Immunohistochemical Application of GATA 3 in Differentiating Urothelial Cancer from Prostatic Cancer. A Clinicopathological Study

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ABSTRACT:

BACKGROUND:

Urothelial carcinoma and prostatic adenocarcinoma are the most common tumors of genitourinary system. Both can demonstrate a broad histology or present as undifferentiated carcinoma that made the distinction of which is the primary source very difficult. In indeterminate tumor morphology, the immunohistochemical markers will assist the pathologists to avoid misinterpretation.

AIM OF THE STUDY:

To assess immunohistochemical expression of GATA3 in urothelial and prostatic adenocarcinoma with correlation to different clinicopathological parameters.

MATERIALS AND METHODS:

This retrospective study was done on formalin-fixed paraffin embedded tissue blocks from 20 urothelial CA and 20 prostatic adenocarcinoma cases. GATA3 was used and semi-quantitatively scored.

RESULTS:

90% of urothelial CA cases were GATA3 positive with 75% strong expression. While 85% of prostatic CA cases were GATA3 negative, there was no significant correlation between GATA3 expression and clinicopathological parameters except for gender in urothelial CA.

CONCLUSION:

GATA3 has a potential role to confirm the urothelial origin of the tumor in comparison to prostatic carcinoma. There was no significant correlation between GATA3 expression in urothelial carcinoma and prostatic carcinoma cases and clinicopathological parameters except for gender in the first.

KEY WORDS: GATA3, urothelial carcinoma, prostatic adenocarcinoma

INTRODUCTION:

Bladder cancer is the ninth most common cancer in the world. More than 90% are urothelial carcinoma while other morphological types such as squamous cell carcinoma and adenocarcinoma account for less than 5%. (1)

Urothelial carcinoma is 3-4 times more common in male patients than females[1]. Most of the patients are over 60 years of age. Tobacco smoking, chemical exposure and Schistosomiasis are important risk factors[2,3,4].

Urothelial carcinomas morphology ranges from papillary to flat, noninvasive to invasive and low grade to high grade. It is found that there are accumulation of successive genetic alterations determine the tumor's type and, subsequently, the patient's clinical outcome.

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Papillary urothelial carcinoma is divided into low and high grades[5]. It represents 70-80% of newly diagnosed bladder cancer and presents with noninvasive or early invasive disease (stages Tis, Ta, or T1)[6]. The prognosis of these patients depends on tumor grade[7]; while the outcome of invasive tumors depends on tumor stage. Distant metastasis associated with poor prognosis and response to therapy[8].

Prostate carcinoma is the second most common cancer in the world[1]. Its incidence increases substantially with patient age. Environmental and genetic factors play important roles in tumor pathogenesis. Well-documented familial association with 5-10 times increased risk in men with multiple affected 1st degree relatives[9]. Gene amplifications have been shown to play a role in the pathogenesis and prognosis of many cancers including prostatic cancer[10].Tumor grade is one of the strongest predictors of prostatic carcinoma behavior and prognosis and for which, Gleason scoring is used for tumor grading11.

The histologic morphology is usually sufficient for the diagnosis, but certain cases may require immunohistochemical studies. GATA3 is one of the GATA transcription factors family that involved in the luminal differentiation of breast epithelium, development of collecting system / urothelium, trophoblastic differentiation and regulation of type 2 helper T cells[12].

MATERIALS AND METHODS

This is a retrospective study including Formalin fixed, paraffin embedded tissue blocks were collected from archived materials in Medical City Complex /Ghazi-Al Hariri histopathology laboratory department in Baghdad (covering the period from February 2020 to December 2020). The paraffin blocks represent 20 cases of urothelial carcinoma of the urinary bladder and 20 cases of prostatic adenocarcinoma (total of 40 cases).

Two sections of 4 μm thickness were taken from each block. The first stained with hemtoxylin and eosin stain (H&E) for histological revision (reassessed for tumor histologic type, grade (score). The other section was stained immunohistochemically for GATA3. The clinicopathological parameters studied included: age gender, tumor grade and stage.

The 20 cases of urothelial cancer were reported according to WHO/ISUP classification, 2016 [13], and 20 cases of prostatic cancer were graded according to Gleason grade(score), 2014 [11].

