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An observational study on immunohistochemical expression profile of PCNA in urothelial carcinoma of bladder and association with clinicopathological parameters

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ABSTRACT

Background:

Bladder cancer remains as one of the most common cancers of the urinary tract, with higher rates of incidence and heterogenous molecular and histological components affecting its prognosis. Proliferating Cell Nuclear Antigen (PCNA) is a protein and immunohistochemical (IHC) biomarker associated with the G1/S phase of the cell cycle that is essential for the proliferation of neoplastic cells. This study aimed to describe the histomorphological aspects of UC of the bladder. To evaluate the expression of PCNA with clinicopathological parameters of the disease such as age, gender, clinical features, site, configuration, histological grade, stage, and lymphovascular invasion (LVI).

Materials and methods:

Forty histologically confirmed cases of urothelial carcinoma (UC) from the Department of Pathology at a tertiary care centre in South India were analyzed. PCNA immunohistochemistry was performed, assessing staining intensity and percentage of positive tumor cells. Chi-square tests evaluated associations with clinicopathological features such as age, gender, clinical presentation, tumor site, configuration, grade, type, stage, and LVI.

Results:

The expression of the PCNA was found to be significantly associated with sessile tumors ($p=0.023$), higher histological grading ($p=0.001$), advanced stage tumors ($p < 0.001$) and LVI ($p = 0.036$), suggesting possible association as a marker of tumor aggressiveness and progression. No associations were found with the parameters such as patient age, gender, clinical presentation, or location of the tumor concerning PCNA expression.

Conclusions:

Our study demonstrated PCNA overexpression in sessile, high-grade tumors, muscle-invasive tumors, LVI and with advanced tumor stage. This shows that PCNA may serve as a marker of proliferation and aggressiveness aiding in prognosis, treatment planning and decision making. More studies are required for validation of our findings.

Keywords: *PCNA, Urothelial carcinoma, Histopathology, Immunohistochemistry.*

INTRODUCTION

Bladder cancer is one of the most prevalent forms of cancer of urogenital tract, with urothelial carcinoma (UC) making up more than 90% of existing cases. It consists of non-invasive and aggressive muscle-invasive forms, which requires the need for convincing prognostic biomarkers for knowing the aggressiveness and prognosis of patients. The management of bladder cancer is based primarily on the stage of the cancer and may consist of transurethral resection of bladder tumor (TURBT), Bacillus Calmette-Guérin therapy (BCG), intravesical chemotherapy, radiation therapy, systemic chemotherapy, and cystectomy. It is important to grade tumors accurately since higher grade tumors are associated with advanced stages and worse prognosis and survival (1).

PCNA is a nuclear protein which is associated with DNA replication and repair as well as acts as a marker for malignancies. Previous studies suggested that it is overexpressed in a number of cancers, and PCNA in UC correlates with high tumor grade, higher mitotic activity, and higher stage (2). PCNA is found to have elevated concentrations in aggressive malignancies, as suggested in studies conducted on other cancers (3). However, there are very few research done on PCNA in UC in the Indian population, making further exploration into its prognostic nature necessary.

In this study, we aimed to evaluate the histomorphology of UC, including tumor site, histological grade, and lymphovascular invasion (LVI). In addition, the research aimed at quantifying the expression of PCNA by immunohistochemistry (IHC) and to evaluate the association with clinicopathological parameters such as patient age, sex,

tumor configuration, histological grade, pathological tumor stage, and LVI.

MATERIALS AND METHODS

Study Design

In our study we included 40 cases of UC of the bladder, obtained from archived tissue blocks at the Pathology Department from a tertiary care health centre in South India. All the specimens were obtained through TURBT procedure.

Inclusion criteria included cases with complete clinical, radiological, and operative records. Patients who had undergone radiation therapy or chemotherapy prior to tissue collection was excluded from the study. Cases of non-urothelial malignancies or those with incomplete clinical or radiological data in their records and other non-urothelial tumors like adenocarcinoma, squamous cell carcinoma, neuroendocrine tumors and lymphomas were not included. Cases with inadequate material or blocks with exhausted tissue samples were excluded. Clinicopathological data, including patient age, gender, clinical feature, tumor configuration, tumor grade, LVI and pathological t stage were recorded to analyse their association with IHC findings.

