Research Article

Immunohistochemical Expression of Livin in Urinary Bladder Cancer

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Abstract
Background: Urinary bladder cancer (UBC), the most common urinary tract cancer and the ninth most common cancer worldwide. Livin is the most recently identified member of the inhibitor of apoptosis family. High expression levels of Livin may influence the prognosis of different types of cancer. The aim of current study is to investigate the relation between Livin expression and the clinicopathological features of UBC. **Methods:** Immunohistochemical staining for Livin was performed on 60 cases of urinary bladder cancer divided into; 43 cases of transitional cell carcinoma and 17 cases of squamous cell carcinoma. **Results**: As regarding transitional cell carcinoma, Livin high expression was detected in 48.8% of cases. A statistically significant association was observed between Livin high expression and higher tumor grade, advanced tumor stage (P value < 0.001, 0.001 respectively), larger tumor size and tumor multifocality (P value = 0.001, 0.035 respectively). As regarding squamous cell carcinoma, Livin high expression was detected in 52.9% of cases. A statistically significant association was observed between Livin high expression and higher tumor grade (P value < 0.001), advanced tumor stage, larger tumor size and tumor multifocality (P value = 0.018, 0.012, 0.019 respectively). **Conclusion**: Livin high expression could be considered as poor prognostic marker in the evaluation of patients with urinary bladder cancer, Livin can play essential role in the pathogenesis, aggressiveness, invasion, and progression of UBC.
KeyWords: Immunohistochemistry - Livin- Transitional cell carcinoma- Squamous cell carcinoma.
All authors have no conflict of interest.

Introduction
Bladder cancer, the most common urinary tract cancer. It is the ninth most common cancer worldwide, (Siegel et al., 2015). In Egypt, UBC is the second most common malignancy in male and the third common cancer for both sex combined. In Middle Egypt (Minya), UBC represents about 17.5% of all malignances, while in Lower Egypt it represents 11.3%, and in Upper Egypt it represents 15.6% (Ibrahim et al., 2014).

Material and Methods
1. Tissue specimens
The present study comprised 60 cases of UBC, 36 radical cystectomy and 24 transurethral resection (TUR) specimens were selected. The presence of muscularis propria in the TUR cases was mandatory for the selection. The cases included; 43 cases of urothelial carcinoma and 17 cases of squamous cell carcinoma. Tumor type and grade were evaluated according to WHO criteria (Moch et al., 2016).Tumor stage was estimated by TNM staging (Edge et al., 2010).

2. Immunohistochemistry
Primary antibody against Livin: Polyclonal mouse antibody (50 µl, concentrated, Lab Vision Laboratories, USA), diluted at (1:100). For negative control the primary antibody was replaced with PBS. Positive control was squamous cell carcinoma of skin.

3. Scoring of Immunostaining
Livin was expressed mainly in the cytoplasm. Ten high power fields were counted per section in each case and the average of counted fields was calculated. The median was taken as a cut off point for Livin expression. The expression was divided into 2 categories; low expression and high expression. For statistical purposes, negative cases are included among low expressions and considered as one category.

4. Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS software version 16).

Results

1. Clinicopathological Features

3. Immunohistochemical expression of Livin in urothelial carcinoma cases and its association with patients' clinicopathological features Table (1):

