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The role of P27 as a prognostic factor in association with grading and staging of urothelial bladder cancer

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Abstract
Background: Bladder cancer (BC) is the tenth most common cancer worldwide with urothelial carcinoma (UC) being the main histological subtype. P27 is a cyclin-dependent kinase inhibitor that negatively regulates cell proliferation.
Objective to analyze the clinical significance of p27 expression in Iraqi urothelial bladder cancer patients.
Methods: This retrospective cross-sectional analysis of 53 formalin-fixed, paraffin-embedded bladder biopsy samples from December 2022 to July 2023, Teaching hospitals and private labs, these were transurethral biopsies. Clinicopathological parameters like age, sex, tumor morphology, muscle invasion, clinical stage, and histological grade were obtained from histopathological reports and clinical data. P27 expression was analyzed, and p27 expression was correlated with patients' clinical parameters.
Results: In 31% of patients, nuclear p27 protein expression was positive. Loss of p27 expression was associated with higher tumour clinical stage (P=0.006) and muscle invasion (P=0.024). Reducing p27 expression score was associated with high grade (p<0.001), clinical stage (P=0.035), and older age > 69 (P=0.05). The link between p27 immunohistochemical expression and histological morphology and sex was not significant.
Conclusion: This paper describes p27 expression in Iraqi bladder cancer patients and shows that p27 is involved in bladder carcinogenesis. p27 immunopostivity was inversely associated to bladder tumour muscle invasiveness and clinical stage, making it a prognostic marker. p27 low score is linked to high tumour stage and grade.

Keywords: P27, A prognostic factor, grading, staging, urothelial, bladder, cancer.

Introduction
Urothelial carcinoma, traditionally known as transitional cell carcinoma, accounts for approximately 90% of primary urinary bladder tumors. The development of this carcinoma is influenced by both genetic and environmental factors, with some factors being modifiable and others not [1]. This type of cancer ranks among the top ten most common cancers globally and in Iraq. According to the 2021 Iraqi Cancer Registry, urothelial carcinoma is the fifth most prevalent cancer overall. Among males, it is the second most common, while it does not appear in the top ten for females. It predominantly affects individuals over 60 years of age, with a higher incidence in males [2]. The most common presentation of urothelial carcinoma is the exophytic form, which includes patterns such as villo-glandular, papillary, or nodular and is the dominant type [3]. Non-muscle invasive bladder cancer (NMIBC) represents the majority of bladder cancer cases, accounting for about 75% [4]. Characterized by a high likelihood of recurrence and progression, NMIBC shows 5-year recurrence rates of 50 to 70% and progression rates of 10 to 30% [4]. Early detection and appropriate treatment are crucial in reducing the risk of recurrence and progression. The management of NMIBC involves transurethral resection of the bladder tumor (TURBT), intravesical chemotherapy and immunotherapy, and ongoing monitoring [4]. However, there is a noTable shortage of Bacillus Calmette-Guérin (BCG) for NMIBC immunotherapy in primary medical facilities [5]. The 2021 guidelines for NMIBC treatment and surveillance in China encompass several aspects, including TURBT, postoperative chemotherapy, BCG immunotherapy, combination treatments, management of carcinoma in situ (CIS), radical cystectomy, and handling recurrences [6]. Aim of the Study The objective of this study is to evaluate the clinical significance of p27 expression in Iraqi patients with urothelial bladder cancer.

