Original Article

Evaluation of GATA3 tumor marker expression in the progression of urothelial bladder cancer in patients referred to Golestan Hospital in Ahvaz

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ABSTRACT

The transcription factor GATA-binding protein 3 (GATA3) is helpful as a marker to distinguish urothelial bladder carcinoma (UBC) from other cancers. Hence, the current study was done with the purpose of evaluating the expression of the tumor marker GATA3 in the progression of UBC. In this cross-sectional study, the total number of UBC samples were collected in the period of 2018-2019. First, the demographic and clinical data of each sample, such as age and gender of the patients, depth of tumor, grade of tumor, and type of tumor variant, were extracted from the patient record and recorded. Samples were stained in this manner to examine GATA3 immunohistochemistry (IHC) with labeled antibodies. Finally, 39 patients were included. There was no statistically significant association observed ($P > 0.05$) between age, gender, tumor grade, muscular invasion, and GATA3 expression. The relation was performed, and depending on the level of significance, it could be said that there was no significant association ($P=0.54$). 2 individuals were GATA3 expression negative and high grade, 16 individuals were diffuse strong and high grade, 20 individuals were diffuse strong and low grade, and one individual was heterogeneous strong.

In conclusion, a higher percentage of positive expression of GATA3 was observed in the present study compared to other studies performed in this field. However, due to the lack of correlation between its expression and demographic information, as well as the clinicopathological factors of bladder urothelial carcinoma, GATA3 is not a reliable marker to determine prognosis in this cancer type.

Keywords: Neoplasms, Urinary bladder neoplasms, Cancer, Biomarkers, GATA binding protein 3

Introduction

Bladder cancer (BC) is more common in men than women, it is usually more detected in older subjects [1], which is the fourth most common cancer in Iranian men, as well as the second most common cancer of the genitourinary system after the prostate [2].

Most of these tumors are superficial, but in disparity cases, more aggressive tumors are treated with urinary diversions, such as radical cystectomy. Chemotherapy is also commonly prescribed as add-on therapy after cystectomy for patients with progressive disease [3]. Regardless of advances in invasive treatment choices, the prognosis of muscle-invasive bladder cancer has not improved significantly. Therefore, identifying molecules that play an essential role in the development of BC can effectively determine the prognosis of this cancer [4].

GATA Binding Protein 3 (GATA3) is a zinc transcription factor in T-Helper 2 (TH2) differentiation. In particular, GATA3 has been extensively studied in mammary gland morphogenesis and the pathophysiology of breast cancer [5]. Based on animal models, GATA3 plays an essential role in BC, and its loss is related to oncogenesis. A series of studies show that low expression of GATA3 is associated with higher tumor grade in BC, which indicates a poor prognosis of the disease. Therefore, GATA3 can be considered a tumor suppressor in breast cancer. GATA3 is considered a differentiation marker in urine [6, 7].

Consequently, GATA3 expression is widely used to discriminate primary or metastatic urothelial bladder carcinoma (UBC) from its mimics [8, 9]. Downregulation of GATA3 expression has recently been reported in the UBC and high-grade muscle-invasive carcinomas [10]. Nevertheless, the GATA3 function in BC progression is not well defined. Therefore, this study aims to evaluate GATA3 tumor marker expression in the progression of UBC.

Materials and Methods

Study Design and Setting

This study was based on The Strengthening the Reporting of Observational Studies in Epidemiology (**STROBE**) Statement [11]. This retrospective cross-sectional study was based on a series of UBC specimens collected between 2019 and 2020 from Golestan and Imam Khomeini Hospitals, Ahvaz, Iran, who underwent trans urethral resection of bladder tumor (TURBT) procedure. The ethical code for this study was secured from the ethics committee of Jundishapur University of Medical Sciences (AJUMS), Ahvaz (IR.AJUMS.HGOLESTAN.REC.1400.058). Moreover, all patient data obtained was handled with strict confidentiality.

Participants

Data from patients with a minimum of 18 years with sufficient tumor mass, absence of necrosis/hemorrhagic, and complete medical records were included in this study. Incomplete recordings, or without laboratory results were excluded from this study.

Variables

The demographic and clinical characteristics of each specimen, including patient age and gender, tumor depth, and grade of tumor were extracted from the patient record and recorded. The slides stained with hematoxylin-eosin (H&E) were examined.