Clinicopathological features	*Total* 43 (100%)	Livin expression		*P* value
		Negative/low expression (52.2%)	High expression (48.8%)	
Age				
<60	22 (51.2)	11 (57.9)	11 (45.8)	0.317
≥60	21 (48.8)	8 (42.1)	13 (54.2)	
Gender				
Male	36 (83.7)	16 (72.7)	20 (95.2)	0.054
Female	7 (16.3)	6 (27.3)	1 (4.8)	
Bilharziasis				
Negative	35 (81.4)	19 (86.4)	16 (76.2)	0.322
Positive	8 (18.6)	3 (13.6)	5 (23.8)	
Location				
unifocal	31 (72.1)	19 (61.3)	12 (38.7)	0.035*
multifocal	12 (27.9)	3 (25)	9 (75)	
Tumor size				
<3	27 (62.8)	19 (70.3)	8 (29.6)	0.001*
≥3	16 (37.2)	3 (18.7)	13 (81.2)	
Tumor grade				
Low grade	22 (51.2)	20 (90.9)	2 (9.5)	< 0.001*
High grade	21 (48.8)	2 (9.1)	19 (90.5)	
Nodal status				
Negative	26 (92.7)	26 (100)	0 (0)	0.448
Positive	2 (7.3)	1 (50)	1 (50)	
TNM stage				
Ta	21 (48.8)	19 (86.4)	2 (9.5)	
T1	2 (4.7)	0 (0)	2 (9.5)	< 0.001*
T2	17 (39.5)	3 (13.6)	14 (66.7)	
T3	3 (7)	0 (0)	3 (14.3)	
T4	0 (0)	0 (0)	0 (0)	
Tumor infiltrating lymphocyte				
Absent	5 (11.6)	2 (9.1)	3 (14.3)	
Mild	19 (44.2)	8 (36.4)	11 (52.4)	0.126
Moderate	14 (32.6)	8 (36.4)	6 (28.6)	
Marked	5 (11.6)	4 (18.2)	1(4.8)	

**** P - value <0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.***

Association between Livin expression and clinicopathological features for patients with squamous cell carcinoma (n=17) Table (2):

Clinicopathological features	*Total* 17 (100%)	Livin expression		*P* value
		Negative/low expression (47.1%)	High expression (52.9%)	
Age				
<60	7 (41.2)	3 (37.5)	4 (44.4)	0.581
≥60	10 (58.2)	5 (62.5)	5 (55.6)	
Gender				
Male	13 (76.5)	7 (87.5)	6 (66.7)	0.335
Female	4 (23.5)	1 (12.5)	3 (33.3)	
Bilharziasis				
Negative	10 (58.8)	5 (62.5)	5 (55.6)	0.581
Positive	7 (41.2)	3 (37.5)	4 (44.4)	
Location				
unifocal	9 (52.9)	6 (70.0)	3 (33.3)	0.019
multifocal	8 (47.1)	2 (25)	6 (66.7)	
Tumor size				
<3	8 (47.1)	5 (62.5)	3 (33.3)	0.012
≥3	9 (52.9)	3 (37.5)	6 (66.7)	
Tumor grade				
Grade I	0 (0)	0 (0)	0 (0)	< 0.001*
Grade II	9 (52.1) 8	8 (100)	1 (11.1)	
Grade III	(47.1)	0 (0)	8 (88.9)	
Nodal status				
Negative	5 (62.5)	5 (100)	0 (0)	0.553
Positive	3 (38.5)	0 (0)	3 (100)	
TNM stage				
Ta	0 (0)	0 (0)	0 (0)	
T1	2 (11.8)	2 (25.0)	0 (0)	0.018*
T2	8 (47.1)	5 (62.5)	3 (33.3)	
T3	6 (35.3)	1 (12.5)	5 (55.6)	
T4	1 (5.9)	0 (0)	1 (11.1)	
Tumor infiltrating lymphocytes				
Absent	8 (47.1)	3 (37.5)	4 (44.4)	
Mild	6 (35.3)	4 (50)	5 (55.6)	0.824
Moderate	3 (17.6)	1 (12.5)		
Marked	0 (0)	0 (0)		

**** P - value <0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.***

Discussion

In the present study, as regard TCC cases, Livin high expression was detected in 48.8%. This was in close relation with Wang et al., 2014 who reported that high expression was in 53.5% of cases. In the current study, a statistically significant positive association between Livin high expression and tumor multifocality. However Gazzaniga et al., 2003; Chen et al., 2013, reported no significant association between tumor multifocality and Livin high expression. A statistically significant association between Livin high expression and larger tumor size, However Chen et al., 2013,

reported no significant association between larger tumor size and Livin high expression.

As regarding SCC cases, Livin high expression was detected in 52.9% of cases. Gazzaniga et al., 2003, reported that Livin high expression in 62.6% of cases. This result which is nearly in accordance with Gayyed et al., 2015, who reported that Livin high expression, was 48% of cases.

The present study reported a statistically significant association between Livin high expression and tumor grade. The present study reported a statistically significant association between Livin high expression and advanced tumor stage.

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