cm in their greater dimensions, most of them were high stages T3 (60%) and T4 (21.7%).

Most cases; 65%; were transitional cell carcinoma, 30% were squamous cell carcinoma and 5% were adenocarcinoma. This is almost similar to the relative frequencies stated by Salem and Mahfouz [15] and Ashley *et al.* [19]. Also our findings were nearly equal to the results of the Cancer Pathology Registry of National Cancer Institute, Cairo University during the years 2003-2004 reported that 2/3 of bladder cancers were TCC (64.20%), SCC (28.30%) and adenocarcinoma (4.50%) [2]. The present study showed divergent differentiation in TCC represented 33.3 % of the total cases (51.3% of TCC cases), with the most common being squamous differentiation followed by glandular differentiation and lastly mixed squamous and glandular differentiation. This finding was in concordance with that reported by Lopez-Beltran *et al.* [20] and Wasco *et al.* [21]. In the present study, 2 cases (3.3%) were classified as grade I, 21 cases (35%) as grade II and 37 cases (61.7%) as grade III. These findings were not in harmony with Su *et al.* [3], who showed grade I tumors were 84 cases (39%), grade II were 82 cases (38%) and grade III were 50 cases (23%).That may be contributed to the larger sample size and that all cases of the latter were of urothelial (transitional) cell carcinoma while our study included SCC and adenocarcinoma which was the reason of using grading of the tumors as grade I, II and III while other studies classified urothelial tumors high grade and low grade tumors; high-grade being equivalent to grade III [22]. Thalitados Reis *et al.* [23] detected high grade tumors in 75% of cases and Malats *et al.* [24] found that 76% of patients had high-grade tumors; both are in concordance with our current study.

Our study showed that most cases were pT3 (60%) and pT4 (21.7 %). That was different from what was recorded by Su *et al.* [3] that T2-4 tumors were 26% of all cases. We also found that most of the cases were classified as N0 (65%); 10 cases as N1 (16.7%); and 11 cases as N2 (18.3%). That was different from that found by Su *et al.* [3] as N0 cases were (91%) and N1-2 cases were (8%), which might be attributed to higher tumor grades and more aggressive behavior of tumor among Egyptians or delayed seeking of medical advice. Our results showed that 56.7% of our cases showed positive vascular invasion and 31.7% had evidence of nerve involvement. This is quite close to the results of Thalitados Reis *et al.* [23], who reported microvascular invasion in 50% of cases and Leissner *et al.* [25], who reported that lymphatic, blood vessel and perineural tumor invasion were present in 54.1%, 13.1% and 47.7% of specimens, respectively. Our results revealed that 41.7% of our cases showed schistosomalova in their specimens. This agrees with Salem and Mahfouz [15], who found that the incidence of associated schistosomiasis in bladder carcinoma decreased from 80% to 50%. Gouda *et al.* [12] also reported a significant decline of Bilharzial association in bladder carcinoma from 82.4% to 55.3%.

Total ALDH1A1 expression score ranged from 0.6 % to 300% with a mean of 174.4%. 55 cases (91.7%) showed high score (>10%). That was higher than that observed by Su *et al.* [3], who mentioned that high ALDH1A1 expression was found in 26% (56 of 216) of human bladder tumor specimens of his cases. This might be attributed to a larger sample size of the latter and that the majority of our cases were of higher grades and stages than theirs. We observed that normal adipose tissue and nerve bundles within tumor specimens showed strong positive ALDH1A1expression with agrees with Levi *et al.* [26]. In our study, no significant correlations were found between ALDH1A1expression and clinicopathological features such as patient's age, sex and tumor size. That was similar the results of Su *et al.* [3]. ALDH1A1 score was not significantly related to tumor shape or tumor site. These two correlations were not thoroughly evaluated by other comparative studies. There was highly significant correlation between ALDH1A1 expression and the three histological types of tumor among cases with high score, where the highest expression of ALDH1A1 was among cases of adenocarcinoma followed by the cases of TCC and SCC which showed the lowest expression. A significant difference between of TCC and adenocarcinoma cases on one hand and SCC cases on the other hand was evaluated by post hoc Tukey's test. Non-significant correlation in ALDH1A1 expression among cases with high score with the presence of divergent differentiation of TCC was concluded though highest expression was noticed in cases of TCC with squamous differentiation and lowest expression in mixed differentiation. As far as we know, the correlation between ALDH1A1 expression and various histological tumor types was not thoroughly evaluated in other studies.

The study revealed highly significant correlation between high tumor grades and higher mean ALDH1A1 expression scores. However, this was concluded between grade III on hand and grades I &II on the other hand by post hoc Tukey's test. That agreed with the results of Su *et al.* [3], where high ALDHA1 expression was found in 14%, 23% and 50% in grades I, II and III, respectively.

As regard TNM stage, a significant positive moderate correlation (among patients of high score) was observed between tumor stage (T) and ALDH1A1 expression; i.e. they are directly proportionate but no significant difference between T3 and T4. That was supported by the results of Su *et al.* [3], who observed elevated ALDH1A1 expression in 21% of noninvasive urothelial carcinomas and 41% of advanced bladder carcinomas (T2-4). A gradual increase in ALDH1A1 expression was observed with the increase of N and M stage, but without statistical significance. This didn't agree with Su *et al.* [3], who found a significant correlation of ALDH1A1 expression with lymph node metastases. This may be related to different sample sizes, tumor behavior affected by geographic and genetic variability between different races. There was a significant relation between vascular invasion and ALDH1A1 expression among cases of high score. That agrees with the hypothesis that Cancer stem cells are involved in tumor progression and metastasis. As regarding the presence of nerve invasion, more expression was noticed with the presence nerve invasion among patients of high score but not statistically significant. Non-significant correlation was found between ALDH1A1 expression and schistosomal association among cases with high score. As far as we know, no other study evaluated the correlation between ALDH1A1expression and vascular, neural invasion. In the current study, most cases (5/7) gradual increase in ALDH1A1expression from normal urotheliumto dysplasia, then CIS and finally the invasive cancer expressing the highest score. This agrees with the dictum; cancer stem cells are responsible for tumor self-renewal and progression.

In conclusion, ALDH1A1 expression profile was closely correlated with important histopathological characteristics (tumor types, grades, stages and vascular invasion) of bladder carcinomas and showed significant correlations to prognostically bad parameters and thus can be an indicator to biological behavior of individual cases, predict recurrence and progression and help identify tumors that could benefit the most effective treatments. In addition, the development of an established specific marker to isolate and characterize stem-like cancer cells of bladder carcinoma would greatly enrich our understanding of tumor biology in bladder.

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