Histopathological (HP) Technique

For all the cases tissue samples were obtained through TURBT in 10% buffered formalin. Standard grossing procedures were followed, and tissue samples underwent routine processing in an automatic tissue processor (Leica TP1020). Thin sections of 3 – 4 microns were made using a rotary microtome (Leica RMT2125RT) and stained using Hematoxylin and Eosin (H&E) using standard staining techniques and protocols.

Histopathological (HP) examination

Microscopic examination included pattern of arrangement of tumour cells, degree of pleomorphism, histological grade, histological type and LVI. The diagnosis, grading, and staging of UCs were carried out using the latest 2022 WHO criteria (4). UCs were classified based on their grades as high-grade (Grade III) tumors and low-grade (Grade I-II) tumors. UC was divided into stages of invasion as Ta for superficial non-invasive tumors, T1 for lamina propria invasive tumors and T2 for deep, muscle-invasive tumors.

Immunohistochemical (IHC) Technique

IHC was performed using the peroxidase-antiperoxidase (PAP) method on 3 - 4µm thick sections from paraffin-embedded TURBT specimens, mounted on positively charged slides and incubated overnight. The slides were deparaffinized, rehydrated and underwent antigen retrieval with microwave heating in Tris-EDTA buffer (pH 9). Blockage of endogenous peroxidase activity was done using 3% H_2O_2. Primary antibody PCNA (EP91) rabbit monoclonal was applied and incubated for one hour. After buffer washes, Polyexcel Horseradish Peroxidase (HRP)-conjugated secondary antibody was applied for 15 minutes. Visualization was done using 3,3'-diaminobenzidine (DAB) chromogen, followed by counterstaining with Harris hematoxylin and mounting with distyrene plasticizer xylene (DPX). Colonic tissue was used as positive control for PCNA and phosphate buffered saline (PBS) in place of primary antibody served as the negative control.

Scoring and Data Analysis

Immunostaining for PCNA was assessed blindly, without knowledge of clinicopathological data. PCNA nuclear stains were graded as 1+ if less than 25% cells stained positive, 2+ if 25 –75% of tumor cells were positive, and 3+ if more than 75% were stained positive (5) .

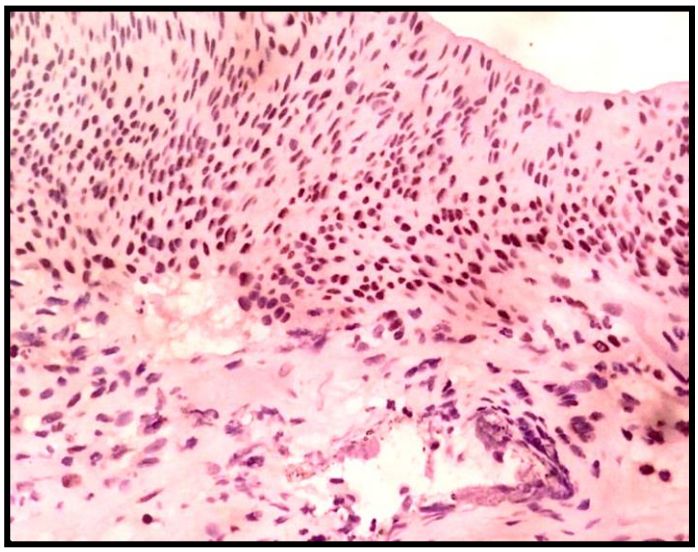

Figure 1: PCNA 1+ (nuclear stain) (IHC x100)

