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# Assessment of survivin and p27 expression as potential prognostic markers in urothelial cell carcinoma of urinary bladder in Egyptian patients

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## Abstract

**Background:** Bladder cancer (BC) is the tenth most common cancer worldwide with urothelial carcinoma (UC) being the main histologic subtype. Survivin is an apoptosis inhibitor that is associated with tumor proliferation and invasion. P27 is a cyclin-dependent kinase inhibitor that negatively regulates cell proliferation. The expression of both proteins was variable among different solid tumors including UC.

**Methods:** We aimed to investigate the expression of survivin and P27 in UC of urinary bladder and correlate their expressions with histopathological parameters in an attempt at studying the possibility of their use as targeted therapies. The investigation was performed through immunohistochemical staining for both proteins on sections belonging to 60 UCs and 12 mild chronic cystitis cases (controls). Immunopositivity (number of positive cases) and expression score (percentage of positive urothelial cells) were evaluated.

**Results:** Both survivin and P27 were absent in urothelial cells of mild chronic cystitis lesions while expressed in 60% and 43.3% of UCs, respectively. High score of survivin and low score of P27 were associated with poor prognostic factors of UC (solid pattern, high grade, and deep tumors). By logistic regression test, survivin expression can be a predictive risk factor associated with solid pattern and high-grade UC, while P27 expression can be a predictive risk factor associated low-grade UC.

**Conclusion:** High survivin and low P27 expression scores were associated with the studied prognostic factors of UC. Both proteins may play a role in UC progression and can have a value as prognostic and/or diagnostic markers of UC, as well as targeted therapies.

**Keywords:** Survivin, p27, Urothelial carcinoma, Papillary, Grade, Stage

## 1 Background

Bladder cancer (BC) is the tenth most frequent cancer globally and is the eighth leading cause of cancer-related deaths, being the fourth most prevalent cancer in men and the twelfth most frequent cancer in women, with mortality rates almost four times higher in men than

in women [1]. In Egypt, it is the fourth most common cancer and the fifth cause of cancer-related deaths [2]. Urothelial carcinoma (UC) is considered the most common histologic subtype of BC [3].

Clinicopathological variables, such as grading and staging systems, have long been recognized as prognostic factors in UC patients; however, more recent insights into molecular pathogenesis of UC had identified potential markers that can predict the progression and behavior of UC. It was observed that UCs of the same grade and

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stage can behave differently, in addition to that, there is variability in the likelihood of progression, recurrence, and survival [4].

Survival, growth, and proliferation of malignant cells are affected by changes in both pro-apoptotic and anti-apoptotic signaling pathways. Survivin belongs to the family of inhibitors of apoptosis proteins (IAPs) that plays a pivotal role in mitotic progression, apoptosis inhibition, angiogenesis, progression of cancer, and invasion [5]. Survivin gene is expressed during embryonic life but not in terminally differentiated adult tissues. It is expressed in cancer cells during the G2/M phase of the cell cycle and counteracts apoptosis activation during mitosis by interfering with caspase activity [6].

The interactions of cyclins, cyclin-dependent kinases (CDKs), and their inhibitors regulate cell cycle progression. P27 is a cyclin-dependent kinase inhibitor (CKI) that controls protein synthesis during the cell cycle. In a quiescent state, it negatively regulates cell proliferation through the inhibition of the G1 to S phase transition to allow repair of DNA damages [7]. P27 is inactivated in cancer cells by various mechanisms including impaired synthesis, accelerated degradation, and mislocalization. Its expression in UC is contradictory, as it can exhibit reduced or increased expression and distinct nuclear or cytoplasmic location [8].

In the present study, we investigated the significance of survivin and P27 expressions in UC of the urinary bladder, by correlating their expression with clinicopathological features, including tumor pattern (papillary or solid), grade of differentiation, and stage of invasion (non-muscle or muscle-invasive).

