

The Role of Immunohistochemical Expression of Annexin A6 in High Grade Urothelial Carcinoma and Cystitis

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ABSTRACT:

BACKGROUND:

Urothelial carcinoma is the most common histological type of bladder cancer, it accounts for 90% of bladder cancers. Annexin A6 (AnxA6) is a member of a superfamily of Ca²⁺-dependent membrane-binding proteins.

AIM OF STUDY:

To study the expression of Annexin A6 in high grade urothelial carcinoma and cystitis using anti-Annexin A6 antibody; and to correlate the results with some clinico-pathological parameters such as age, sex, presence of necrosis, inflammation, hemorrhage and muscle invasion.

MATERIAL AND METHODS:

A retrospective case control study included a total of 60 paraffin embedded samples were divided into two groups: 30 cases of high grade urothelial carcinoma and 30 cases of cystitis. The samples were collected from the archives of teaching labs of Al Yarmouk Teaching Hospital and the archives of Almustansiryah College of medicine for a period from November 2019 to November 2020.

RESULTS:

Annexin A6 was positive in 73.3% of urothelial carcinoma cases. Males were 86.3% while female were 13.6% of positive cases. Annexin A6 was positive in 40% in cystitis samples.

CONCLUSION:

Annexin A6 expression was significant in high grade urothelial carcinoma samples as compared with cystitis samples. In urothelial carcinoma samples, Annexin A6 was significantly associated with the presence of inflammation, hemorrhage, necrosis and muscle invasion, but there was no association with other clinicopathological parameters. Annexin A6 expression shows significant association in primary tumors rather than recurrent tumors.

KEYWORD: Annexin A6, immunohistochemistry, urothelial carcinoma, Iraq.

INTRODUCTION:

Urothelial ca. is the most common histological type of bladder cancers, it accounts for 90% of bladder cancers⁽¹⁾. In Iraq, bladder cancer (90.9% is urothelial ca.) is the seventh most common cancer. It is the eighth cause of cancer death in both sexes and the fourth cause of cancer mortality in males according to the annual report of the Iraqi cancer registry⁽²⁾. Bladder cancer is the 7th most common cancer worldwide⁽³⁾. Cystitis is defined as an inflammatory condition of the urinary bladder. It is a common condition affecting both sexes and all ages, it has no racial predisposition, but females, especially those younger than 50 years, are affected more than males⁽⁴⁾.

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Annexin A6 is a protein that in humans is encoded by the *ANXA6* gene⁽⁵⁾. In the bladder, Annexin A6 expression is observed during urothelial de-differentiation⁽⁶⁾.

MATERIALS AND METHODS:

A retrospective case control study included a total of 60 selected cases (tissue paraffin block). These cases were obtained from urinary bladder of patients with chronic cystitis (30 cases) and high grade urothelial carcinoma (30 cases). The cases were taken by TURBT and collected from the archives of teaching lab of Al-Yarmook Teaching Hospital and private lab in Baghdad. The period of study was from November 2019 to November 2020. Each block was sectioned into 2 slides (1 for H&E and the other for IHC). All cases were reviewed for the diagnosis by consultant pathologists. This study aims to show the immunohistochemical expression of Annexin A6 in urothelial carcinoma and cystitis by using validated Annexin A6 antibody.

IMMUNOHISTOCHEMICAL EXPRESSION OF ANNEXIN A6

Scoring of Annexin A6

The appearance of different brown (cytoplasmic and/or membranous staining) was observed and the cells were scored as (positive/ negative) accordingly. The slides were evaluated with low-power microscopy (10x) to establish the largest staining areas, if no staining seen at low power re-evaluation performed with high power (40x) to assess weak staining region, all areas of every slide were observed and scored semi quantitatively by measuring the percentage of cells that are positively stained over the entire amount of malignant cells "percent".

Annexin A6 slides were reviewed to evaluate staining expression of the marker under light microscope it shows cytoplasmic and/or membranous staining (7). The percentage of cells staining was evaluated using 10% as cut-of value as follows: 10% _ 0.1, 20% _ 0.2, 30% _ 0.3 and so on ...

