Prognostic utility of GATA-3 and CK-14 Immunohistochemical expression in urothelial carcinoma of urinary Bladder and its clinicopathological correlation

Abstract

Background- Bladder urothelial carcinoma, is considered the 7th most common cancer in males. Identifying reliable biomarkers like GATA3 and CK14 through immunohistochemical methods can aid in early detection, risk stratification, and personalized treatment strategies

Aims &Objectives: Assessment of GATA3 and CK14 expression in urinary bladder carcinoma and correlation with clinical and histopathological variables, for both diagnostic and prognostic purposes.

Methods and Materials: This is prospective study, 80 clinically diagnosed cases of urothelial carcinoma were included in one year of duration. All the cases were histopathological evaluated and immunohistochemically stained with GATA binding protein 3 and CK14.

Results: Out of 80 cases of urothelial carcinoma, the majority of patients were over 60 years of age. GATA3 expression was negative in 33 cases (41.25%), weak in 1 case (1.25%), moderate in 18 cases (22.5%), and strong in 28 cases (35%). Immunohistochemical (IHC) expression of CK14 was negative in most patients (82.5%), moderate in 6.25%, and strong in 8.75%. GATA3 expression showed a statistically significant correlation ($P < 0.001$) with high tumor grade and muscle invasion as compared to low-grade, non-invasive tumors. CK14 expression was also significantly associated with muscle invasion, pronounced nuclear pleomorphism, and high mitotic activity (>10/10 HPF). These markers can be effectively used to predict tumor grade and depth of invasion in biopsy samples based on morphological features, aiding in accurate diagnosis and appropriate clinical management.

Conclusion: Combining GATA-3 and CK-14 expression profiles can enhance understanding of urothelial carcinoma's histological subtype and aggressiveness, potentially guiding treatment and management strategies.

Key Words: Urothelial carcinoma, Histopathological examination, Immunohistochemistry GATA3 and CK-14.

INTRODUCTION: Bladder urothelial carcinoma ranks the tenth most frequent cancer world-wide overall for both genders.[1] urinary bladder cancer was the most frequent cancer in urinary tract as about 14.2% of male's malignancies of urothelial origin according to Global Cancer Observatory. [2] Bladder cancer is a rare malignancy in the Indian population. As per the GLOBOCAN 2022 database, bladder cancer is the 17th most common malignancy in India about 3.1%. [3] The 5-year prevalence appears to be 3.57 per 100000 population leading to

about 11000 deaths each year. [3] The incidence of bladder cancer is higher in males compared to females (Relative incidence being 4:1in most urban population-based cancer registries in India).[4] Bladder cancer is a disease with high heterogeneity in its pathology and clinical presentation. Tobacco consumption is the most important risk factor in bladder cancer. Risk for smokers is 3-4fold higher compared to non-smokers and is estimated to cause 31% of bladder cancer deaths among men and 16% among women.[5,6] Generally, urothelial carcinoma is categorized into non-muscle-invasive bladder cancer (NMBC) and muscle-invasive bladder cancer (MIBC) according to bladder wall invasion.[7]While NMBC generally has a low risk of distant metastasis and better out comes, MIBC is more aggressive and is more likely to metastasize. MIBC usually requires intensive management, which includes radical cystectomy with perioperative chemotherapy.[8,9] According to "Bladder Cancer Molecular Taxonomy Group," molecular classification of muscle-invasive bladder carcinoma categorized to two main groups, luminal and basal with difference in biological and histological patterns and clinical manifestation.[10,11] It has been reported as immunohistochemical antibodies are useful indicators for both luminal and basal tumors. Luminal bladder carcinomas express markers of terminal differentiation as CK20, GATA3 and uroplakins; whereas, basal carcinomas can express basal types cytokeratin like CK5, CK6 and CK14 which act as markers of basal urothelial cells progenitor /stem cells.[12,13] In this study we aimed to use immunohistochemical markers GATA binding protein 3 (GATA-3) that has high sensitivity and specificity in identifying urothelial differentiation. Compared to other markers associated with urothelial cells, GATA-3 has a higher sensitivity than uroplakin III and a higher specificity than p63, S100P and thrombomodulin.[14-16] Thus, GATA-3 has been shown to be an important indicator for distinguishing UCs from other types of carcinomas.[17] CK 14, an acidic type I keratin, is a novel immunohistochemical marker found in the mitotically active basal cells of stratified epithelium. In addition, the expression of CK14 indicated the presence of a highly tumorigenic population of stem cells.[18]CK14 immunoreactivity was found to increase in the early stages of carcinogenesis and coincide with the development of malignant lesions in the urinary bladder. Their expression will be correlated with the patient clinicopathological parameters to explore their prognostic role.

