

Diagnostic and Prognostic Implications of Estrogen Receptor and GATA3 Expression in Bladder Cancer: A Clinicopathological Study

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ABSTRACT

Background: The estrogen receptor (ER) and GATA-binding factor 3 (GATA3) are implicated in the pathogenesis and progression of various cancers, including bladder cancer. Although the relationship between GATA3 and ER in bladder cancer remains underexplored, these markers have shown promise in other neoplasms such as breast cancer. This study investigates the expression patterns of ER and GATA3 in bladder cancer and correlates these with clinicopathological parameters to assess their utility as diagnostic and prognostic markers. **Methods:** We conducted a cross-sectional study of 99 bladder cancer patients at a private hospital and histopathology lab in Ramadi, Iraq, from March 2022 to April 2023. Data collected included age, gender, cancer grade, stage, and the status of ER and GATA3. **Results:** Among the bladder cancer patients, the majority were male (70.7%) with predominantly high-grade tumors (70.7%). GATA3 positivity was significantly associated with lower-grade tumors, with 89.7% of low-grade cancers exhibiting positive GATA3 expression. No significant correlations were observed between GATA3 expression and patient age or gender. The interaction between ER and GATA3 and its impact on tumor behavior and progression was analyzed, underscoring their potential roles in the molecular pathology of bladder cancer. **Conclusion:** The expression of GATA3 is significantly associated with lower cancer grades and exhibits a notable relationship with ER status, highlighting their potential as valuable biomarkers for grading and potentially guiding therapy in bladder cancer. This study contributes to the understanding of the molecular mechanisms underlying bladder cancer and reinforces the importance of these markers in clinical pathology.

Keywords: Clinicopathological, Significance, Bladder, Cancer, Estrogen Receptor, GATA3.

INTRODUCTION

Bladder cancer is a significant global health concern, accounting for substantial morbidity, mortality, and healthcare expenditure. It ranks as the tenth most common cancer worldwide, with approximately 550,000 new cases and 200,000 deaths annually [1]. The incidence of bladder cancer is higher in men than in women, and the disease is more prevalent in developed countries. The primary risk factors for bladder cancer include tobacco smoking, exposure to specific occupational chemicals, and chronic inflammation resulting from infections or other causes [2]. Despite advancements in diagnosis and management, the prognosis for patients with advanced bladder cancer remains poor, and the molecular mechanisms underlying the disease remain incompletely understood [3]. Numerous studies demonstrating the therapeutic and prognostic value of biomarkers involved in the bio-molecular mechanism of urothelial carcinoma and other urinary tract tumors, like PSA in prostate cancer, CXR2 and CXR3 in renal cell carcinoma and IL-17 and TNF- α in CLL. Recent evidence has highlighted the critical role of estrogen receptors (ERs) in the development and progression of various malignancies, including breast, endometrial, and ovarian cancers. ERs are nuclear hormone receptors that mediate the cellular effects of estrogen, a hormone with essential functions in the reproductive system and various other physiological processes. Oestrogens are capable of binding to two discrete receptors, ER α and ER β , each of which possesses unique biological activities and tissue distribution patterns [4, 5]. When estrogen is present, it turns on endoplasmic reticulum (ERs), which changes the activation of target genes and impacts cell growth, division, and death [6]. Based on new information, ERs may

possibly play a role in the growth of bladder cancer. Scientists have found that ER α and ER β are present in both healthy and cancerous bladder tissue. However, they are expressed in these two types of tissue in different ways. Bear in mind that the amount of ER β found in bladder cancer has decreased. However, it is still not clear what part ER α plays in the growth of the disease. Also, research has shown that endothelial receptor (ER) signals can be changed by oestrogen, which can change how bladder cancer cells grow and spread [7, 8]. This means that ERs might be able to be used as treatment targets for this cancer. A zinc finger protein called GATA3 controls gene expression during foetal development and tissue differentiation. It is part of the GATA family of proteins. It is important to know that GATA3 helps build and maintain the urothelium, which is the epithelial lining of the urinary system. GATA3 downregulation has been observed to occur frequently in bladder cancer, and the absence of this protein has been linked to aggressive tumour characteristics, an elevated pathological grade, and an unfavourable prognosis. Furthermore, it has been demonstrated that GATA3 functions as a tumour suppressor in bladder cancer through the inhibition of cellular migration, invasion, and proliferation [9]. In bladder cancer, the relationship between ERs and GATA3 is still a subject of active research. GATA3 has been implicated in breast cancer to regulate ER signalling and expression, according to a number of studies; this suggests that a comparable interaction might be at play in bladder cancer as well [10]. Enhancing our comprehension of the correlation between ERs and GATA3 in bladder cancer may yield significant knowledge regarding the molecular mechanisms that drive the ailment and facilitate the development of