Data measurement

Immunohistochemical test

The Immunohistochemistry (IHC) test was performed using a standard protocol of previous studies [12]. Samples were incubated with 1 g/ml diluted GATA3 primary anti-mouse monoclonal antibody (Dako, Carpinteria, CA). Hematoxylin stain (HS) (Yekta Tajhiz Azma, Iran) was used to develop background contrast. Nuclear staining of GATA3 in at least 10% of the cells was considered a positive result.

Statistical analysis

Descriptive statistics including mean and standard deviation (± SD) were used. The Kruskal-Wallis and Spearman coefficient tests were used to check the relationship between different variables. The significance level of $P < 0.05$ was defined and analyzed using SPSS (ver. 22) software.

Results and Discussion

Finally, 39 patients with a mean age of 65.62 ± 13.04 years were evaluated. Most of the study participants were men (82.1 %). Regarding tumor grade, 19 tumors (48.7%) were high grade, and 20 tumors (51.3%) were low grade. Most of the subjects (74.36%) did not have muscle invasion. In this study, two subjects (5.1 %) were negative for GATA3 expression, 36 subjects (92.3 %) had a diffuse strong, and one subject (2.6 %) had a heterogeneous strong (**Table 1**).

Table 1. Patient demographic information, clinicopathological characteristics and GATA3 expression status.

Variables		Patients (N = 39)
Age, Mean ± SD		65.62 ± 13
Gender (Male), N (%)		32 (82.1)
Tumor grade, N (%)	High	19 (48.7)
	Low	20 (51.3)
Muscular invasion, N (%)	Present	10 (25.64)
	Absent	29 (74.36)
GATA3 expression status, N (%)	Negative	2 (5.1)
	Diffuse strong	36 (92.3)
	Heterogeneous strong	1 (2.6)

The relation between age, gender, tumor grade, muscular invasion, and GATA3 expression was measured using the Kruskal-Walli's test, and there was no statistically significant association observed ($P > 0.05$) (**Table 2**). 2 individuals were GATA3 expression negative and high grade, 16 individuals were diffuse strong and high grade, 20 individuals were diffuse strong and low grade, and one individual was heterogeneous strong. The relation was performed, and depending on the level of significance, it could be said that there was no significant association (P=0.54). IHC expression of GATA3 in UBC is shown in **Figure 1**. IHC staining for GATA3 was evaluated for nuclear staining.

Table 2. Relationship between patient demographic information and clinicopathological characteristics with GATA3 expression status.

Variables		IHC GATA3 expression status			P-Value
		Negative	Diffuse strong	Heterogeneous strong	
Age, Mean ± SD		51.5 ± 33.23	66.06 ± 11.71	78 ± 0.00	0.517
Gender (Male), N (%)		1 (2.56)	30 (76.92)	1 (2.56)	0.437
Tumor grade, N (%)	High	2 (5.12)	16 (41.02)	1 (2.56)	0.189
	Low	0	20 (51.28)	0	

Muscular invasion, N (%)	Present	1 (2.56)	32 (82.05)	1 (2.56)	0.246
	Absent	1 (2.56)	4 (10.24)	0	

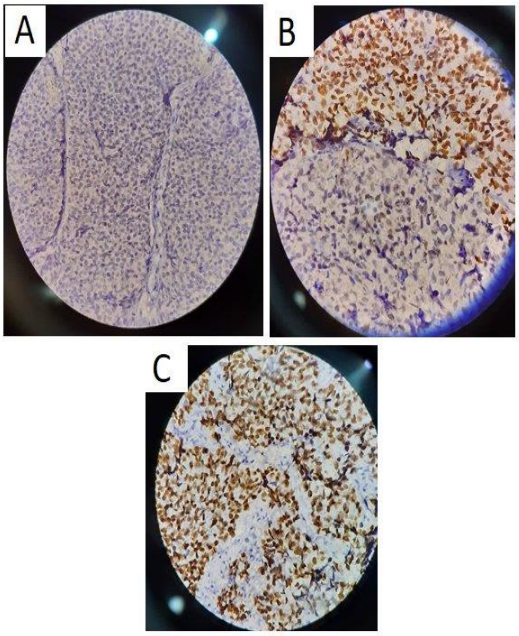

Figure 1. Immunohistochemical expression of GATA3 in bladder urothelial cancer. IHC staining for GATA3 was evaluated for nuclear staining. From top left: (A) negative expression of GATA3. (B) Heterogeneous strong positive expression of GATA3. (C) Diffuse strong positive expression of GATA3.

As a helpful marker for UBC differentiation, the IHC of GATA3 is widely used. In this study, the IHC determination of GATA3 expression levels was carried out in 39 patients. Some results were like those observed in UBC tissue samples and others were different.