AIMS & OBJECTIVES- To study the utility of GATA-3 and CK-14 immunohistochemical expression in urothelial carcinoma of urinary bladder. GATA-3 and CK 14 expression in correlation with clinicopathological aspect of urothelial carcinoma of urinary bladder.

MATERIALS AND METHODS: It was a retrospective prospective study conducted in the department of pathology for 1 year of duration. A total of 80 clinically diagnosed cases of urothelial carcinoma that underwent transurethral resection or radical cystectomy were included. Approval was obtained from the institutional ethical committee, and clinical data were obtained from case sheets. Histologically diagnosed cases of urothelial carcinoma, patients who give consent to enrol in the study and follow up. All poorly preserved slides, retrieval or insufficient tumor tissue and patients with insufficient clinical and radiological details were excluded from the study. Tissue samples were received in our histopathology

laboratory in 10% buffered formalin and were further processed. Haematoxylin and Eosin-stained slides were evaluated and reported as per the WHO/International Society of Urologic Pathology (ISUP) Classification of bladder tumor 2016. Special emphasis was laid on tumor type, grade, muscle invasiveness, divergent differentiation, necrosis, mitotic activity. Immunohistochemistry (IHC) for GATA-3 and CK14 was performed on a 4-5 µm thick section cut from formalin-fixed paraffin-embedded blocks. Staining and evaluation were done using monoclonal primary antibodies for GATA-3 (Clone: L 50-823). We used Bladder transitional carcinoma as a positive control for GATA-3. For negative control, primary antibody was omitted while performing immunohistochemical staining. Cytokeratin 14 (Clone: LL002) Mouse Monoclonal Antibody with positive tissue control is Prostate. Both positive and negative controls were included in every batch of Immunohistochemistry (IHC) staining. A negative tissue controls provide an indication of non-specific background staining

Immunohistochemical staining evaluation GATA3 immunostaining interpretation nuclear staining for GATA3 was graded as weak, moderate, or strong, and negative [14]. CK14 immunostaining interpretation was positive CK14 immunostaining appears as brown cytoplasmic staining. The assessment included the following: Total immunostaining score (TIS) was calculated by multiplying percentage score (PS), and intensity score (IS): PS: 0 = no positive cells, 1 = any positive cell up to 10%, 2 = 10–50%, 3 = 51–80%, and 4 = more than 80%. IS: 0 = no colour reaction, 1 = mild intensity, 2 = moderate intensity, and 3 = strong intensity. TIS: 0–1 = negative, 2–3 = mild, 4–8 = moderate, and 9–12 = strong [15].

Statistical Analysis: Data management and analysis were performed using Statistical Package for Social Sciences (SPSS)23. Numerical data were summarized using means and standard deviations or medians and ranges. Categorical data were summarized as percentages. Comparisons between the 2 groups with respect to normally distributed numeric variables were done. For categorical variables, differences were analysed with (Chi-square) test. All p-values are two-sided. $p < 0.05$ were considered significant.

Results- This study included 80 cases of urothelial carcinoma with majority of patients belonging to age group over 60 years. A strong male predominance was observed, with approximately 75 cases (93.75%). Among these, 47 patients (58.75%) were from rural areas and were predominantly farmers by occupation. Regarding personnel habits, 32 cases (40%) were smokers only, while 28 cases (35%) reported both smoking and tobacco chewing. Alcohol consumption was noted in 13 cases (16.25%) and exclusive tabaco use was seen in 5 cases (6.25%) (Table: 1).

The most common presenting complaint was intermittent haematuria, observed in 78 cases (97.5%), followed by obstructive urinary symptoms in 24 cases (30 %) and burning micturition. in 20 cases (25%)

Tumor location was most frequently in the right posterolateral wall 36 cases (45%) followed by the left posterolateral wall, 24 cases (30%) and left lateral wall, 20 cases (25%). Transurethral Resection of Bladder Tumour (TURBT) was performed in 79 cases (98.75%), while radical cystectomy was performed in only 1 case (1.25%). Most patients received

chemotherapy (approximately 42.50%), followed by BCG therapy (3.75%) and radiotherapy (2.5%). Regarding Outcome, 36 patients (45.0%) had died, while 44 patients (55.0%) were alive.