Immunohistochemical staining evaluation: The slides were examined at 400× magnification. Only nuclear staining was considered positive, the criterion for positive immunohistochemical reaction is dark brown precipitate in the nucleus for GATA3. The score was assessed as following:[14]

- The *percentage of tumor* cells labeled by GATA3 was scored as follows:
  - ***Score 0**: No tumor cells stained
  - ***Score 1**: 1–10%
  - ***Score 2**: 11–50%
  - ***Score 3:** 51–80%
  - ***Score 4**: 81–100%
- The *staining intensity* of tumor cells labeled by GATA3 was scored as follows:
  - ***Staining Score 0**: No tumor cells stained
  - ***Staining Score** 1: Weak
  - ***Staining Score 2:** Moderate
  - ***Staining Score 3:** Strong.

Finally, *immunoreactivity score* for GATA3 expression was calculated by multiplying the number representing the percentage of immunoreactive cells by the number representing staining intensity and the cases were categorized in four groups shown in table below:

Group	Immunoreactivity score	Interpretation
I	0-1	Negative
II	2-4	Weakly positive
III	5-8	Moderately positive
IV	9-12	Strongly positive

Statistical analysis was performed with SPSS v18.88 (Statistical package for social sciences) and also Excel 2010 programs. Data analysis was done using chi-square test for categorical, also calculate frequencies, percentages, ranges, means standard deviation and standard errors of mean. Values were considered statistically significant when p-value is equal or less than **0.05**.

RESULTS:

Group A: Urothelial carcinoma:

1: distribution of cases according to the clinicopathological parameters:

Twenty cases of urothelial carcinoma with age (66 ± 12) years old. 18 (90%) patients were males, regarding the type of biopsy: 19 (95%) of them were (TURBT) , regarding tumor stage: 14 (70%) of patients were in stage T1, and 10 (50%) of cases with high grade of tumor .

Regarding the association between tumor stage and other parameters in patients with urothelial carcinoma, **there was a significant association between tumor stage and grade of tumor**, 100% of T2 and T4 is in high stage of tumor while 100% of T3 is in high with squamous differentiation and 100% of Ta stage in low grade of tumor. There was no significant statistical correlation between stage and age and gender Regarding the correlation between tumor grade and other clinicopathological parameters in patients with urothelial carcinoma, there was no significant statistical correlation between grade of tumor and other parameters including age and gender .

2: GATA3 expression in urothelial carcinoma:

This study results showed positive nuclear staining in 18/20 cases, strong expression in 15/20 (75%) cases and moderate in 3/20 (15%) of cases as shown in figure (3.1), figure(3.3)and figure(3.4).

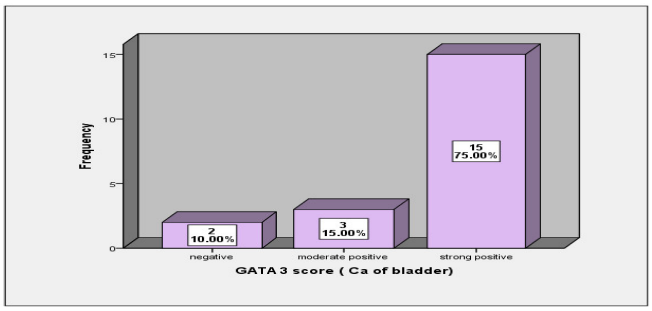
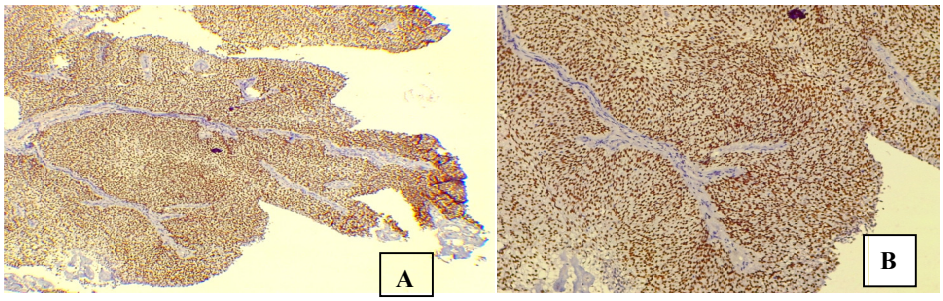


Figure 1: Distribution of GATA3 score in cases with urothelial carcinoma.

Table 1: Correlation between GATA 3 and variables in patients with urothelial carcinoma.

