

GATA3 EXPRESSION IN DIFFERENT MORPHOLOGICAL VARIANTS OF URINARY BLADDER NEOPLASMS

Pathology

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ABSTRACT

Urothelial carcinoma comprises approximately 90% of all primary bladder tumors. The gold standard for diagnosing bladder carcinoma is biopsy of suspicious area. The management and post treatment monitoring of bladder cancers are largely dependent on stage and grade. Urothelial carcinoma has a varied and wide histological spectrum posing a diagnostic challenge on H&E examination alone. Immunohistochemistry is a valuable adjunct for diagnosis. Among various immunohistochemical markers that have been proven to influence disease progression and patient's survival, GATA3 serves as a useful diagnostic and prognostic marker for urothelial neoplasm. We studied morphological spectrum of Urinary Bladder neoplasms with expression of GATA3 in histological variants at L.L.R.M Medical College, Meerut. We examined 60 tissue samples of Transurethral Resection of Bladder Tissue chips or bladder biopsy in 10% buffered formalin. These were analysed for histopathological diagnosis and immunohistochemically stained for GATA3. Out of the total 60 cases, statistically significant correlation was observed between different grades and invasiveness of urothelial carcinoma with GATA3 expression in 80% cases. GATA3 can be used as sensitive and specific marker for Urothelial Carcinoma. It can also be effectively used in the prediction of probable grade and tumor invasion in biopsied material with poor morphological characters; and thus, serves as a useful diagnostic and prognostic marker as well as helps in future appropriate treatment

KEYWORDS

Bladder Neoplasms, Urothelial Carcinoma, GATA3

INTRODUCTION

Approximately ninety percent of all primary bladder tumours are UCs (urothelial carcinoma). In India, bladder cancer ranks as the 17th most common cancer, according to the GLOBOCAN 2020 database¹. The 5-year prevalence is approximately 3.57 per 100,000 people, which results in approximately 11,000 deaths annually¹. Comparatively speaking, men are more likely than women to develop bladder cancer. In the majority of India's urban population-based cancer registries, the relative incidence is 4:1¹. Biopsy of the suspicious area is the gold standard for diagnosing bladder carcinoma. Their further management and post-treatment monitoring are largely dependent on stage and grade. The histological spectrum of urothelial carcinoma is broad and diverse, making a diagnosis based solely on H&E examination difficult. Roughly 70% of superficial urothelial carcinomas have the potential to return, and 15%–25% advance to a more advanced stage, grade, or metastatic condition¹. Immunohistochemistry is a valuable adjunct for diagnosis. IHC markers for urothelial carcinomas include p63, uroplakin III, CK 7, CK 20, high molecular weight cytokeratin, thrombomodulin, S 100 P and GATA 3. CK 7, CK 20 and HMWCK, are known to be expressed in a variety of cancers. For urothelial carcinomas, uroplakin III and S-100 P are very specific. However, researchers have developed novel IHC indicators as a result of their poorer sensitivity, particularly in high grade urothelial carcinomas. GATA3 has proven to influence disease progression and patient survival as well as serves as a useful diagnostic and prognostic marker for urothelial neoplasm. However, the percentage of positive results and their strength can differ depending on the grade and type of primary and metastatic cancer.

Members of the GATA transcription factor family either directly activate or restrict target gene expression by engaging the DNA sequence (A/T) GATA (A/G) in the gene promoters⁷. The GATA family members comprise of two groups. The hematological and nervous system growth is frequently, albeit not always, associated with the first group, that comprises GATA1, GATA2, and GATA3. The second group, which spans GATA4, GATA5, and GATA6, is typically coupled with various organs and tissues of mesodermal as well as endodermal origin^{7,8}. Specifically, The development and maturation of several tissues depends on GATA3, including the luminal glandular epithelial cells found in the breast^{9,10,11} parathyroid gland¹², adipose tissue¹³, kidney¹⁴, sympathetic nervous system^{15,16,17}, and lens fibre cells of the

eye¹⁸. Numerous malignancies that affect humans, notably leukemia, carcinomas of the breast and gastrointestinal tract have been attributed to mutations, loss of expression, or shifts in the altogether expression of GATA factors.

Aim and objectives

1. To study the morphological spectrum of Urinary Bladder neoplasms at Lala Lajpat Rai Memorial Medical College, Meerut.
2. To study the expression of GATA3 in the histological variants of Urinary bladder neoplasms.

Material and method

Study group: The current study was conducted in Department of Pathology at LLRM Medical College, Meerut in partnership with the Department of Surgery at the SVBP Hospital, LLRM Medical College, Meerut. The study included patients with lower urinary tract symptoms (LUTS) whose radiography, cystoscopy, and clinical examination was suspicious. The histopathology lab received the tissue samples of Transurethral Resection of Bladder Tissue (TURBT) chips or bladder biopsy in 10% buffered formalin and processed it routinely.

Study design: Prospective study.