## 2 Methods

### 2.1 Study design

This retrospective study enrolled sixty specimens of UC that were selected from archival blocks at the Pathology Department, Theodor Bilharz Research Institute in the period from 2020 to 2021. Specimens were obtained either as radical cystectomy or transurethral bladder tumor resections. In all cases, we took only samples with histologically identifiable detrusor muscle. None of the patients had received radiation or chemotherapy before sampling.

### 2.2 Histopathological technique and evaluation

One paraffin-embedded block was selected from each case and cut into 4  $\mu$ m sections, then stained by hematoxylin and eosin (H&E) stain for routine histopathological examination and diagnosis.

The histological diagnoses, grading, and staging of UCs were based on WHO criteria. UCs were divided according to the grade of differentiation into low (GI-II) and

high (GIII) grades, and divided according to the stage of invasion into superficial, non-muscle invasive tumors (Ta, T1) and deep, muscle-invasive tumors (T2-T4) [9]. In addition; 12 specimens with mild chronic cystitis were taken as controls.

The inclusion criteria include cases of primary UC and with properly recorded clinical, radiological, and operative data in their files. Exclusion criteria were cases of non-urothelial malignancy or those with inadequate data in their files.

### 2.3 Immunohistochemical (IHC) technique

IHC was performed on the formalin fixed-paraffin embedded tissues [10]. The tissue sections were put in the oven at 60 °C for 4 h, deparaffinized in xylene, and rehydrated in a graded ethanol series. Antigen retrieval was performed with 10 ml sodium citrate buffer, pH 6.0, at 90 °C for 30 min. Sections were incubated in 0.03% hydrogen peroxide (EnVision/HRP, Dako) for 10 min at room temperature, to remove endogenous peroxidase activity, then were rinsed in wash buffer. The sections were incubated with survivin antibody (code M3624, Dako, Copenhagen, Denmark) at a dilution of 1:100, and P27 antibody (F-8): sc-1641, Santa Cruz Biotechnology, CA, USA) overnight at 4 °C. Sections were then washed three times for 5 min in PBS. Slides were rinsed in wash buffer and incubated for 30 min in blocking serum (0.04% bovine serum albumin, A2153, Sigma-Aldrich, Shanghai, China, and 0.5% normal goat serum X0907 (EnVision/HRP, Dako)). The chromogenic reaction was carried out with 3,3'-diaminobenzidine chromogen solution for 10 min. Finally, sections were counterstained with hematoxylin, dehydrated with graded ethanols, and mounted.

For each setting; positive and negative controls were routinely used. Duodenal tissue was used as a positive control for survivin, while lymphocytes in the used section served as an internal control for P27. Negative controls were carried out in which phosphate-buffered saline was used instead of the primary antibody.

### 2.4 Scoring and data analysis

The tissue sections were examined by using light microscope (Scope A1, Axio, Zeiss, Germany). Photomicrographs were taken using a microscope camera (AxioCam, MRc5, Zeiss, Germany).

Scoring of survivin and P27 immunostaining was performed in a blind fashion to the patients' clinicopathological data using  $\times 40$  objective.

Both immunopositivity (number of positive cases) and expression score (percentage of positive urothelial cells) were evaluated. Expression in  $>10\%$  of urothelial cells was considered positive. Positive expression in 10–50%

of urothelial cells = low score, expression in  $\geq 50\%$  of urothelial cells = high score [11].

## 2.5 Statistical analysis

Analyses were performed using SPSS version 23 (IBM Corp., Armonk, New York, USA). The significance of differences in means was calculated using t-test. Fischer's exact test was used to assess the significance of differences between clinicopathological variables. Logistic regression test was performed to identify prognostic factors in relation to studied clinicopathological variables. Differences were considered statistically significant whenever  $p < 0.05$ .

## 3 Results

Our study sample included 60 specimens of UC belonging to 54 males and 6 females. The majority of them had papillary architecture (55%) and were high-grade (61.6%), whereas half of them were superficial. The mean age of UC patients was  $62.47 \pm 10.85$  years, meanwhile, the mean age of controls was  $52.75 \pm 14.34$  years with a significant difference between them ( $p < 0.01$ ).