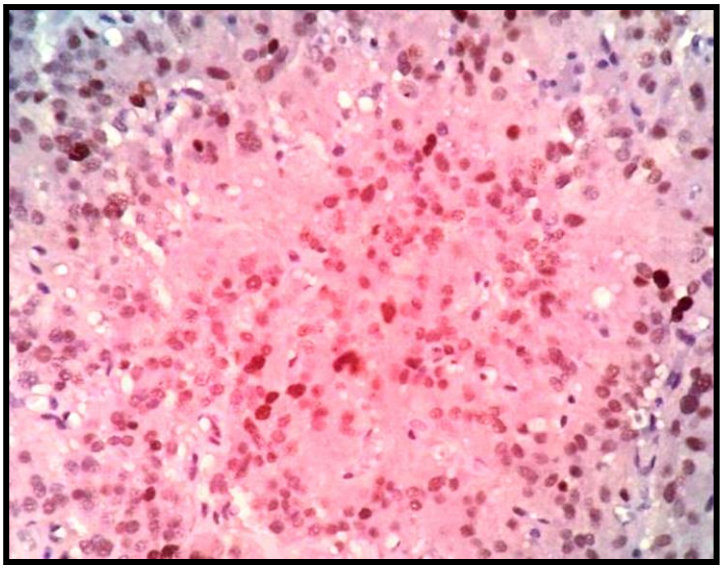

Figure 2: PCNA 2+ (nuclear stain) (IHC x400)

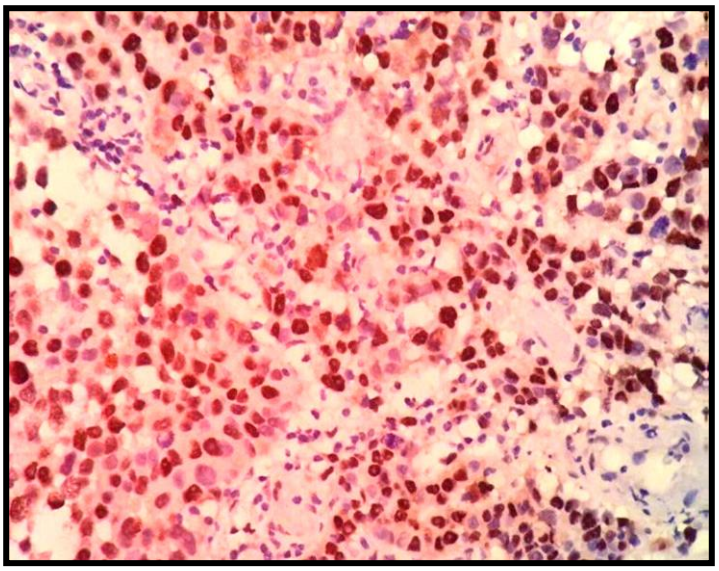

Figure 3: PCNA 3+ (nuclear stain) (IHCx400)

Statistical analysis

Statistical analysis was carried out using SPSS software 23.0. IHC expression and its association were compared with clinicopathological parameters through Chi-square test. Statistically significant association was considered if p value was less than 0.05

RESULT

Demographic and clinical characteristics of the study population

Most patients were between 61 and 80 years old (55%), with a predominance of males (75%). Hematuria was the most common clinical presentation (82.5%), and the specimens were received from TURBT in all cases. Tumors were most frequently located on the right lateral wall 22.5%. The papillary configuration was more common (57.5%). LVI was present in 10% of cases. Tumor staging revealed that 45% of cases were in stages T2, indicating muscle invasion. IHC analysis of PCNA showed strong expression (3+) in 40% of cases (as shown in Figure 1), moderate expression (2+) in 17.5% (Figure 2), and low expression (1+) in 42.5% (Figure 3). Table 1 displays the distribution of individual clinicopathological parameters among the cases.

Table 1: Distribution of clinicopathological parameters among cases

Variables	Frequency	Percentage
Age		
21-40	1	2.5
41-60	13	32.5
61-80	22	55
81-100	4	10
Sex		
Male	30	75
Female	10	25
Clinical Presentation		
Hematuria	33	82.5
Dysuria	3	7.5
Increased frequency	4	10
Procedure		
TURBT	40	100
Tumour site		
Anterior	5	12.5
Base	4	10
Left lateral wall	8	20
Posterior	7	17.5
Right lateral wall	9	22.5
Superior	1	2.5
Neck	6	15
Histological grade		
High grade	22	55
Low grade	18	45
Tumour Configuration		
Papillary	23	57.5
Sessile	17	42.5
LVI		
Absent	36	90
Present	4	10
Tumour stage		
Ta	11	27.5
T1	11	27.5
T2	18	45
PCNA		
1+	17	42.5
2+	7	17.5
3+	16	40

Association of PCNA expression among clinicopathological parameters

The association among PCNA expression and various clinicopathological parameters was analyzed and shown in table 2. There was no statistically significant association with PCNA expression and age ($p = 0.178$), sex ($p = 0.967$), clinical presentation ($p = 0.403$), or tumor site ($p = 0.119$).