Study period: One Year from September 2022 to August 2023

Sample size: 60

Methodology:

The histopathology lab collected tissues of bladder biopsies following a TURBT in 10% buffered formalin, processed them exactly as per protocol, and produced paraffin-embedded blocks. Hematoxylin and Eosin was used to stain successive sections that were 3–5 μ thick. These were examined for histopathological diagnosis.

Two separate pathologists assessed and reported on each case. Besides this, sections 2–4 μ thick were also obtained on slides coated with Poly-L lysine for immunohistochemistry and the antigen was extracted using a microwave heat method. Primary antibodies were used for GATA3 immunohistochemical staining followed by incubation with the secondary antibody conjugated with HRP for 45 minutes. The reaction products were revealed using diaminobenzidine (DAB) as the chromogen. These were subsequently counterstained with Harris' Hematoxylin to enhance nuclear detection. Sections of the

uterine cervix have used as positive controls. As a negative control, duplicate sections were treated with phosphate buffer saline instead of the primary antibody.

On a bluish background, nuclear positivity was visible in a dark brown colour. The strength of nuclear staining was assessed on a scale of 0 to 3: 0 (not stained), 1 (weak), 2 (moderate), and 3 (intense). The percentage of cells that stained favorably was determined for each staining intensity and recorded as: 4+ (>75%), 3+ (51–75%), 2+ (26–50%), 1+ (5–25%), and negative ie. 0 (tumor cells stained less than 5%). Lastly, the intensity score and percentage positivity were multiplied to determine the immunoreactivity score for GATA3: 0–1 = Negative, 2–4 = Weakly Positive, 5–8 = Moderately Positive, and 9–12 = Strongly Positive.

Inclusion criteria:

Symptomatic patients with LUTS and hematuria alongwith individuals suspicious of bladder neoplasms on cystoscopy, radiology or clinical examination.

Exclusion criteria:

Individuals who previously underwent biopsy.

Additional causes of hematuria including upper tract transistional cell carcinoma and renal cell carcinoma.

All insufficient or inadequately preserved specimens.

Patient who lacked the necessary radiological and clinical information were eliminated.

$P < 0.05$ is regarded as statistically significant in the Pearson's Chi-Square Test, which was used for statistical analysis.

Observation and results

The study involved analysis of histopathology and immunohistochemistry of 60 patients with urinary bladder neoplasms identified by clinical examination, radiography, and cystoscopy. The morphological spectrum of urinary bladder neoplasms is shown in Table 1.

Table 1.: Morphological Spectrum of cases according to final histological diagnosis (n=60)

S.No.	Histological diagnosis	No. Of cases	Percentage (%)
1)	Urothelial Papilloma	2	3
2)	Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP)	3	5
3)	Low-Grade Urothelial Carcinoma (LGUC)	7	12
	Non-invasive	7	12
	Invasive	0	0
4)	High-Grade Urothelial Carcinoma (HGUC)	44	73
	Non-invasive	14	23
	Invasive	30	50
5)	Urothelial Carcinoma with squamous differentiation	4	7
	Total	60	100

The study encompassed 60 cases, out of which maximum 44 (73%) had High grade Urothelial carcinoma (HGUC); 7 cases (12%) had Low-grade Urothelial carcinoma (LGUC); 4 cases (7%) had Urothelial carcinoma with squamous differentiation; 3 cases (5%) had Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP); and 2 cases (3%), Urothelial papilloma.

Out of the 44 cases of HGUC, muscle invasion was seen in 30 cases (50%) while non-invasive cases were 14 (23%).

Out of the 7 cases of LGUC, all were non-invasive; 7 cases (12%). Table 1.

Table 2. Age-wise Distribution of total cases (n=60)

Age distribution	Number of cases	Percentage (%)
31-40	5	8.3

41-50	14	23.3
51-60	24	40
61-70	15	25
71-80	2	3.3
Total	60	100

The maximum number of cases in the current study group were of the age group 51–60 years; 24 (40%), followed by 15 cases (25%) in between 61–70 years, 14 cases (23.3%) in between 41–50 years, 5 cases (8%) in between 31–40 years, and 2 cases (3.3%) in 71–80 years age group. (Table 2.)

Table 3 Sex wise Distribution of total cases (n=60)

Gender distribution	Number of cases	Percentage (%)
Male	55	92
Female	5	8
Total	60	100

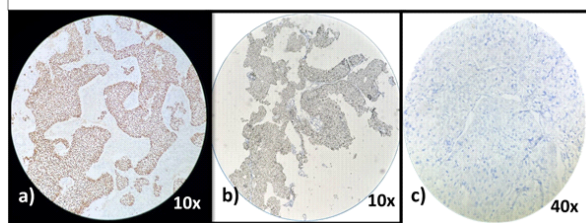
With a male-to-female ratio of 11:1, males accounted for the majority of cases in the current study, 55 (92%), followed by 5 (8.3%) in females. (Table 3.)