### 3.1 Survivin immunoreactivity

Positive survivin immunoreactivity was detected as nuclear staining. Table 1 shows that survivin expression was not detected in chronic cystitis cases, while 36/60 (60%) of the studied UCs showed expression.

Survivin immunopositivity was significantly more frequent in tumors with solid growth pattern (92.6%), high-grade of differentiation (83.8%), and deep tumors (80%)

compared to papillary tumors (33.3%), low-grade (21.7%), and superficial tumors (40%), respectively.

High and low expression scores were seen in 64 and 36% of survivin-positive UCs. High expression score was significantly higher in solid tumors (80%) and deep tumors (79.2%) compared to papillary tumors (27.3%) and superficial ones (33.3%). Also, high score was more prevalent in high-grade tumors (67.7%) compared to low-grade ones (40%) without significant difference.

Furthermore, all papillary, low-grade, and superficial UCs had low expression scores (Fig. 1).

Regarding patients' sex, survivin was detected in 35/60 (64.8%) of UCs in males, 23 of them (65.7%) were of high expression score and the remainder were of low score. Only 1/6 (16.7%) of females was positive for survivin with low expression score.

### 3.2 P27 immunoreactivity

P27 immunoreactivity was confirmed in 26/60 (43.3%) of UCs which was expressed as nuclear and cytoplasmic staining of UCs cells. However, in chronic cystitis cases, P27 was detected only in scattered nuclei of lymphocytes.

Table 2 shows that P27 immunopositivity was observed in 45.5% of papillary tumors compared to 40.7% of solid ones, without statistical significance. P27 was significantly more frequent in low-grade tumors (74%) and superficial tumors (63.3%) compared to high-grade (24.3%) and deep tumors (23.3%), respectively.

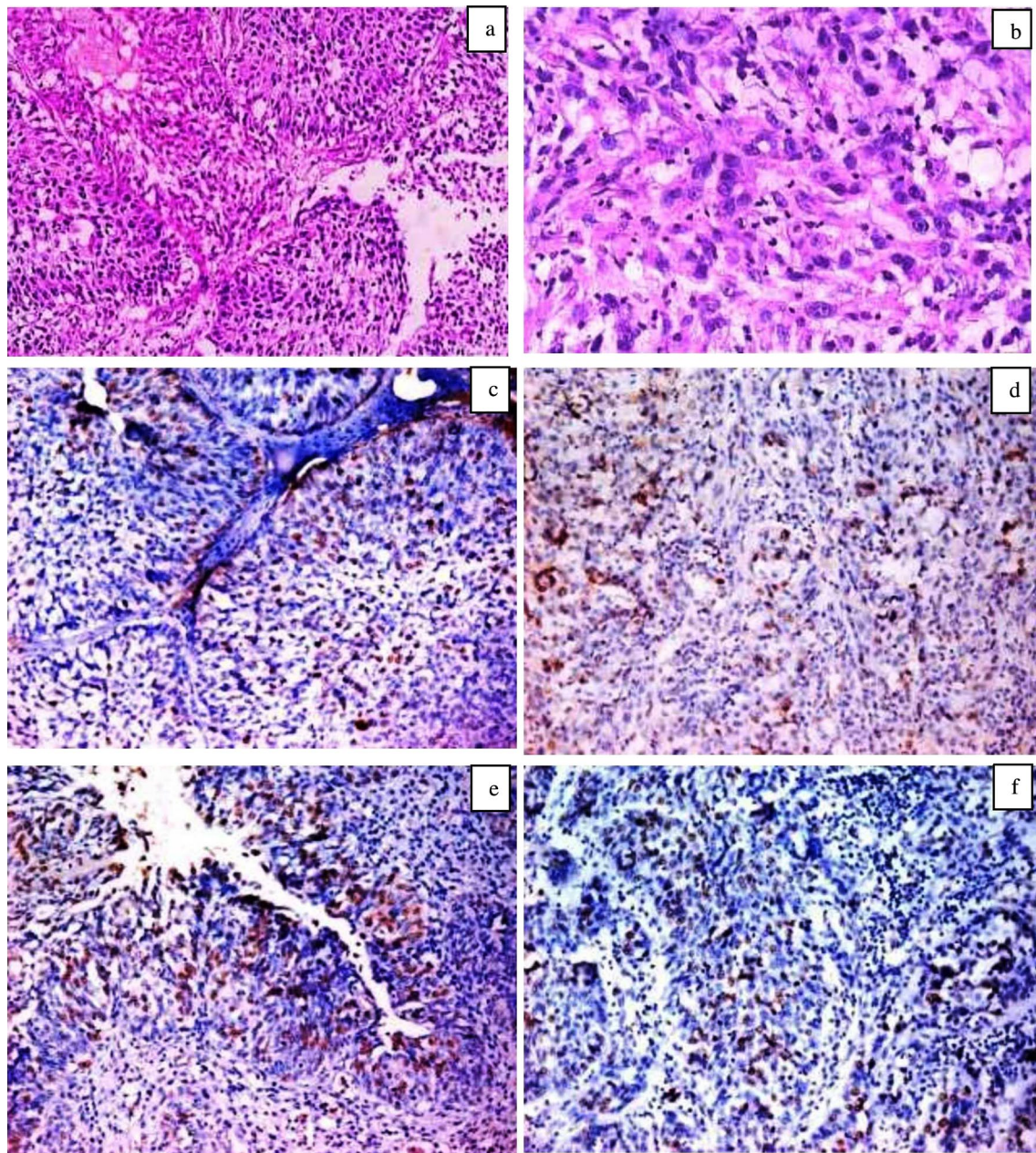
High and low expression scores were seen in 27% and 73% of P27-positive UCs, respectively. High score was predominant in papillary (64.7%), low-grade (64.7%), and