Histopathologically 66.25% of cases diagnosed as high-grade urothelial carcinoma and 33.75% were low-grade urothelial carcinoma. Regarding the depth of invasion,19 cases (23.75%) showed lamina propria invasion, 34 cases (42.5%) had muscle invasion and 27 cases (33.75%) were non-invasive. Lymphovascular invasion (LVI) was present in 23 cases (28.75%), perineural invasion (PNI) in 11 cases (13.75%) and necrosis in 52.50% patients . (Figure1)

GATA3 expression was negative in 33 cases (41.25%), moderate in 18 cases (22.50%), and strong in 28 cases (35.0%) of urothelial carcinoma (UC). CK-14 expression was negative in 82.5%, moderate in 6.25%, and strong in 8.75% of UC.(Table 2)

Low-grade tumors without lymphatic invasion (LVI), perineural invasion (PNI), or necrosis were significantly associated with moderate to strong GATA3 expression. Negative and weak GATA3 expression was observed in high grade tumors with marked nuclear pleomorphism and high mitotic activity (>10/10 HPF) (Figure2)

Alive patients had significantly higher GATA3 expression were detected. A strong statistical association was observed between GATA3 expression and histopathological parameters with grades, invasion, LVI, necrosis, and survival status (P-value of <0.001) (Table 3)

Muscle invasive tumors showed variable CK14 expression (weak to strong). Low grade tumors with absence of lymphatic invasion (LVI), perineural invasion (PNI), and necrosis was associated with higher CK14 expression. (Figure3) (Table 4)

In deceased patients, significant CK14 expression was observed with LVI, perineural invasion (PNI) and necrosis with a p-values of <0.001, while other parameters such as grade, nuclear pleomorphism, mitosis and overall outcome did not show a statistically significant association.

Comparison of GATA3 and CK14 expression with tumor grade, lamina propria invasion, muscle invasion, necrosis and PNI showed a statistically significant association (p-value of <0.001). In muscle invasive tumors there was a higher prevalence of negative and weak GATA3 expression and a higher prevalence of weak to strong CK14 expression.

Among deceased patients with high-grade urothelial carcinoma (UC) showed 81.82% had negative GATA3 expression and remaining patients showed weak expression (18.18%). All deceased patients exhibited moderate CK14 expression and 57.14% showed strong CK14 expression. The comparison of GATA3 and CK14 expression with survival outcomes also demonstrated a statistically significant association (p-value of <0.001)

Discussion- Bladder cancer can be categorized into different molecular subtypes, reflecting the heterogenicity of the disease. Gene expression profiling has identified at least three main

subtypes: luminal, basal and double-negative. Luminal tumours are characterized by the high expression of terminally differentiated urothelial cell markers such as GATA3, CK20 and uroplakin, indicating differentiation towards umbrella cells.[19,20]) Basal subtype tumour express markers like CK5/6 and CK14, typically found in mesenchymal stem cells and display characteristics of squamous and sarcomatous differentiation.[21] Recent studies have shown that the expression of GATA3 and CK5/6 can identify molecular subtypes in approximately 80-90% of cases.[19,22] The absence of either GATA3 or CK5/6 expression is linked to poorer survival, and the absence of both markers is strongly predictive of an adverse outcome.

Miyamoto et al, first highlighted the prognostic role of GATA3 in urothelial neoplasm, showing that its loss correlates with high-grade or muscle-invasive tumours, whereas strong GATA3 expression was independently associated with poor prognosis. [23] Our study also found a statistically significant correlation ($P < 0.001$) between histological grade and GATA3 expression. Notably, patients with high grade or strong GATA3 expression showed better survival outcomes.

In our study, 53 (66.25%), had a high-grade tumour, and 27 (33.75%) had a low-grade tumour, consistent with meta-analysis done by Lin et al. (2019) who found that squamous differentiation in UC was associated with high grade features and advanced stages pT3/T4.[24]

GATA3 expression in our study was absent in 33 patients (41.25%), weak in 1 (1.25%), moderate in 18 (22.5%), and strong in 28 (35%). Elzohery et al. (2021) similarly reported that 70% of UC cases lacked GATA3 expression, while 30% were positive. [25] Muscle-invasive tumours in our study showed weak GATA3 expression, while non-invasive and lamina-invasive tumours typically exhibited moderate to strong expression. These findings are consistent with those of Miyamoto et al., reinforcing that GATA3 loss is associated with muscle-invasive disease.[26]

Additionally, weak or absent GATA3 expression was significantly associated with adverse histopathological features, including marked nuclear pleomorphism ($P = 0.002$), high mitotic activity (>10/10 HPF; $P < 0.001$), necrosis ($P = 0.019$).