GATA3 has a primary function in the progress and differentiation of tissues [13]. Mutations, downregulation, or overexpression of GATA relate to human tumors, such as leukemia, breast cancer, and gastrointestinal cancer. GATA3 is one of the most critical and sensitive IHC markers for distinguishing UBC [6, 14-17].

Higgins et al. 2007 proposed GATA3 expression as a marker for transitional epithelium and UBC and suggested that it was peculiar for that [18]. However, this marker is not always specific to high-grade invasive UBC. It may morphologically resemble other high-grade metastatic tumors such as prostate cancer, renal cell carcinoma, and squamous cell carcinoma, which is vital for differentiation because treatment and management vary depending on the primary malignancy [19]. Recently a study showed that in challenging cases, GATA3 expression is correlated with high grade UBC and invasive behaviour, suggesting its utility as a diagnostic marker [20].

Some investigators have studied the application of GATA3 IHC expression in several cancers containing urothelial carcinoma, and this marker is a sensitive marker for urothelial cancer [21-24]. Few studies in the past have demonstrated the absence of GATA3 expression in renal cell carcinoma [5]. Lack of GATA3 expression in primary and metastatic renal cell carcinoma was demonstrated as a function of GATA3 in the discriminational diagnosis of primary renal tumors and a possible value in unknown primary metastatic cancers in the presence of renal tumor disease [25]. However, *Chang et al., 2012* showed that none of the high-grade prostate cancers had GATA3 expression. However, weak positive expression has been observed in some benign prostate cancers [26].

GATA3 is a sensitive marker for UBC and this marker should be used routinely in the IHC when evaluating high-grade genital tract carcinoma which the primary location is unknown. In addition, GATA3 exhibits similar staining properties in both primary and metastatic regions [6]. Moreover, expression of GATA3 in UBC was reported between 63% and 93% [27].

In the present study, 94.7% expression of GATA3 was reported in UBC, which is slightly higher than the reported level. Nonetheless, GATA3 expression was detected in 77% of 74 UBC cases, which was lower than in the present study [28]. This study showed no significant association between muscle invasion and GATA3 expression (P=0.246). A study has shown that high levels of GATA3 were associated with increased tumor size in invasive UBC. [27]. The loss of GATA3 was linked with invasive tumors and high-grade [29]. In contrast, the independent tumor marker of poor prognosis is high expression; this is not consistent with the present study. Most tumors positive in this study for GATA3 expression were low grade, although no significant correlation between grade and GATA3 expression was observed (P=0.189). Although, a significant relationship was observed between GATA3 expression and histological grade (P<0.001) [28]. Furthermore, similar results were obtained in other study [18]. GATA3 expression was significantly lower (95%), which may show the role of GATA3 expression in progression to higher grades [18]. Conversely, 66% of muscle-invasive had GATA3 positive compared [28].

This study has many limitations. One of the most important reasons for the difference in GATA3 expression and the lack of correlation of its expression with patient demographic information and clinicopathological factors can be cited as the difference in sample size and the type of antibody clone used. The restrictions of the current study also comprised the small sample size and the lack of investigation of the association between GATA3 expression and other clinicopathological factors.

Conclusion

In conclusion, a higher percentage of positive expression of GATA3 was observed in the present study compared to other studies performed in this field. However, due to the lack of correlation between its expression and demographic information, as well as the clinicopathological factors of bladder urothelial carcinoma, GATA3 is not a reliable marker to determine prognosis in this cancer type.

Informed consent

This was a retrospective study which informed consent of patients was waived by the Institutional Review Board (IRB) and Ethics Committee of the of Ahvaz Jundishapur University of Medical Sciences.

Authors' contributions

H.J, L.F: designed data collection tools, monitored data collection, wrote the statistical analysis plan, cleaned, and analyzed the data, and drafted and revised the paper. P,KH, and M.S: wrote the statistical analysis plan, cleaned, and analyzed the data. H.J, L.F, and P,KH: implemented the study, analyzed the data, drafted, and revised the paper.

Data availability statements

The datasets generated during and/or analysed during the current study are not publicly available due to privacy ethical but are available from the corresponding author on reasonable request.

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Conflict of interest: The authors declare that they have no conflict of interest.

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Ethics statement: The initial proposal of the work was approved by the Institutional Review Board (IRB) and Ethics Committee of the of Ahvaz Jundishapur University of Medical Sciences, Iran (code: IR.AJUMS.HGOLESTAN.REC.1400.058).

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