PCNA expression showed a significant association with high grade tumors ($p < 0.001$), tumor configuration also showed a statistically significant relation ($p = 0.023$), with sessile tumors having a greater prevalence of strong PCNA expression (3+). Additionally, PCNA expression and LVI showed a significant association ($p = 0.036$), with all four LVI-positive cases showing strong PCNA positivity (3+). The expression of PCNA was also found to be strongly associated with tumor stage ($p < 0.001$), in which higher expression levels (3+) were noted in muscle invasive and advanced stage tumors (T2). These

findings indicate that higher PCNA expression is significantly associated with more aggressive tumor features, such as high histological grade, sessile growth pattern, LVI presence and advanced tumor stage.

Table 2: Association of PCNA expression among clinicopathological parameters

Variables		PCNA				p value
		1+	2+	3+	Total	
Age	21-40	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0.178
	41-60	6 (46.2%)	0 (0%)	7 (53.8%)	13 (100%)	
	61-80	10 (45.5%)	5 (22.7%)	7 (31.8%)	22 (100%)	
	81-100	1 (25%)	1 (25%)	2 (50%)	4 (100%)	
Sex	Male	13 (43.3%)	5 (16.7%)	12 (40%)	30 (100%)	0.967
	Female	4 (40%)	2 (20%)	4 (40%)	10 (100%)	
Clinical presentation	Hematuria	15 (45.4%)	7 (21.2%)	11 (33.4%)	33 (100%)	0.403
	Dysuria	1 (33.3%)	0 (0%)	2 (66.7%)	3 (100%)	
	Increased frequency	1 (25%)	0 (0%)	3 (75%)	4(100%)	
Tumour site	Anterior	1 (20%)	3 (60%)	1 (20%)	5 (100%)	0.119
	Base	0 (0%)	0 (0%)	4 (100%)	4 (100%)	
	Left lateral wall	4 (50%)	1 (12.5%)	3 (37.5%)	8 (100%)	
	Posterior	4 (57.1%)	1 (14.3%)	2 (28.6%)	7 (100%)	
	Right lateral wall	5 (55.5%)	2 (22.25%)	2 (22.25%)	9 (100%)	
	Superior	1 (100%)	0 (0%)	0 (0%)	1 (100%)	
	Neck	2 (33.3%)	0 (0%)	4 (66.7%)	6 (100%)	
Histological grade	High grade	2 (9.1%)	4 (18.2%)	16 (72.7%)	22 (100%)	<0.001
	Low grade	15 (83.3%)	3 (16.7%)	0 (0%)	18 (100%)	
Tumour Configuration	Papillary	13 (56.6%)	5 (21.7%)	5 (21.7%)	23 (100%)	0.023
	Sessile	4 (23.5%)	2 (11.8%)	11 (64.7%)	17 (100%)	
LVI	Absent	17 (47.2%)	7 (19.5%)	12 (33.3%)	36 (100%)	0.036
	Present	0 (0%)	0 (0%)	4 (100%)	4 (100%)	
T Stage	Ta	10 (90.9%)	1 (9.1%)	0 (0%)	11 (100%)	<0.001
	T1	5 (45.5%)	4 (36.4%)	2 (18.1%)	11 (100%)	
	T2	2(11.1%)	2(11.1%)	14 (77.8%)	18 (100%)	

DISCUSSION

As reported by the Global Cancer Observatory (GLOBOCAN) in 2022, UC is the ninth most common worldwide and the thirteenth most deadly cancer. There are approximately 614,298 new cases of bladder cancer a year. Bladder cancer incidence exhibits significant disparity between the genders, with much higher rates in men than women. The risk of bladder cancer increases with advancing age, peaking at 65 and older. It is more common in Europe, Asia and North America (6). In our research of 40 cases of UC of the bladder, we found that the disease was more common with older age with 55% of patients in the 61-80 age group. One case (2.4%) was found in the 21-40 age range. The mean age was 65 years. These findings were similar to Inagaki et al. (7), having a mean patient age of 66.3 years, suggesting bladder cancer is primarily a disease of the elderly age group due to increased exposure to risks such as smoking, chemical workplace exposure, and hereditary factors. Predominately male gender was most affected, with 75% of the patients being male and 25% female.