CK14 expression, assessed through immunohistochemistry, showed weak to strong positivity in muscle-invasive tumours. In deceased patients, CK14 expression ranged from weak to strong. Elzohery et al. (2021) reported significant associations between histological subtype and CK14 expression ($P < 0.001$), with positive staining in 64.3% of UC with squamous differentiation, 100% of pure squamous cell carcinoma (SCC), and none of the UC cases without squamous features. CK14 was 100% sensitive for SCC and 64.3% sensitive and 100% specific for UC with squamous differentiation. Gulmann et al. (2013) similarly reported CK14 expression in 100% of SCC, 74% of invasive UC with squamous features, and 27% of pure UC. [27]

In our study, CK14 expression significantly correlated with tumour stage (P = 0.001), consistent with Jangir et al. (2019), who found that advanced-stage, muscle-invasive bladder cancers expressing basal markers CK14 and CK5/6 often exhibited squamous differentiation and shorter survival. [28]

In summary, high-grade malignancy in bladder cancer is frequently associated with GATA3 loss. Numerous studies, including ours, suggest that GATA3 is a valuable prognostic biomarker for muscle invasive bladder carcinoma. [29,30]

Conclusion- This study highlights the diagnostic and prognostic significance of GATA3 and CK14 immunohistochemical markers in urothelial carcinoma. GATA3 expression was significantly associated with lower tumour grade, non-invasiveness, and improved survival outcomes, making it a valuable marker for favourable prognosis. In contrast, CK14 expression correlated with high-grade, muscle-invasive tumours and adverse histopathological features such as LVI, PNI, necrosis, and high mitotic activity. The inverse relationship between GATA3 and CK14 expression underscores their potential utility in tumour subtyping. Incorporating these markers into routine histopathological assessment can improve the accuracy of tumour grading and staging, particularly in limited biopsy samples, and guide more effective treatment strategies.

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FIGURE LEGENDS

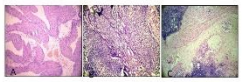
Fig1: Histopathology of urothelial carcinoma A. Low grade (H&E Stain,100X) B. High grade(H&E Stain,400X) C.Mucle Invasive (H&E Stain,400X)

Fig2: Immunohistochemical expression of GATA3 in Urothelial Carcinoma A. Strong expression in low grade B. Moderate expression in high grade C. weak expression in high grade(IHC Stain,100X)

Fig3: Immunohistochemical expression of CK-14 in Urothelial Carcinoma A. Negative expression in low grade B. Moderate expression in high grade C. Strong expression in high grade(IHC Stain,100X)

FIGURES

Fig1: Histopathology of urothelial carcinoma A. Low grade (H&E Stain,100X) B. High grade(H&E Stain,400X) C.Mucle Invasive (H&E Stain,400X)



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315 Figure2: Immunohistochemical expression of GATA3 in Urothelial Carcinoma A. Strong
316 expression in low grade B. Moderate expression in high grade C. weak expression in high grade(IHC
317 Stain,100X)

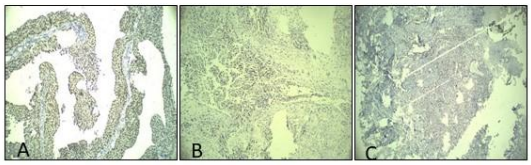

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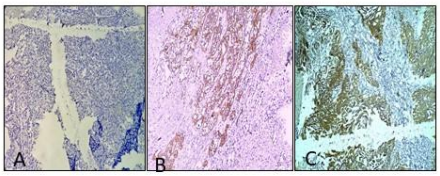
Figure3: Immunohistochemical expression of CK-14 in Urothelial Carcinoma A. Negative expression in low grade B. Moderate expression in high grade C. Strong expression in high grade(IHC Stain,100X)



TABLES:

Table 1: Demographic baseline characters of patients

	Demographic Profile	Number(N)	%
Age	21-40 years	2	2.50
	41-60 years	25	31.25
	>60 years	53	66.25
Gender	Male	75	93.75
	Female	5	6.25
Personal habits (addiction)	Smoking and tobacco	28	35.00
	Smoking only	32	40.00
	Tobacco only	5	6.25
	Alcohol	13	16.25
Clinical Features	Obstructive symptoms	24	30.00
	Intermittent hematuria	78	97.50
	Burning micturition	20	25.00
	Pain abdomen	19	23.75