The intensity of staining: 0_ none, 1_ mild intensity, 2 _ moderate intensity, 3_strong intensity. The final score was calculated by multiplying the intensity by percentage of total cells staining (0_3).

Statistical analysis

Data were analyzed by using statistical package for the social sciences (SPSS version 23) computer software program. Descriptive statistics presented as frequency tables, Continuous variables were expressed as mean \pm standard deviation and categorical variables as numbers and percentages. The P-value below or equal to 0.05 was considered to be significant.

RESULTS:

This study included 60 samples were taken from 30 patients with high grade urothelial carcinoma and 30 patients with cystitis. Annexin A6 was positive in 73.3 % of urothelial carcinoma samples (Table 1).

Table 1: Annexin A6 characteristics of urothelial carcinoma samples.

Expression	No.	Percentage %
Positive	22	73.3
Negative	8	26.6
Total	30	100.0

Annexin A6 expression according to the age group was : 4.5% of cases were positive in < 50 years old , 9% were positive in 50_59 years, 40.9% were positive in 60_69 years, 36.3% were positive in 70-79 years and 9% were positive in

80 years and more age group. Males were 86.3% while female were 13.6% of cases. The mean age and gender shown no significant association with Annexin A6 (Table 2) .

Table 2: The association between age, gender and Annexin A6 expression in urothelial carcinoma sample.

Variables	Annexin A6 expression	Percentage%	P value
<u>Age (Mean\pmSD)</u> (67.2 \pm 9.4)			0.73*
<50	1	4.5	
50_59	2	9.0	
60-69	9	40.9	
70_79	8	36.3	
\geq 80	2	9.0	
<u>Gender</u>			0.58**
Male	19	86.3	
Female	3	13.6	
Total	22	100.0	

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Annexin A6 was positive in 79.2% of samples that had necrosis, inflammation and hemorrhage, while 69.2% of cases that had squamous metaplasia and glandular differentiation were positive, 75% of samples with muscle invasion were positive and 87.5 of recurrent tumors were

positive. There was significant association between Annexin A6 expression and the presence of necrosis, inflammation, hemorrhage status, muscle invasion and primary tumors (**Table 3**).

Table 3: Relation of Histopathological features with Annexin A6 expression .

Histopathological result		Annexin A6		P value
		Negative	Positive	
<u>Necrosis, inflammation and hemorrhage</u>	Absent	3(50%)	3 (50%)	0.004*
	Present	5(20.8%)	19 (79.2%)	
<u>Glandular differentiation and squamous metaplasia</u>	Absent	4(23.5%)	13 (76.5%)	0.16
	Present	4(30.8%)	9 (69.2%)	
<u>Muscle invasion</u>	Absent	3(30%)	7 (70%)	0.025*
	Present	5(25%)	15 (75%)	
<u>Tumor type</u>	Primary	7(31.8%)	15 (68.2%)	0.034*
	Recurrent	1(12.5%)	7 (87.5%)	0.088*

Annexin A6 was positive in 40% of cystitis samples (**Table 4**). The percentage of Annexin A6 expression in cystitis samples according to age group was 33.3 % were positive in the age

group between 50-59 years old, 25% were positive in age group 60-69 years old. There was no significant difference in the mean age and gender between patients and Annexin A6 expression (**Table 5**).

Table 4: The characteristics of Annexin A6 expression in cystitis samples.

Expression	No.	Percentage%
Positive	12	40.0
Negative	18	60.0
Total	30	100.0

Table 5 : The association between age, gender and Annexin A6 expression in cystitis samples .

Variables	Annexin A6 expression	Percentage%	P value
<u>Age(Mean±SD)</u>			0.50*
<50	1	8.3	
50-59	4	33.3	
60-69	3	25.0	
70-79	2	16.6	
≥80	2	16.6	
<u>Gender</u>			0.44**
Male	9	75.0	
Female	3	25.0	
Total	12	100.0	

The Annexin A6 expression was compared

between two groups and there were a significant association between them (**Table 6**).