			GATA3 score		P-value
variables		negative	moderate positive	strong positive	
	45 - 54 years	0 (0.0%)	0 (0.0%)	4 (26.7%)	0.46
Age	55 - 64 years	1 (50.0%)	1 (33.3%)	2 (13.3%)	
	65-74 years	0 (0.0%)	2 (66.7%)	6 (40.0%)	
	> 75 years	1 (50.0%)	0 (0.0%)	3 (20.0%)	
gender	female	1 (50.0%)	1 (50.0%)	0 (0.0%)	0.03
	male	1 (50.0%)	2 (66.7%)	15 (100.0%)	
tumor stage	T1	0 (0.0%)	3 (100.0%)	11 (73.3%)	0.93
	T2	1 (50.0%)	0 (0.0%)	2 (13.3%)	
	T3	0 (0.0%)	0 (0.0%)	1 (6.7%)	
	T4	1 (50.0%)	0 (0.0%)	0 (0.0%)	
	Ta	0 (0.0%)	0 (0.0%)	1 (6.7%)	
tumor grade	high	2 (100.0%)	1 (33.3%)	7 (46.7%)	0.6
	high with squamous diff.	0 (0.0%)	0 (0.0%)	1 (6.7%)	
	low	0 (0.0%)	2 (66.7%)	7 (46.7%)	

P-value ≤ 0.05 (significant).



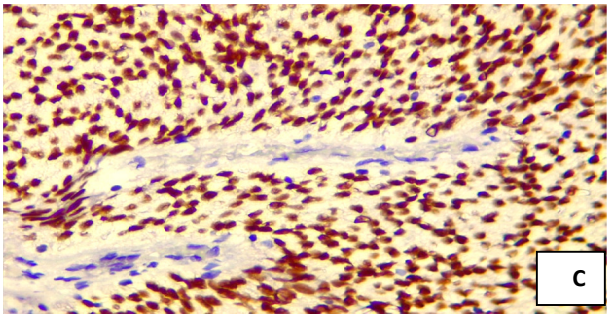


Figure 2: Microphtographs for strong positive GATA3 staining for low grade papillary urothelial carcinoma, bladder biopsy: A: 40X**, B:** 100X**, C:** 400X.

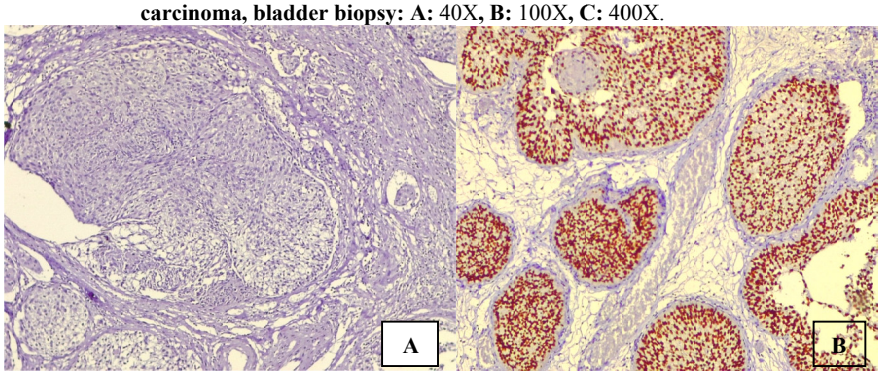


Figure 3: Microphtographs of GATA3 IHC for high grade papillary urothelial carcinoma, bladder biopsy: A: strong positive staining, 100X , B: negative staining , 100X.

Group B: prostatic Adenocarcinoma:

This study included 20 male cases of prostatic carcinoma with age (69 ± 7) years old. In 16/20 (80%) cases, the type of biopsy was (TRUS) and in 3/20 (15%) of cases the type of biopsy was true cut biopsy. With reference to Gleason grades in patients suffered from prostatic carcinoma, 5/20 (25 %) of patients were in grade 4, while 4/20 (20%) of them in grades (1, 3 and 5) respectively. There is no significant correlation between tumor grade and age of patients with prostatic carcinoma.

GATA3 expression in prostatic carcinoma:

17/20 (85%) of cases were GATA3 negative, while the other 3/20 (15%) of cases were GATA3 positive as shown in figure(4) , figure (5).

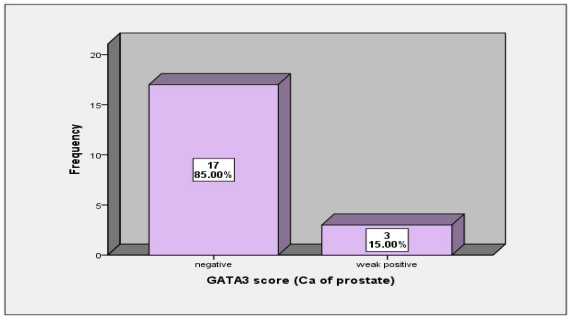


Figure 4: Distribution of GATA3 score in patients with prostatic adenocarcinoma.