Table 2: Distribution of patients with Histopathology & Immunohistochemistry (GATA3 and CK-14 Expression)

	Grade	Number(N)	Percentage (%)
Urothelial carcinoma	Low	27	33.75
	High	53	66.25
	Intensity		
IHC (GATA 3)	Negative	33	41.25
	Weak	1	1.25
	Moderate	18	22.50
	Strong	28	35.00
IHC (CK14)	Negative	66	82.50
	Weak	2	2.50
	Moderate	5	6.25
	Strong	7	8.75

Table 3: Comparison of GATA3 expression with histopathological parameters

		IHC (GATA 3)									
		Negative (n=33)		**Weak (n=1)**		**Moderate (n=18)**		**Strong (n=28)**		**Chi Sq.**	**p-Value**
Grade	Low	0	0.00	0	0.00	9	50.00	18	64.29	31.12	<0.001
	High	33	100.00	1	100.00	9	50.00	10	35.71		
Invasion	Lamina	0	0.00	0	0.00	9	50.00	10	35.71	81.60	<0.001
	Muscle	33	100.00	1	100.00	0	0.00	0	0.00		
	Noninvasive	0	0.00	0	0.00	9	50.00	18	64.29		
Nuclear pleomorphism	Weak	0	0.00	0	0.00	8	44.44	18	64.29	69.18	<0.001
	Moderate	1	3.03	0	0.00	8	44.44	8	28.57		
	Marked	32	96.97	1	100.00	1	5.56	1	3.57		
Mitosis	Score 1	0	0.00	0	0.00	9	50.00	18	64.29	69.83	<0.001
	Score 2	1	3.03	0	0.00	8	44.44	9	32.14		
	Score 3	32	96.97	1	100.00	1	5.56	1	3.57		
LVI	Present	21	63.64	0	0.00	1	5.56	1	3.57	33.40	<0.001
	Absent	12	36.36	1	100.00	17	94.44	27	96.43		
PNI	Present	11	33.33	0	0.00	0	0.00	0	0.00	18.16	<0.001
	Absent	22	66.67	1	100.00	18	100.00	28	100.00		
Necrosis	Present	32	96.97	1	100.00	2	11.11	7	25.00	47.93	<0.001
	Absent	1	3.03	0	0.00	16	88.89	21	75.00		
Outcome	Alive	5	15.15	1	100.00	15	83.33	23	82.14	33.63	<0.001
	Dead	27	81.82	0	0.00	3	16.67	6	21.43		

Table 4: Comparison of IHC (CK 14) expression with histopathological parameters

		Immunohistochemistry (CK-14)									
		Negative (n=66)		**Weak (n=2)**		**Moderate (n=5)**		**Strong (n=7)**		**Chi Sq.Test**	**p-Value**
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**		
Grade	Low	27	40.91	0	0.00	0	0.00	0	0.00	8.65	0.034
	High	39	59.09	2	100.00	5	100.00	7	100.00		
Invasion	Lamina	19	28.79	0	0.00	0	0.00	0	0.00	22.96	0.001
	Muscle	20	30.30	2	100.00	5	100.00	7	100.00		

	Noninvasive	27	40.91	0	0.00	0	0.00	0	0.00		
Nuclear pleomorphism	Mild	26	39.39	0	0.00	0	0.00	0	0.00	20.23	0.003
	Moderate	14	21.21	2	100.00	0	0.00	1	14.29		
	Marked	24	36.36	0	0.00	5	100.00	6	85.71		
Mitosis	Score1	27	40.91	0	0.00	0	0.00	0	0.00	19.18	0.004
	Score2	17	25.76	0	0.00	0	0.00	1	14.29		
	Score3	21	31.82	2	100.00	5	100.00	6	85.71		
LVI	Present	14	21.21	1	50.00	4	80.00	4	57.14	11.44	0.010
	Absent	52	78.79	1	50.00	1	20.00	3	42.86		
PNI	Present	3	4.55	1	50.00	5	100.00	2	28.57	46.95	<0.001
	Absent	63	95.45	1	50.00	0	0.00	5	71.43		
Necrosis	Present	29	43.94	2	100.00	5	100.00	6	85.71	11.37	0.010
	Absent	37	56.06	0	0.00	0	0.00	1	14.29		
Outcome	Alive	40	60.61	1	50.00	0	0.00	3	42.86	7.39	0.061
	Dead	26	39.39	1	50.00	5	100.00	4	57.14		