**Table 1** Relation between survivin immunoreactivity and clinicopathological variables of the studied cases

Item (n)	Survivin immunopositivity		p-value	Survivin expression score		p-value
	Positive n (%)	Negative n (%)		Low score n (%)	High score n (%)	
Mild chronic cystitis (12)	0	12 (100)	<0.001	0	0	<0.001
Urothelial carcinoma (60)	36 (60)	24 (40)		13 (36)	23 (64)	
Age	< 60 years (21)	12 (57)	0.476	9 (75)	3 (25)	<0.001
	$\geq 60$ years (39)	24 (61.5)		4 (16.7)	20 (83.3)	
Sex	Male (54)	35 (64.8)	0.033	12 (34.3)	23 (65.7)	0.361
	Female (6)	1 (16.7)		1 (100)	0	
Pattern	Papillary (33)	11 (33.3)	<0.001	8 (72.7)	3 (27.3)	0.004
	Solid (27)	25 (92.6)		5 (20)	20 (80)	
Grade	Low (23)	5 (21.7)	<0.001	3 (60)	2 (40)	0.239
	High (37)	31 (83.8)		10 (32.3)	21 (67.7)	
Stage	Superficial (30)	12 (40)	0.002	8 (66.7)	4 (33.3)	0.01
	Deep (30)	24 (80)		5 (20.8)	19 (79.2)	

NMIBC Non-muscle invasive bladder cancer, MIBC Muscle-invasive bladder cancer, n Number, % Percentage





**Fig. 1** Hematoxylin and eosin staining of urothelial carcinoma (**a, b**) and immunohistochemical expression of survivin (**c, d**) and P27 (**e, f**). **a** Low-grade papillary urothelial carcinoma ( $\times 200$ ), **b** High-grade urothelial carcinoma ( $\times 400$ ), **c** Low-grade papillary urothelial carcinoma with low survivin score, nuclear expression ( $\times 200$ ), **d** High-grade solid urothelial carcinoma with high survivin score, nuclear expression ( $\times 200$ ), **e** Low-grade papillary urothelial carcinoma with high P27 score, nuclear expression ( $\times 200$ ), **f** High-grade urothelial carcinoma with high P27 score, cytoplasmic expression ( $\times 200$ )

superficial (58%) tumors, whereas all solid, high-grade, and deep UCs have low expression scores (Fig. 1).