Methods

This retrospective cross-sectional study evaluated 53 formalin-fixed, paraffin-embedded tissue blocks from patients diagnosed with urothelial carcinoma (TCC) between 2018 and 2023. The specimens, collected from various medical centers including Al-Hilla Teaching Hospital, Imam AL-Sadiq Teaching Hospital, and private labs, were reassessed for tumor morphology, grade, and invasiveness using hematoxylin and eosin staining (H&E) and immunohistochemical staining for p27. The study was conducted at Babylon University College of Medicine's pathology laboratory from December 2022 to July 2023. The inclusion criteria for the study were broad, covering all age groups, both sexes, all tumor grades and stages, and patients before and after receiving chemotherapy or deep x-ray therapy. Exclusion criteria included incomplete clinicopathological data, non-TCC bladder tumors, and cystectomy cases. For quality control, liver tissue served as a positive control (recommended by the manufacturer's leaflet) and a negative control by omitting the primary antibody. Fifty-three patients, 37 males and 16 females, were included, and their tumor specimens were obtained via transurethral resection of bladder tumors (TURBT). Key equipment used included an Olympus light microscope, Kardelen cotton swabs, AFCO cover slides, a Histo lab microtome, and various other laboratory tools from international sources, illustrating the study's broad equipment base. The primary immunohistochemical marker used was p27 KIP1, detected using a polydetector HRP/ DAB detection system. This system involves multiple steps, including deparaffinization, rehydration, antigen retrieval, and staining, culminating in a 3-step polymeric detection staining method for the immunohistochemical analysis. Specimens were first deparaffinized, rehydrated through graded alcohols, and stained with Harris hematoxylin and eosin. For immunohistochemical staining, slides underwent antigen retrieval, were blocked for peroxidase activity, and then incubated with primary antibodies against p27. This was followed by application of a link solution and a label before visualization using DAB and counterstaining with Mayer's hematoxylin. Scoring of p27 expression was assessed only for nuclear expression under a high-power field (×400), with the criteria for positivity based on the percentage of tumor cells showing nuclear staining. Less than 50% positivity was categorized as low expression, while more than 50% was considered high expression. Cases with less than 10% of positive nuclei were deemed negative [7, 9]. Data analysis was performed using SPSS version 27, with categorical variables presented as frequencies and percentages, and continuous variables as means±SD. Statistical significance was assessed using Student's t-test for continuous variables and Pearson Chi-Square or Fisher's Exact test for categorical variables, with a significance threshold of $p \leq 0.05$. Overall, this study provides comprehensive insights into the clinicopathological characteristics of urothelial carcinoma in an Iraqi cohort, with a specific focus on the expression of the p27 biomarker, which may offer potential diagnostic and prognostic implications.

Results

Distribution of patients with bladder cancer according to age groups including (< 50 years, 51-60 years, 61-70 years and ≥ 71 years). Patients with age (< 50 years) represent (N=6, 11.3%), patients with age (51-60 years) represent (N=11, 20.8%), patients with age (61-70 years) represent less than half of patients (N=23, 43.4%) and patients with age (≥ 71 years) represent (N=13, 24.5%) of total patients. Mean age of patients was (62.70±11.44) older patient was 92 years and younger patient was 36 years. Distribution of patients with bladder cancer according to sex. Male patients represent majority of patients (N=37, 69.8%) and female patients represent only (N=16, 30.2%) of total patients. as shown in Table (2)

Table 1: Distribution of patients with bladder cancer according to age (N=53)

Age (years)	Number	%
< 50 years	6	11.3%
51-60 years	11	20.8%
61-70 years	23	43.4%
≥71 years	13	24.5%
Total	53	100.0%

Table 2: Distribution of patients with bladder cancer according to sex

Sex	Number	%
Male	37	69.8%
Female	16	30.2%
Total	53	100.0%

Distribution of patients with bladder cancer according to clinical staging including (stage 0, stage 1, stage 2, stage 3 and stage 4) and grading. Regarding staging of bladder cancer stage 0 represent (N=9, 17.0%), stage 1 represent (N=20, 37.7%), stage 2 represent (N=16, 30.2%), stage 3 represent five patients (9.4%) and stage 4 represent three patients (5.7%). Regarding grading of bladder cancer patients with high grade represent (N=30, 56.6%) and those patients with low grade represent (N=23, 43.4%). As in Table 3.

Table 3: Distribution of patients with bladder cancer according to clinical staging and histological grade of cancer (N=53)

Study variables	Number	%
Clinical Stage of bladder cancer		
Stage 0	9	17.0%
Stage 1	20	37.7%
Stage 2	16	30.2%
Stage 3	5	9.4%
Stage 4	3	5.7%
Total	53	100.0%
Grade of bladder cancer		
High	30	56.6%
Low	23	43.4%
Total	53	100.0%

Distribution of patients with bladder cancer according to immunohistochemical study of p27 marker and the patients with positive P27 nuclear expression score. Positive P27 marker represents 31 patients (58.5%) and patients with negative P27 marker represent 22 patients (41.5%). For those with positive P27marker, high P27 nuclear expression score represent 21 patients (67.7%) and those with low P27 nuclear expression score represent only 10 patients (32.3%). Figure (1) represent positive stain (high score) X400 and Figure (2-4) represent positive stain (low score) X400 and in Table 4.