innovative therapeutic approaches [11]. Aim of this study: The purpose of this investigation is to examine the relationship between estrogen receptors (ER) and GATA3 in bladder cancer especially in stage 2 (Pt2) tumors. We will investigate the correlation between the expression patterns of ERs and GATA3 in bladder cancer specimens and their clinicopathological characteristics. In addition, we will explain the expression of ERs and GATA3 in bladder cancer cells and its implications for the progression of the disease.

Method

Cross-sectional study of 99 cases of bladder carcinoma, the study conducted in a private hospital and private histopathology laboratory in Iraq / Ramadi from the period March 2022- April 2023. The data collected include; Age group (years), Gender of patients, Grade of the tumor, Stage of the tumor, Estrogen Receptors status, and GATA3. Inclusion criteria: we include both sex in this study, both low and high-grade tumors, stage (Pt2) only because in this stage the tumor has grown through connective tissue into the muscle which is considered serious and experience more post-operative complications, regarding tumor type only transitional cell carcinoma was concerned as it is the commonest bladder tumor. Exclusion criteria: we exclude other types of bladder cancer (adenocarcinoma, squamous cell carcinoma, sarcoma & other rare types). The samples were represented by formalin-fixed, paraffin –embedded blocks. The blocks were sectioned

at 4-micron thickness and stained by ordinary stain (H & E), then the slides were examined by pathologist. The criteria for diagnosis of transitional cell carcinoma were based on presence of papillae and sheets of neoplastic urothelium invading the submucosa and muscular layer with increased urothelial thickness, loss of cell polarity and orientation, marked pleomorphism, hyperchromasia & numerous mitosis. After that IHC markers (ER & GATA-3) were done on tissue sections using mouse monoclonal antibodies, peroxidase –conjugate and DAB chromogen. Version 22 of SPSS was used to conduct statistical analyses. Categorical data were expressed using frequency and percentage, while continuous data were expressed using mean, median, and standard deviation (SD). The Chi-square test was used to evaluate the relationship between variables, and Pearson's correlation was used to determine the relationship between continuous data. A P-value of 0.05 or lower was statistically significant.

Results

Cross sectional study of 99 patients with a bladder Cancer Mean age of patients 51.6 ± 13.4 years' old. 29.3% of patients at age group ≥ 60 while 28.3% of patients at age group 40-49 years old. Most of patients are males (70.7%) and have high grade cancer (70.7%), 37.4% of patients have positive estrogen receptors response and 29.3% of patients have positive GATA3, all patients (100%) at stage Pt2. As shown in table 1.

Table 1: Distribution of Patients According to the Variables of Study.

Variables		Frequency	Percentage
Age group (years)	20-29	4	4.0
	30-39	15	15.2
	40-49	28	28.3
	50-59	23	23.2
	≥ 60	29	29.3
Gender	Females	29	29.3
	Males	70	70.7
Grade	High grade	70	70.7
	Low grade	29	29.3
Stage	Pt2	99	100.0
Estrogen receptors	Negative	62	62.6
	Positive	37	37.4
GATA3	Negative	70	70.7
	Positive	29	29.3

Table 2: Association between GATA3 and the Variables of Study.