By considering patients' sex, P27 was detected in 24/54 (44.4%) of UCs in males, 17 of them (70.8%) were of low

score and the remainder 7/24 (29.2%) were of high score, while P27 was detected in 33.3% of females with UCs and were of low score. These relations did not achieve a statistical significance.

**Table 2** Relation between p27 immunoreactivity and clinicopathological variables of the studied cases

Item (n.)	P27 immunopositivity		p-value	P27 expression score		p-value
	Positive n (%)	Negative n (%)		Low score n (%)	High score n (%)	
Mild chronic cystitis (20)	0	12 (100)	0.01	0	0	< 0.001
Urothelial carcinoma (60)	26 (43.3)	34 (56.7)		19 (73)	7 (27)	
Age						
< 60 years (21)	13 (62)	8 (38)	0.032	11 (84.6)	2 (15.4)	0.500
≥ 60 years (39)	13 (33.3)	26 (66.7)		8 (61.5)	5 (38.5)	
Sex						
Male (54)	24 (44.4)	30 (55.6)	0.472	18 (75)	6 (25)	0.526
Female (6)	2 (33.3)	4 (66.7)		2 (100)	0	
Pattern						
Papillary (33)	15 (45.5)	18 (54.5)	0.459	4 (26.7)	11 (64.7)	< 0.001
Solid (27)	11 (40.7)	16 (59.3)		11 (100)	0	
Grade						
Low (23)	17 (74)	6 (26)	< 0.001	6 (35.3)	11 (64.7)	0.002
High (37)	9 (24.3)	28 (75.7)		9 (100)	0	
Stage						
Superficial (30)	19 (63.3)	11 (36.7)	0.002	8 (42)	11 (58)	0.01
Deep (30)	7 (23.3)	23 (76.7)		7 (100)	0	

NMIBC Non-muscle invasive bladder cancer, MIBC Muscle-invasive bladder cancer, n Number, % Percentage

**Table 3** Pattern of survivin and p27 staining in urothelial carcinomas

Pattern of staining in UCs	n (%)
Survivin <sup>+</sup> /p27 <sup>+</sup>	12 (20)
Survivin <sup>+</sup> /p27 <sup>-</sup>	24 (40)
Survivin <sup>-</sup> /p27 <sup>+</sup>	14 (23.3)
Survivin <sup>-</sup> /p27 <sup>-</sup>	10 (16.7)
Total	60 (100)

UC Urothelial carcinoma, n Number, % Percentage

### 3.3 Correlations between survivin and P27 immunoreactivity with clinicopathological variables of the urothelial carcinomas

Coexpression of survivin and P27 was seen in 12/60 (20%) of UCs. Seven out of these 12 cases (58.5%) showed high score of survivin/low score of P27, meanwhile, both proteins were negative in 10/60 (16.7%) of UCs (Table 3). A significant negative correlation was found between survivin and P27 scores ( $r = -0.336$ ,  $p = 0.009$ ).

According to Logistic regression test (Table 4); survivin expression showed a significant correlation with solid pattern and high-grade UC, while P27 expression