Table 4: Distribution of patients with bladder cancer according to immunohistochemical study of p27 marker and the patients with positive P27 nuclear expression score.

Study variables	Number	%
P27 Immunohistochemically marker		
Positive	31	58.5%
Negative	22	41.5%
Total	53	100.0%
P27 Nuclear expression score		
High	21	67.7%
Low	10	32.3%
Total	31	100.0%

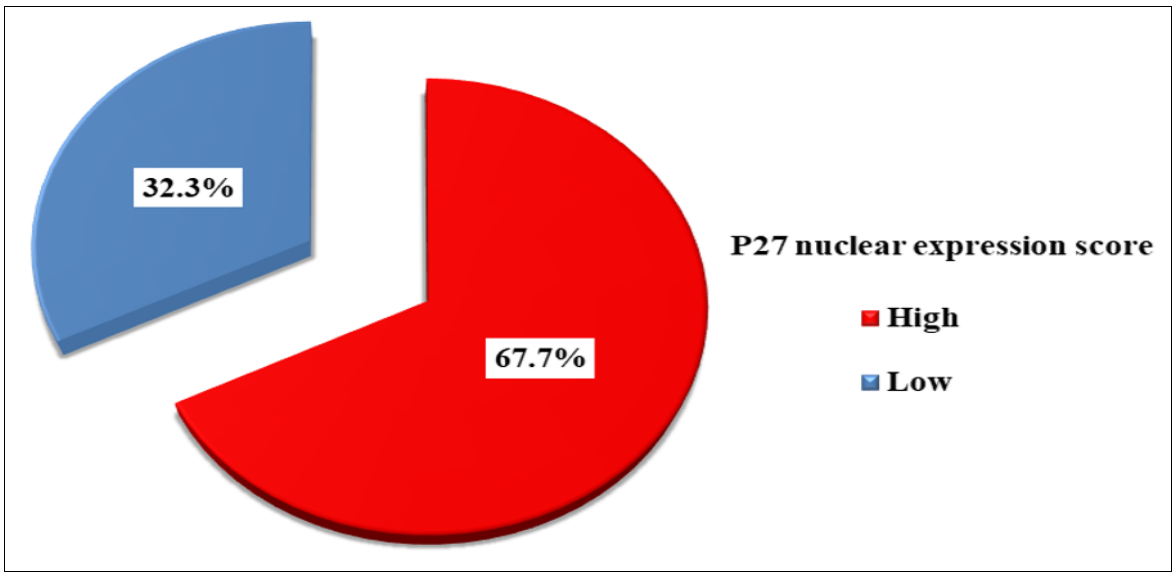

Fig 1: Distribution of patients with bladder cancer according to P 27 nuclear expression score (N=31).