GATA3 IHC expression and clinicopathological parameters correlation in prostatic carcinoma, table (2):

1.Age: there was no significant statistical correlation between GATA3 expression and the age of patients. Out of 3 positive cases, two of them were at age group 65-74 years and one at age group >75 years.

2.Gleason grade: two positive cases were grade 4, and one case was grade 3. There was no significant statistical correlation between GATA3 score and tumor grade.

Table 2: Association between GATA 3 score and variables in patients with prostatic carcinoma.

			GATA3 score	P-value
variables		negative	weak positive	
	55 - 64 years	5 (29.4%)	0 (0.0%)	
Age	65 - 74 years	8 (47.1%)	2 (66.7%)	0.56
	> 75 years	4 (23.5%)	1 (33.3%)	
Gleason score	grade 1	4 (23.5%)	0 (0.0%)	0.32
	grade 2	3 (17.6%)	0 (0.0%)	
	grade 3	3 (17.6%)	1 (33.3%)	
	grade 4	3 (17.6%)	2 (66.7%)	
	grade 5	4 (23.5%)	0 (0.0%)	

P-value ≤ 0.05 (significant).

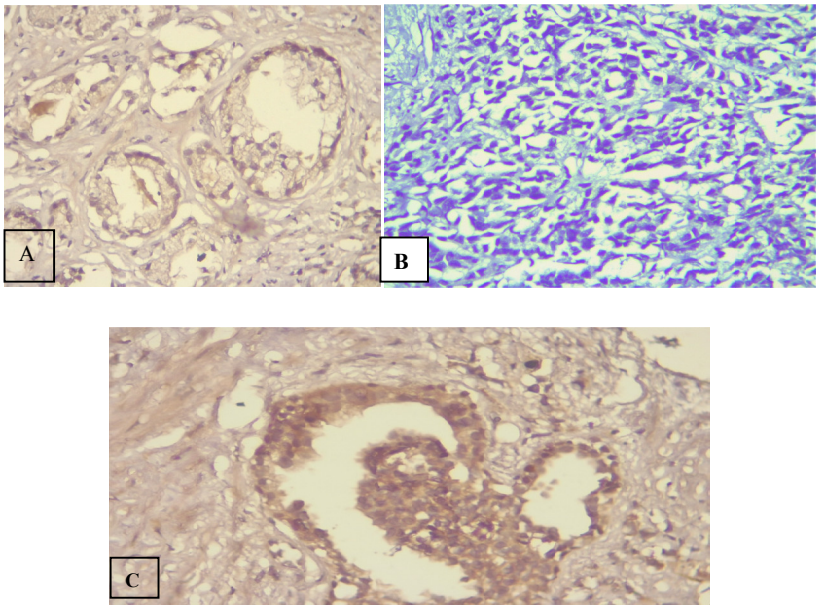


Figure 5: Microphotographs for GATA3 IHC, prostatic adenocarcinoma, prostate biopsies: A: negative staining, gleason (3+3), 400X. B: negative staining, gleason (5+4), 400X. C: weak positive staining, gleason (4+3), 400X.

There was a significant correlation between GATA3 score and tumor type with (p value 0.0001). 100% of patients who have strong positive GATA3 and 100% of moderate positive GATA3 expression had urothelial carcinoma, while 89.5% of cases with negative GATA3 and 100% with weak positive GATA3 had prostatic carcinoma, as illustrated in table (3).

Table 3: Correlation between GATA3 score and tumor type.

			GATA3 score		P-value
Tumor type	negative	weak positive	moderate positive	strong positive	
Urothelial Ca	2 (10.5%)	0 (0.0%)	3 (100.0%)	15 (100.0%)	
Prostatic Carcinoma	17 (89.5%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	0.0001
Total	19 (100.0%)	3 (100.0%)	3 (100.0%)	15 (100.0%)	

P-value ≤ 0.05 (significant).

DISCUSSION:

In the few past years, many markers used to determine the prostatic origin in adenocarcinoma such as prostatic specific antigen (PSA), Prostatic specific alkaline phosphatase (PSAP), Racemase and NKX3.1, in addition to the cytokeratin and p63. on the other hand, no specific marker was used for urothelial carcinoma. For that reason, applying this study using GATA3 to discriminate both tumors and help to determine the urothelial origin of malignant lesion.