**Table 4** Analysis of risk clinicopathological variables of urothelial carcinoma by Logistic regression test

Clinicopathological variables of UC		B	S.E	Wald	df	Sig	Exp(B)	95% CI for EXP(B)	
								Lower	Upper
Papillary pattern	Survivin expression	2.750	0.986	7.781	1	0.005	15.640	2.265	107.988
	P27 expression	-0.192	0.525	0.134	1	0.714	0.825	0.295	2.309
Grade	Survivin expression	2.471	1.076	5.273	1	0.022	11.839	1.436	97.583
	P27 expression	-2.176	0.610	12.723	1	0.000	0.113	0.034	0.375
Stage	Survivin expression	1.010	0.921	1.204	1	0.272	2.747	0.452	16.692
	P27 expression	-1.321	0.883	2.239	1	0.135	0.267	0.047	1.506
Age	Survivin expression	-2.055	1.122	3.355	1	0.067	0.128	0.014	1.155
	P27 expression	-0.587	0.823	0.509	1	0.475	0.556	0.111	2.788
Sex	Survivin expression	0.278	1.376	0.041	1	0.840	1.320	0.089	19.593
	P27 expression	-2.773	1.183	5.491	1	0.019	0.062	0.006	0.635
Survivin expression	P27 expression	1.030	0.544	3.576	1	0.053	2.800	0.963	8.139

UC Urothelial carcinomas; B Coefficient of regression; SE Standard error; Wald, statistic of Wald test coefficient; df Degree of freedom; Sig P-value; Exp(B), odds ratio; 95% CI, 95% confidence interval



was associated with low-grade UC. Moreover, survivin expression was associated with P27 expression.

#### 4 Discussion

Infiltrating UC is the most common variant of urinary bladder cancer. Muscle-infiltrating tumors affect around 25–30% of UC patients, with a serious risk for further progression, metastasis, and death [12].

Although grading and staging systems are the most reliable predictors of bladder cancer development, they are insufficient to predict the course of most invasive tumors [13]. Furthermore, conventional therapies, such as chemotherapy or radiation, are only effective in a minority of individuals due to disease heterogeneity. In this study, we examined the expression patterns of survivin and P27 proteins seeking to find prognostic markers that may be employed in the clinical setting.

Since the discovery of survivin more than two decades ago, its use as a target for cancer therapeutics has been a major goal in cancer research. This is because it is not detected in normal adult tissues [14]. In BC, survivin expression was shown to be an indicator of poor prognosis in some studies, but not in others [15].

In the current study, survivin was not detected in urothelial cells of the mild chronic cystitis lesions, but it was detected in UC cells. We detect survivin positivity in 60% of UCs, although Makboul et al. [16] and Arafat et al. [17] reported positivity in a slightly higher percentage (78 and 71%, respectively). Although each of these studies was conducted on Egyptian patients, however, higher percentage may be related to use of different antibody clones.

We found that the difference in survivin immunopositivity between solid and papillary UCs, low and high grades, as well as superficial and deep UCs was significant. Furthermore, high score of survivin was associated with solid pattern, high-grade and deep tumors with no significant difference between them and their counterparts. Our findings matched those of Arafat et al. [17] and Stec et al. [18]. These findings can reflect the role of survivin in the differentiation and invasiveness of UC and indicate the association between survivin expression with a poor prognosis in patients with UC. Other studies by Srivastava et al. [19] and Lv et al. [20] did not find a statistically significant relationship between survivin immunopositivity and grade or stage of UCs. These conflicting findings indicate the need for more study in this area.

In our study, no P27 expression was detected in the urothelial cells of the mild chronic cystitis lesions, the same finding was reported by Al-Fakhar et al. [21]. This can be explained as P27 is high during quiescence and low during S phase, as cells start to proliferate, so with

presence of cystitis; levels of P27 can be decreased or absent.

We found that P27 was positive in 43.3% of UCs, which was close to that reported by Al-Fakhar et al. [21] (50%), while Kami et al. [22] and Kapur et al. [23] reported a greater percentage (83.4 and 71%, respectively). This higher percentage can be attributed to type of specimens examined in both studies, being recurrent or progressing UCs, and adenocarcinomas, respectively.