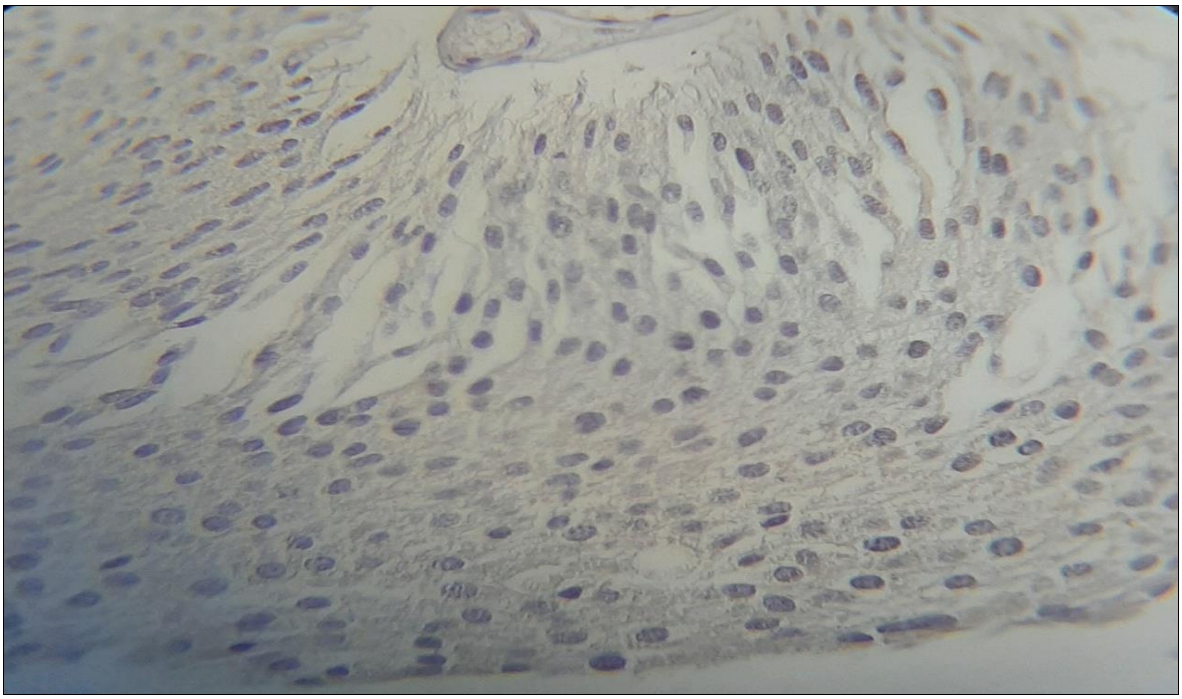

Fig 2: Urothelium with negative p27 expression (x400)

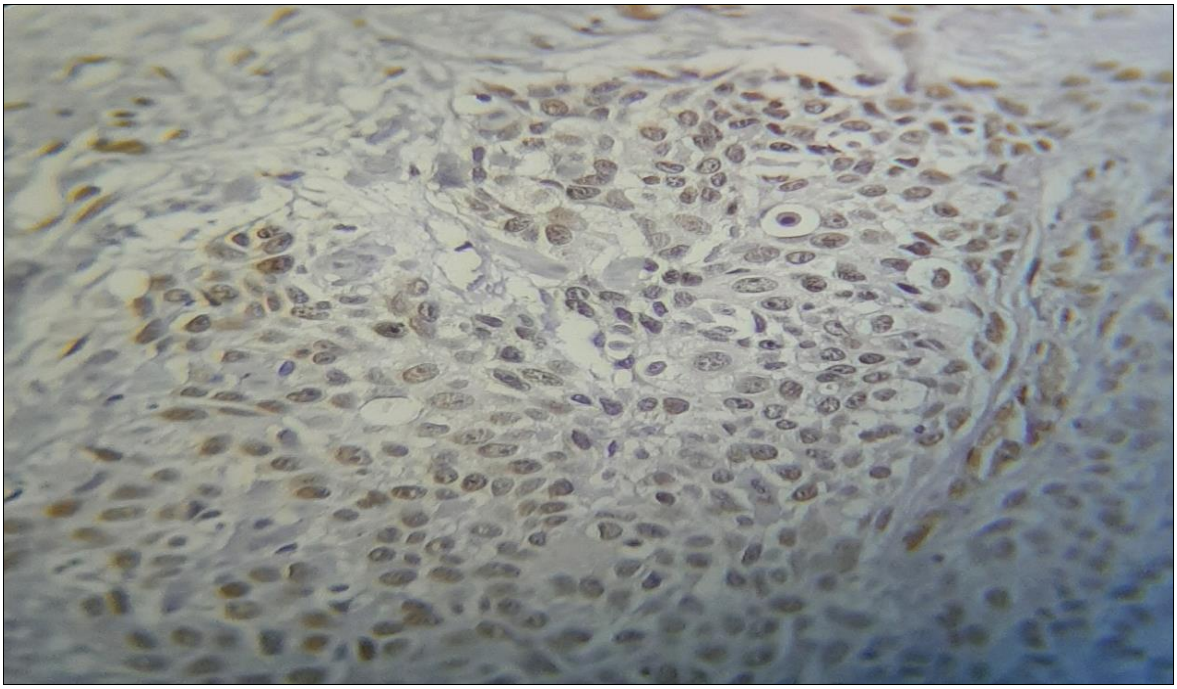

Fig 3: Positive p27 staining with low score (<50% of tumour field are stained with moderate intensity) (X400)