Table 4: Expression of GATA3 in histological variants of Urinary bladder neoplasms (n=60)

S.No.	Histological diagnosis (total cases)	Positive		Negative	
		No.	(%)	No.	(%)
1)	Urothelial Papilloma (2)	2	3	0	0
2)	Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP) (3)	3	5	0	0
3)	Low Grade Urothelial Carcinoma (LGUC) (7)	7	12	0	0
	Non-invasive	7	12	0	0
	Invasive	0	0	0	0
4)	High-Grade Urothelial Carcinoma (HGUC) (44)	34	57	10	17
	Non- invasive	14	23	0	0
	Invasive	20	33	10	17
5)	Urothelial Carcinoma with squamous differentiation (4)	2	3	2	3
	Total (60)	48	80	12	20

All three cases of PUNLMP (5%) and 7 cases of LGUC (12%) in the current investigation, as well as two cases of Urothelial Papilloma (3%), tested positive for GATA 3 expression. While out of the 44 cases of HGUC 34 cases (57%) were GATA-3 positive and 10 cases (17%) were negative. Among the four Urothelial Carcinoma with squamous differentiation cases, two (3%) exhibited GATA-3 positivity. In total, 48 of 60 cases (80%) tested positive for GATA 3. (Table 4. Fig. 1.)

Fig.1: Expression of GATA3 in histological variants- a) PUNLMP b) Low grade Urothelial Carcinoma c) High grade Urothelial



DISCUSSION

This study focused on the different morphological subtypes of bladder neoplasms and their GATA 3 expression. The study included 60 cases of Urinary bladder neoplasms. Among the total 60 cases, Urothelial Papilloma were 03%, PUNLMP 05%, LGUC 12%, HGUC 73% and Urothelial Carcinoma with squamous differentiation 07%.

All 12% of cases of LGUC were non-invasive. Of the 73% cases of HGUC, 23% were non-invasive and 50% were invasive Urothelial Carcinoma.

GATA 3 positivity was seen in 48 (80%) out of the 60 cases in the current study. Researches conducted in the past for GATA3 have demonstrated that GATA3 expression in urothelial carcinoma varies with values ranging from 63% to 93%. First investigated by Higgins et al. in 2007, the expression of GATA3 as a marker for urothelial cancer

was found to be very specific. In their study of 321 UC, 67% showed GATA-3 positivity³⁶. A handful of other researchers have also investigated the utility of GATA3 immunohistochemistry expression across multiple tumors, including urothelial carcinoma, and concluded that it is a sensitive indicator for the disease.^{19,20,21,22,23,24,25}

GATA 3 was noted positive in 65% of cases of invasive urothelial carcinoma and in all cases (100%) of non-invasive urothelial carcinoma. GATA3 positivity was observed in 86% of urothelial neoplasms, including 72–80% of high-grade and/or muscle-invasive tumors and 98 percent of low-grade and/or non-muscle-invasive tumors, according to Miyamoto et al. (2012).⁷

In the present study, GATA3 positivity was observed in 100% of LGUC cases and 75% of HGUC cases. Mienittien et al. (2014) observed that the bladder's urothelium showed significant GATA-3 positivity. GATA-3 was reported to be strongly positive in 49 out of 54 (90.74%) cases of UC, but negative in high-grade, poorly differentiated, and UC with squamous differentiation³⁷. 77% of UC patients had GATA-3 positive, according to Agarwal et al.'s 2019 research. High-grade, invasive tumors had minimal or no GATA-3 expression, whereas low-grade, non-invasive tumors had moderate to robust GATA-3 expression.¹⁹

In 2021, Naik M et al. discerned GATA-3 expression in 101/126 (80.15%) of UC²⁴. GATA-3 expression was downregulated from non-invasive to invasive UC²⁴, from low-grade to high-grade UC, and from normal urothelium to UC. In line with many other studies, we found that GATA3 expression was present in 80% of the cases in our investigation.^{19,20,21,22,23,24,25}

GATA3 elimination has been correlated with high-grade and/or muscle-invasive urothelial neoplasms, according to Miyamoto et al.⁷. Our investigation identified a strong connection ($P < 0.001$) between GATA3 expression and histological grade. GATA3 immunoreactivity was present in 100% of low-grade tumors, compared to 75% of high-grade tumors and all non-invasive UC cases had strong GATA3 expression. Therefore, it was found that invasive urothelial tumours had lost GATA3 expression, which is consistent with the outcomes of Miyamoto et al., Agrawal H et al., Naik M et al., and Mienittien et al.^{7,19,24,25}

Table 5.

Table 5. Comparison of GATA3 positivity in current study to previous studies

Previous Studies	GATA 3 Positivity (%)
Higgins et.al (2007)	67
Miyamoto et al. (2012)	86
Mienittien et al. (2014)	90.74
Agarwal et al. (2019)	77
Naik M et al. (2021)	80.15
Present Study	80

CONCLUSION

Based on our current investigation, GATA 3 is a helpful tool for identifying urothelial neoplasms. With the progression of the cancer characteristics from normal urothelium to urothelial carcinoma, higher grade, and invasiveness, the GATA 3 percentage positivity and intensity declined. Our findings support the hypothesis that, in addition to histological grading and staging, the use of the GATA 3 immunomarker in urothelial carcinoma may provide extra prognostic information.

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