This is consistent with previous studies by Shiina et al. (8), Hattori et al. (9), Bozlu et al. (10), and Agarwal et al. (11), all of which noted similar gender distributions. Hematuria was the most common presenting symptom, seen in 82.5% of cases. Other symptoms included increased urinary frequency (10%) and dysuria (7.5%). These findings correspond with those of Agarwal et al. (11) and Inagaki et al. (7) who also noted hematuria as the leading symptom. In our study, the most frequent tumor locations were the right lateral wall (22.5%) and left lateral wall (20%) with posterior wall coming in third (17.5%). The infrequently encountered sites included neck at (15%), anterior wall (12.5%), base (10%), and superior wall (2.5%). Mahak Agarwal et al. (11) also reported varied tumor localization, highlighting the diversity in tumor site distribution. Papillary tumors were slightly more common (57.5%) than sessile tumors (42.5%). These findings are in line with Helal et al. (12) and Agarwal et al. (11). High-grade UC was seen more frequently, 55% compared to low-grade, 45%. This observation aligns with the other studies conducted by Helal et al. (12) and Agarwal et al. (11). On the contrary, Yıldırım et al. (5) noted a predominance of low-grade papillary urothelial neoplasms LG-PUN. Regarding invasion, 45% of the tumors were muscle-invasive T2, 27.5% were at the Ta non-invasive stage, and 27.5% showed invasion to the lamina propria T1 stage. Kumar et al. (13) also reported 70% muscle invasive tumors and 30% to be superficial, which includes L.P and non-Invasive cases (13). In our study, LVI was absent on 90% of the cases and present on 10% of cases. Bai et al. (14) noted LVI in 12 cases and 96 cases were negative. Miyake et al. (15) detected LVI in 39% of the cases, and Brimo et al. (16) detected 13% LVI positivity in pT1 bladder cancer. PCNA (Proliferating Cell Nuclear Antigen) expression was assessed in relation to various parameters. No significant association was found between PCNA expression and sex (0.967) or age group ($p = 0.178$). Similar results were reported by Bozlu et al. (10), Shiina et al. (8), and Hattori et al. (9), suggesting that PCNA expression is independent of demographic factors.

Histological grade ($p < 0.001$) was significantly associated with PCNA expression. These findings are supported by studies conducted by Agarwal et al. (11), Bozlu et al. (10), and Shiina et al. (8), all of which demonstrated similar findings with tumor grade. A significant association was also noted between PCNA expression and tumor configuration ($p = 0.023$). PCNA expression was significantly associated with LVI ($p = 0.036$). This indicates that vascularly invaded tumors can potentially have greater proliferative indices. Our findings need to be verified through additional research studies. Lastly, PCNA expression and tumor stage were significantly related ($p < 0.001$). This result concords with that of Yıldırım et al. (5), who also described increasing PCNA expression along with tumor stage, suggesting its potential as a prognostic marker of tumor progression and aggressiveness.

CONCLUSION

In this study we emphasized the importance of PCNA expression in UC of the bladder and its association with aggressive clinicopathological and poor prognostic factors. PCNA overexpression demonstrated a significant association with tumor configuration, high-grade tumors, muscle-invasive tumors, LVI and advanced tumor stage. These results imply that PCNA plays a significant role as a marker for tumor proliferation and aggressive behavior. Its prognostic value may be helpful to provide risk stratification and inform clinical decision-making. Additional research using bigger cohorts and molecular studies are advised to confirm PCNA's potential in individualized treatment strategies for bladder cancer.

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