Variables		GATA3		Total	P-value
		Negative	Positive		
Age group (years)	20-29	3	1	4	0.47
		75.0%	25.0%	100.0%	
	30-39	10	5	15	
		66.7%	33.3%	100.0%	
	40-49	17	11	28	
		60.7%	39.3%	100.0%	
Gender	50-59	16	7	23	0.63
		69.6%	30.4%	100.0%	
	60	24	5	29	
		82.8%	17.2%	100.0%	
	Females	22	7	29	
		75.9%	24.1%	100.0%	
Grades	Males	48	22	70	0.0001
		68.6%	31.4%	100.0%	
	High	67	3	70	
Stage		95.7%	4.3%	100.0%	-
	Low	3	26	29	
		10.3%	89.7%	100.0%	
Pt2		70	29	99	-
		70.7%	29.3%	100.0%	

P-value ≤ 0.05 (significant).

As shown in table 2, there is significant association between GATA3 and grades of bladder cancer, 89.7% of patients with low grade have positive GATA3 (as shown in figure-1) while 4.3% only of patients with high grade have positive GATA3.

No significant association between GATA3 and age groups, gender. As shown in table 3; we will investigate the functional interaction

between ERs and GATA3 in bladder cancer cells and its implications for the progression of the disease. And the association between GATA3 and estrogen receptors is significant association between GATA3 and estrogen receptors, 13.8% of positive GATA3 have positive estrogen receptors (as shown in figure -2) while 52.9% of Negative GATA3 have Negative estrogen receptors.

Table 3: Association between GATA3 and Estrogen Receptor

Variables		Gata3		P-value
Estrogen Receptor		Positive	Negative	0.003
	Positive	4	33	
		13.8%	47.1%	
	Negative	25	37	
		86.2%	52.9%	
Total		29	70	
		100.0%	100.0%	

P-value ≤ 0.05 (significant).

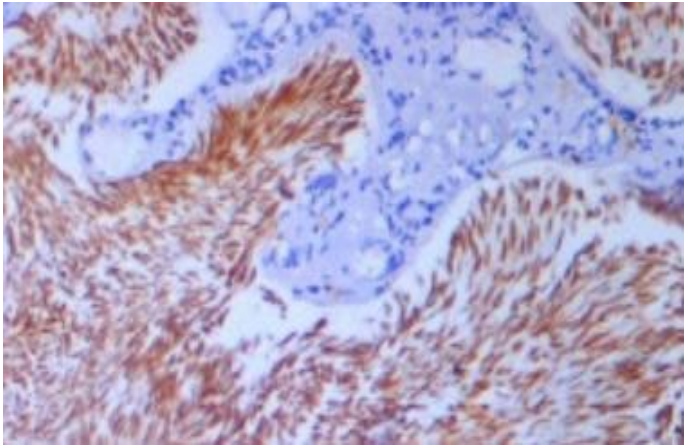


Figure 1: Invasive Transitional Cell Carcinoma of Bladder Stained with Anti-GATA3 Antibody Using Peroxidase-Conjugate & DAB Chromogen. High Power View Revealed Strongly Positive Tumor Cells.

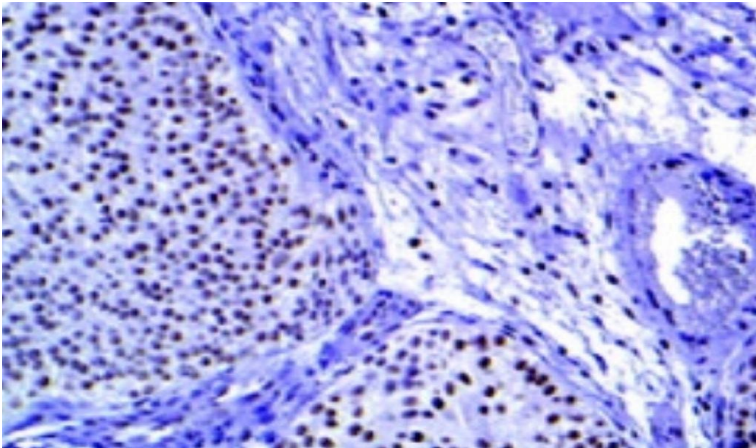


Figure 2: Immunohistochemical Stain of ER for Invasive Urothelial Carcinoma, High Power View Revealed Strongly Positive Nuclear Stain.