GATA3 immunohistochemical expression:

GATA 3 is a transcription factor important in differentiation of breast epithelia, urothelia, and a subset of T-lymphocytes. It has been suggested useful in the evaluation of mammary or urothelial origin or metastatic carcinoma. [15] It has been suggested useful in the distinction between urothelial versus prostatic adenocarcinoma, and metastatic urothelial versus squamous cell carcinoma in the lung, with 80% of metastatic urothelial carcinoma but none of pulmonary squamous cell carcinoma or high grade prostatic carcinoma being positive. [16]

In the current study, a semiquantitative method has been used to calculate the expression of GATA3, While other studies used different scoring method as in **Oh WJ, et al, 2016** [17], who required more than 10% of cells stained to be positive regardless intensity while **Liu H, et al**, **2012** [18] study regarded the results with less than 5% of cells stained to be negative. Other studies used the H-score with different cutoff value ranging from greater than 5 to greater than 20 as in **Clark BZ, et al, 2014** [19].

1.GATA3 IHC expression in urothelial carcinoma: In this study, diffuse strong staining reaction was seen in 75% of urothelial carcinoma cases. This percentage was close to **Abdulla, W.H, 2018** [20] , where 72% of 51 case revealed strong nuclear stain . Also**, Liu H, et al, 2012** [18] found 65% of 72 cases and **Chang A, et al, 2012 (**[16] found 89% of 35 cases revealed strong intensity of staining.

This variation in degrees of expression may be due to limited number of cases in this study and different clones of the markers used by different authors.

50% of high grade urothelial carcinoma is with squamous differentiation and stained strongly for GATA3. **Helmy, et al,2015** [21] found that 70% of 10 urothelial carcinoma with squamous differentiation cases were GATA3 positive while **Miettinen M, et al ,2003**[22] found 5/54 GATA3 negative were urothelial carcinomas with squamous differentiation. This might be due to different selection method or antibody that had been used.

In this study, no significant correlation between GATA3 and other clinicopathological parameters except for gender was found; this might be due to limited number of cases in this study and higher incidence of urothelial carcinoma among male patients.

This interrelation is in keeping with **Leivo MZ, et al, 2016**[23] and **Abdulla, W.H, 2018** [20] studies. On the other hand **Higgins JP, et al**, **2007**[24] reported that higher GATA3 expression in low grade (95%) compared with high grade (57%) urothelial carcinoma.

Also, in **Naik, M.,ettal**, **2020** [25] study showed GATA-3 positivity decreased from normal urothelium (100%) to UC; low-grade UC (100%) to high-grade UC (78.4%) ; non-invasive (100%) to invasive UC(73.4%). **Kamel, N.A., et al, 2019**[26] found down-expression of GATA3 was significantly associated with high grade tumors and tumor progression.

In reviewing previous studies, the sensitivity of GATA3 in urothelial carcinoma was 99%, 96%, 95% in **Leivo MZ, et al,2016** [23], **Abdulla ,W.H, 2018** [20], **Clark BZ, et al, 2014** [19], **Miettinen M, et al, 2003** [22] studies respectively. On the other hand, the sensitivity in studies with larger number of patients was 67% of 308 cases in **Higgins JP, et al, 2007** [24] study and 84.8% of 138 cases **in Oh WJ, et al, 2016** [17] .

2.GATA3 IHC expression in prostatic carcinoma:

In this study, 17/20 (85%) of prostatic adenocarcinoma cases were GATA3 negative, while the other 3/20 (15%) of cases were GATA3 positive, two of them were Gleason grade 4, while **Miettinen**, **2003** [22] study reported 2% (2/95) of prostatic adenocarcinoma cases with positive reaction and the intensity expression was irrespective of Gleason grade. None of **Oh WJ, et al, 2016**[17] , **Liu H, et al, 2012**[18] , **Higgins JP, et al,2007** [24] , **Chang A, et al,2012**[16] and **Abdulla ,W.H, 2018** [20] studies showed GATA3 positivity in prostatic adenocarcinoma.

There was no significant statistical correlation between GATA3 expression and clinicopathological parameters in this study.

McDonald et al, 2020[27,28], studied rare cases of prostatic adenocarcinoma showed focal or diffuse strong staining for GATA3, in this study GATA3 positivity was strong and diffuse in four cases , patchy in two cases and strong focal in three cases; all cases were positive for NKX3.1. In order to avoid this diagnostic pitfall, undifferentiated carcinoma involving the prostate, bladder neck or trigone should be evaluated not only for GATA3, but also prostatic specific markers .[27]

CONCLUSION:

GATA3 is a reliable marker to confirm the urothelial origin of the tumor. There was significant correlation between GATA3 score and tumor type (urothelial versus prostatic). In urothelial carcinoma, no significant correlation was found between GATA3 expression and clinicopathological parameters except for gender.

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