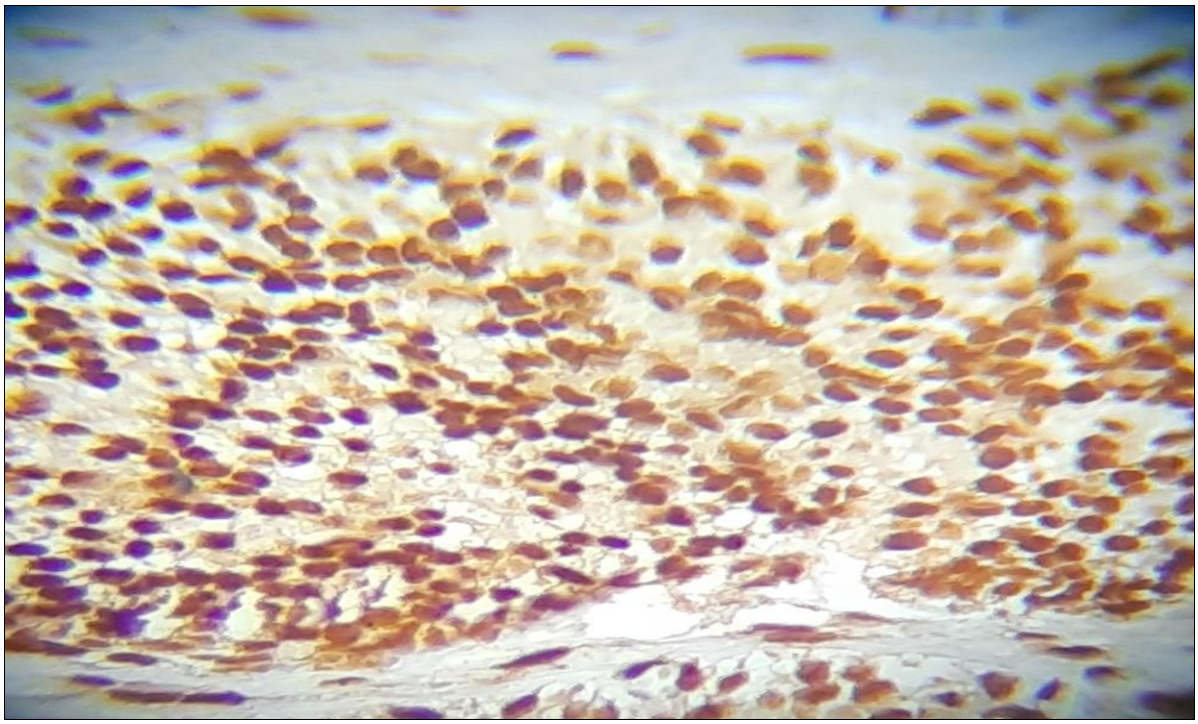

Fig 4: Positive p27 staining with high score (>50% of tumour field are stained with strong intensity), (X400)

The association between P27 immunohistochemically marker and clinical stage of bladder cancer including (stage 0, stage 1, stage 2, stage 3 and stage 4) and association between P27immunohistochemical marker and histological grade of bladder cancer. There was significant association between P27 immunopostivity and clinical stage of bladder cancer. As shown in Table 5.

Table 5: The association between P27immunohistochemical marker and clinical stage and histological grade of bladder cancer (N=53)

Study variables	P27 Immunohistochemically marker		Total (N=53)	P-Value
	Positive (N=31)	Negative (N=22)		
Clinical Stage of bladder caner				
Stage 0	9 (29.0)	0 (0.0)	9 (17.0)	0.006
Stage 1	13 (41.9)	7 (31.8)	20 (37.7)	
Stage 2	5 (16.2)	11 (50.0)	16 (30.2)	
Stage 3	3 (9.7)	2 (9.1)	5 (9.4)	
Stage 4	1 (3.2)	2 (9.1)	3 (5.7)	

Total	31 (100.0)	22 (100.0)	53 (100.0)	
Grade of bladder cancer				
High	15 (48.4)	15 (68.2)	30 (56.6)	0.152
Low	16 (51.6)	7 (31.8)	23 (43.4)	
Total	31 (100.0)	22 (100.0)	53 (100.0)	