Discussion

The Clinic Pathological Significance of stage 2 (Pt2) Bladder Cancer Estrogen Receptor-GATA3 was performed in this study & provides several valuable insights into the demographics and clinical characteristics of patients with bladder cancer. Our study revealed a strong association between GATA3 and bladder cancer grades, with higher GATA3 positivity in low-grade bladder cancer & a significant connection was observed between GATA3 and estrogen responses in this type of tumor. As the ER was used as part of hormonal therapy in many

tumors, especially breast cancer, accordingly ER and GATA3 can be used in the treatment and follow-up of the patient. The predominance of male patients and high-grade cancers observed in this study is consistent with previous research. For example, Ferlay et al. [12] reported that bladder cancer is more common in males and often presents as high-grade disease. The strong correlation between bladder cancer grade and GATA3, a transcription factor critical for T-cell maturation and differentiation, is one of the most remarkable results of our research. Notable because it lends credence to previous studies that identified GATA3 as a useful marker for distinguishing between

low-grade and high-grade bladder cancer [13]. By demonstrating that GATA3 positive is substantially more prevalent in individuals with low-grade bladder cancer, our work adds to the growing body of knowledge about GATA3 and bladder cancer. A study conducted by Chenglu et al. [14] using mRNA data from the TCGA database and bladder cancer cell lines lends credence to our findings. Their findings indicate a notable upregulation of GATA3 mRNA expression when contrasted with normal tissues and cells. When comparing bladder cancer cell lines to normal urothelial cell lines, a Western blot experiment shows that GATA3 protein levels are dramatically decreased. This finding contradicts the previous findings. Furthermore, our research demonstrated a strong correlation between increased GATA3 expression and certain clinical characteristics. Some specific factors that were found to be significantly associated with higher GATA3 expression include: tumour subtype, earlier clinical stage, lower grade tumours, and formalin-fixed paraffin embedded (FFPE) tissues. These associations were significant in both TCGA and FFPE, while marginally significant in TCGA. Ching et al. [15] found no significant connection between GATA3 positive and the grade of bladder cancer, which contradicts our findings. This discrepancy may be due to differences in study populations or methodologies and warrants further investigation. Interestingly, no significant association was found between GATA3 and age groups or gender. This aligns with the findings of several previous studies, including McConkey et al., [16] who reported that GATA3 expression did not differ significantly across age or gender. Our study also revealed a significant association between GATA3 and estrogen responses. This is particularly intriguing, as there are currently conflicting views in the literature. Ching et al. [15] found no correlation between GATA3 and estrogen receptor status. However, a study by Kandalaft et al. [17] reported an association between GATA3 and estrogen receptor status, suggesting a potential role for hormone therapy in bladder cancer. The research by Bernardo

et al. [18] supports the notion that oestrogen receptor alpha (ER) expression is markedly downregulated in lower-grade tumours. In particular, urothelial carcinomas were discovered to have high levels of the collaborating protein GATA3. Lower-grade and stage tumours showed increased GATA3 expression. The information was inadequate to assess the predictive significance of either oestrogen receptor beta (ER-b) or ER [19].

However, GATA3 positive was linked to a greater chance of avoiding recurrence. The expression of ER or ER was shown to be negatively correlated with GATA3. Additionally, a number of reasons of variation in the data were found. In this study, expression of these markers in tumor recurrence/progression can't be assessed which is considered a limitation in this study. According to our findings, can these markers be used as tumor markers in differentiating high-grade recurrent tumors from low-grade tumors?

Conclusion

There is a significant association between GATA3 expression and bladder cancer grade, with a notably higher prevalence of positive GATA3 in low-grade cancers compared to high-grade cancers. No significant correlation was found between GATA3 expression and patient age groups or gender, indicating that GATA3's role in bladder cancer may be independent of these demographic factors.

Recommendation

However, these relationships, especially the potential link between GATA3 and hormone therapy, require further investigation. The study also confirmed the high prevalence of bladder cancer among males and high-grade cancer, emphasizing the need for focused preventive and treatment strategies for these groups. Despite these insights, the complexity of bladder cancer warrants continued research.

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