IMMUNOHISTOCHEMICAL EXPRESSION OF ANNEXIN A6

Table 6: Annexin A6 expression between two groups.

Variable		Participants		Total	P value
		<u>Cystitis</u>	<u>Urothelial carcinoma</u>		
Annexin A6	Negative	18(60%)	8(26.7%)	26	0.009*
	Positive	12(40%)	22(73.3%)	34	
Total		30	30	60	

For the patients with positive Annexin A6, intensity and final score were evaluated and showed that: there was significant difference in mean intensity and final score between two groups($p<0.001$)(Table 7).

Table7: Difference in mean intensity and final score between two groups.

Annexin A6		No.	Mean± SD	Min.	Max	P value
Intensity	<u>Cystitis</u>	12	1.41±0.51	1	2	<0.001*
	<u>Urothelial ca.</u>	22	2.3±0.49	2	3	
	Total	34	0.46±0.11	0.20	0.70	
Final score	<u>Cystitis</u>	12	0.64±0.26	0.30	1.20	<0.001*
	<u>Urothelial ca.</u>	22	1.11±0.35	0.40	1.60	
	Total	34	0.94±0.39	0.30	1.60	

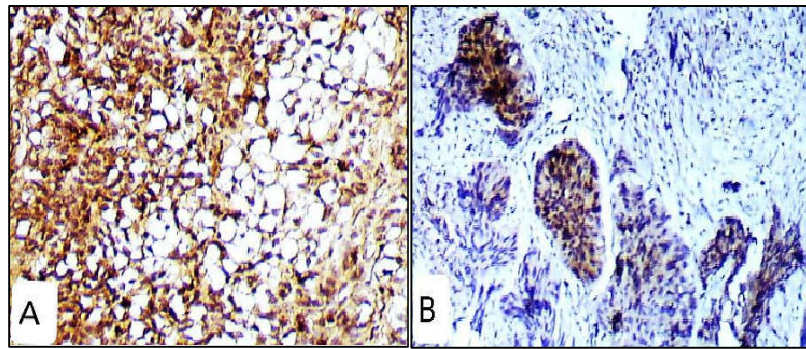


Figure 1: (A)&(B): Microphotography showing +ve IHC expression of Annexin A6 in high grade urothelial ca., brownish discoloration of the cytoplasmic and/or membranous stain, moderate intensity, 20x objective.(B) shows muscle invasion.

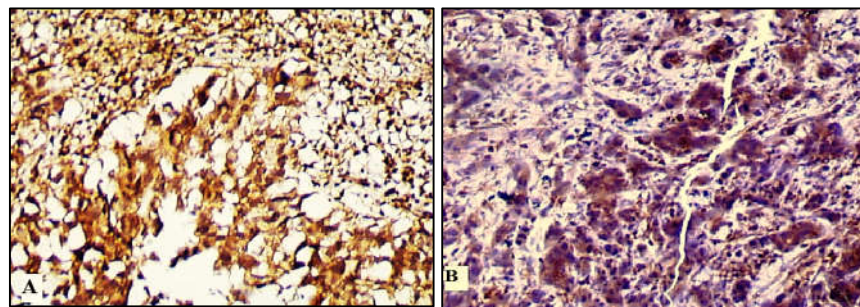


Figure 2: (A), (B):Microphotography showing +ve IHC expression of Annexin A6 in high grade urothelial ca., brownish discoloration of the cytoplasmic and/or membranous stain, strong intensity, 20x objective.

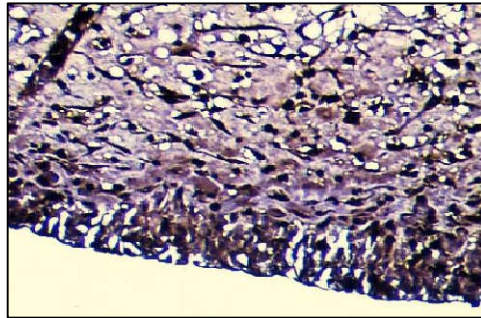


Figure 3 : Microphotography showing +ve IHC expression of Annexin A6 in cystitis, brownish discoloration of the cytoplasmic and/or membranous stain of the lining urothelium, moderate intensity, 20x objective.