The association between P27 nuclear expression score and clinical stage of bladder cancer including (stage 0, stage 1, stage 2, stage 3 and stage 4) and association between P27 nuclear expression score including (high and low) and histological grade of bladder cancer including (high and low) among patients with positive P 27 immunopositivity. There was significant association between P27 nuclear expression score and clinical stage and histological grade of bladder cancer. As in Table 6

Table 6: The association between P27 nuclear expression score and clinical stage and histological grade of bladder cancer (N=31)

Study variables	P27 nuclear expression score		Total (N=31)	P-Value
	High (N=21)	Low (N=10)		
Clinical Stage of bladder caner				
Stage 0	8 (38.1)	1 (10.0)	9 (29.0)	0.035
Stage 1	10 (47.5)	3 (30.0)	13 (41.9)	
Stage 2	1 (4.8)	4 (40.0)	5 (16.2)	
Stage 3	1 (4.8)	2 (20.0)	3 (9.7)	
Stage 4	1 (4.8)	0 (0.0)	1 (3.2)	
Total	21 (100.0)	10 (100.0)	31 (100.0)	
Grade of bladder cancer				
High	5 (23.8)	10 (100.0)	15 (48.4)	<0.001
Low	16 (76.2)	0 (0.0)	16 (51.6)	
Total	21 (100.0)	10 (100.0)	31 (100.0)	

Discussion

This retrospective cross-sectional study analyzed 53 Iraqi patients with urothelial carcinoma (UC), focusing on the expression of p27, a key regulator in the G1-S transition of the cell cycle, known to correlate with tumor grading and staging. The study aimed to evaluate p27 as a prognostic marker in the context of UC's clinical and histopathological parameters. The specimens were graded and staged according to the WHO 2022 classification and AJCC/ISUP 2016 version, respectively, and p27 expression was assessed immunohistochemically. The expression of p27 was found in 41.5% of the cases, aligning with findings from similar studies, where p27 expression varied between 43.3% to 83.4% in UC cases. These variations could be attributed to differences in the type of specimens (e.g., recurrent or progressing UCs) and sample sizes [4, 7-9]. Age distribution in the study ranged from 36 to 92 years, with a mean of 62.70±11.44 years, predominantly in the sixth decade. The relationship between p27 expression and age showed significant differences, contrasting with other studies where no significant correlation was found [7, 9, 10]. Sex distribution analysis revealed no significant correlation between p27 expression and gender, consistent with other findings, suggesting that p27 expression is more closely related to tumor stage and behavior rather than gender [7, 9]. Histologically, 96.2% of the UC cases were papillary, and the correlation between tumor morphology and p27 expression was not statistically significant, aligning with other studies [7, 11, 12]. Regarding tumor grade, 56.6% of the cases were high-grade UC. There was no significant statistical correlation between p27 expression and tumor grade, which contrasts with other studies where a significant correlation was noted [7, 8]. This study found an inverse relationship between low p27 expression and high-grade tumors, similar to findings in other populations [9]. Clinically, p27 expression varied significantly with tumor stage. Stages 0 and 1, considered superficial bladder cancers, showed higher p27 positivity compared to stages 2, 3, and 4, which are considered deeper invasions. This significant association between p27 expression and early-stage tumors suggests its potential as a prognostic marker for less aggressive disease stages [7-9, 13]. The muscle invasion status of bladder cancer revealed that non-muscle invasive bladder tumors (NMIBT) had a higher p27 expression score compared to muscle invasive bladder tumors (MIBT), although the difference was not statistically significant in this study. This finding aligns with the clinical significance noted in other studies, suggesting that higher p27 positivity is associated with non-muscle invasive status [7, 9]. Overall, this study supports the potential of p27 as a prognostic marker in UC, indicating its relevance in predicting tumor behavior based on histopathological and clinical stages. Further studies with larger sample sizes could provide more definitive insights into the role of p27 in UC management and therapy decision-making.

Conclusion

P27 immunopositivity was significantly inversely related to muscle invasiveness of bladder tumor and clinical stage so p27 can be used as prognostic marker, while p27 low score is associated with high clinical stage tumors and grade.

Conflict of Interest:
Not available

Financial Support:
Not available

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