Papillary, low-grade, and superficial UCs had higher P27 positivity than their counterparts. Furthermore, more than half of papillary, low-grade, and superficial UCs had high P27 scores, whereas all solid pattern, high-grade, and deep stage UCs had low P27 scores. Thus, there was an association between P27 expression and better prognostic factors in UC patients, while its lack was linked to poor prognostic factors. Our findings confirm the results of previous studies [24, 25]. Contrary to our findings, Santos et al. [26] and Doganay et al. [27] found high P27 levels in some highly aggressive bladder carcinoma cases and concluded that there was no correlation between P27 and pathological data in UC of the bladder. These conflicting findings were attributed to the fact that P27 tumor suppressor function is mediated through its association with the cyclin E/CDK2 complex, which inhibits the G1 to S phase transition during the cell cycle. When low molecular weight cyclin E isoforms (which are resistant to P27 inhibition) are overexpressed, P27 can no longer function as a CDK inhibitor [21]. As a result, P27 function can be altered by its interactions with other proteins during the cell cycle.

In this study, we detected P27 as nuclear or cytoplasmic expression. Papillary, low-grade, and superficial UCs exhibited nuclear localization, whereas solid, high-grade, and deep UCs exhibited cytoplasmic expression. According to Serres et al. [28], the cytoplasmic location of P27 prevents it from binding to and inhibiting nuclear cyclin-CDK targets, contributing to tumor development through collaboration with other genes including the Ras oncogene. This can explain why cytoplasmic expression was more common in high-grade and deep UCs in our study.

In the current study, 20% of UC patients co-expressed survivin and P27, allowing them to benefit from targeted therapies targeting both proteins, whereas 16.7% of UC patients were negative for both proteins, preventing them from benefiting from such therapies. Survivin score had a significant positive correlation with UC grade and stage of UCs, while P27 score had a significant negative correlation with them. Similar to our results, Chen et al. [29] found a close relation between survivin immunopositivity and UC grade and stage, and

Yang et al. [30] found a significant negative correlation between P27 score with UC grade and stage.

Data in our study revealed that high survivin scores and low P27 scores dominate in elderly patients ( $\geq 60$  years). Age can affect the immune response against a therapeutic protein and the pharmacokinetic response to drugs [31]. According to Johnson et al. [32]. Within precision medicine, age must be considered for therapies to be tailored to a person's specific genetic and molecular composition.

Our work has the advantage of demonstrating survivin and P27 expressions in the same patients with UC, which might lead to more significant results; nevertheless, the study was limited by the small number of cases investigated.

## 5 Conclusion

Both survivin and P27 were upregulated in UCs but not in mild chronic cystitis lesions. High score of survivin and low score of P27 predominate in UCs with poor prognostic factors (solid pattern, high grade, and deep tumors), while low score of survivin and high score of P27 predominate in UCs with better prognostic factors (papillary, low-grade, and superficial UCs). High survivin score and low P27 score dominate in elderly cases ( $\geq 60$  years). By logistic regression test, survivin expression is a predictive risk factor associated with solid pattern and high-grade UC, while P27 expression is a predictive risk factor associated low-grade UC.

Thus, survivin and P27 can be useful prognostic and/or diagnostic tumor markers that can be used to predict UC progression in conjunction with traditional grading and staging systems, as well as may be employed as targeted therapies.

## Abbreviations

BC: Bladder cancer; UC: Urothelial carcinoma; IAPs: Inhibitors of apoptosis proteins; CDKs: Cyclin-dependent kinases; CKI: Cyclin-dependent kinase inhibitor; IHC: Immunohistochemical.

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## Author contributions

NSH interpreted all data, conceived, designed, and wrote the manuscript, ZO designed the structure and drafted the manuscript, and MM designed and critically revised the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

The protocol of this study was approved by the Institutional Review Board (IRB) of Theodor Bilharz Research Institute, for the protection of human subjects and adopted by the 18th world medical assembly, Helsinki, Finland (2013). The IRB waived the need for informed consent from the participants because the study was performed on stored archival tissue blocks. Personal information of these blocks' owners was anonymous and cannot reasonably be used by anyone.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no conflict of interest.

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