DISCUSSION:

In the present study, the age of patients with urothelial carcinoma ranged from 46 - 83 years old with a mean of 66.9 ± 8.8 years. In urothelial carcinoma cases, male patients were more than female patients with a ratio of 5:1. This was in agreement with a study conducted in Iraq, in which the mean age was 69.52 ± 11.17 years and male to female ratio of 5:1 (MOHAMMED *et al.*, 2019) ⁽⁸⁾. There was also an agreement with many studies including a study in Turkey (Koyuncuer *et al.*, 2017) ⁽⁹⁾, the mean age was 68.9 years with a male to female incidence ratio of 4.8:1. Another agreement was with a study conducted in China (Shen *et al.*, 2016) ⁽¹⁰⁾, in which the mean age was 65.1 and male to female ratio was 4.9:1. It was slightly higher than a study conducted in Iraq, in which the mean age was 65.1 ± 10.4 years. Male to female ratio of 3:1 (Mohammed-Najeeb *et al.*, 2020) ⁽¹¹⁾. All these studies indicate a higher prevalence of urothelial ca. in elderly patients and the predominance of male in relation to female patients although there are differences in the ratio between studies which might be attributed to the difference in the size of samples and environmental factor.

To the best of our knowledge, this is the first study evaluating the immunohistochemical expression of Annexin A6 in high grade urothelial carcinoma and cystitis. In this study, there is no significant association between Annexin A6 expression and age or sex of the patients. This was in agreement with a study conducted in Iraq for evaluating Annexin A6 expression on breast carcinoma (Aziz *et al.*, 2020) ⁽¹²⁾, in which there was no association between Annexin A6 expression and age of patients. In this study, there was a significant association of Annexin A6 expression between

urothelial carcinoma and cystitis samples (p value 0.009). Also, the mean of both final score and intensity of Annexin A6 in urothelial carcinoma samples are significantly higher than the mean of final score and intensity of cystitis samples (p value 0.001).

There was an agreement with a study of Annexin A6 expression in ovarian carcinoma (Noreen *et al.*, 2020) ⁽¹³⁾, stated that: Annexin A6 expression was significantly higher in the ovarian carcinoma than the control tissue samples (normal ovarian tissue). This was also in an agreement with a study made in Switzerland (Monastyrskaya *et al.*, 2013) ⁽¹⁴⁾, stated that: a significant Annexin A6 expression is observed during urothelial de-differentiation and another study (Langbein *et al.*, 2006) ⁽¹⁵⁾ state that: Annexin A6 was upregulated in invasive bladder cancer. However, this was different from a study conducted in Iraq (Aziz *et al.*, 2020) ⁽¹²⁾, stated that there is no significant association in Annexin A6 expression between breast carcinoma samples and fibroadenoma samples (P value 0.34).

In our study, there was a significant association between Annexin A6 expression and the presence of inflammation, hemorrhage and necrosis (P value 0.004). Also, there was a significant association with muscle invasion (P value 0.025). In our study, there was no significant association between Annexin A6 expression and the presence of glandular differentiation and squamous metaplasia (P value 0.16). In our study, there was a significant association between Annexin A6 expression and primary urothelial tumors (P value 0.034) while there was no association with recurrent tumors (P value 0.088). Annexin A6 could be one of the potential markers that

helps us understand urothelial cancer pathogenesis and progression as well as its prognosis and possibility to response to therapy.

CONCLUSION:

1. Annexin A6 expression was significant in high grade urothelial carcinoma in relation to cystitis samples. For the patients with positive Annexin A6, the mean of both final score and intensity of Annexin A6 in urothelial ca. samples are significantly higher than that of cystitis samples.
2. In urothelial carcinoma samples, Annexin A6 was significantly associated with the presence of inflammation, hemorrhage, necrosis and muscle invasion.
3. There was a significant association between Annexin A6 expression in primary tumors rather than recurrent tumors.

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