

# Diagnosis of Bladder Cancer by Urine Survivin, an Inhibitor of Apoptosis

## A Preliminary Report

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**Introduction:** Survivin is an inhibitor of apoptosis that is expressed in undifferentiated tissues like tumors. Detection of survivin in urine has been proposed as a diagnostic marker for bladder cancer. We evaluated the urine samples of patients with bladder cancer for survivin and compared them with healthy controls.

**Materials and Methods:** The urine specimens of 20 patients with transitional cell carcinoma (TCC) of the bladder (group 1) and 18 controls without cancer (group 2) were collected before cystoscopy and assessed for survivin by reverse transcriptase polymerase chain reaction.

**Results:** All patients except 1 in group 1 were men. Urine specimens were positive for survivin in 18 (90%) and 9 (50%) patients of groups 1 and 2, respectively ( $P = .007$ ). Sixteen patients with TCC had urine cytology, of which 6 (37.5%) were positive. Urine survivin was positive in all 10 patients with negative cytology. Nine patients in this group had low-grade tumors.

**Conclusion:** Urine survivin seems to have a higher sensitivity than urine cytology, especially in low-grade bladder cancer. The quantitative measurement of survivin in urine by advanced techniques may provide a better diagnostic and prognostic tool. However, the clinical use of survivin and its association with different stages and grades of TCC still requires more studies.

Keywords: transitional cell carcinoma, bladder, survivin, tumor marker, cytology, urine

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## INTRODUCTION

Although we lack a precise statistic, it can be estimated that bladder cancer is the most common urogenital cancer among the Iranian men. Early diagnosis is crucial and patients with bladder cancer must be followed every 3 to 6 month. The current modalities for diagnosis and follow-up of the bladder cancer are cystoscopy and urine cytology; one an invasive high-cost method and the other a low-sensitive and operator dependent method.<sup>(1)</sup> To date, many tumor markers have been proposed for bladder cancer, but none have

had the potential of surpassing the conventional methods. Bladder tumor antigen (BTA), nuclear matrix protein-22, telomerase, and hyaluronic acid hyaluronidase are some of the markers which were proposed.<sup>(2,3)</sup> Suboptimal diagnostic accuracy, technology dependency, and high costs have made them abandoned. An ideal bladder tumor marker requires being feasible and easily and rapidly interpreted, like the prostate-specific antigen in the prostate cancer.

Apoptosis is a genetic cell death program regulated by 3 families of proteins: B-cell leukemia/lymphoma 2

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(BCL2), FLICE-like inhibitory proteins (FLIPs), and inhibitor of apoptosis proteins (IAPs).<sup>(1,4)</sup> The latter consists of 9 apoptosis regulating proteins, one of which is survivin.<sup>(5)</sup> Survivin was first discovered by Ambrosini in 1997.<sup>(5)</sup> It is a 16.5-kd protein expressed by the *BIRC5* gene at chromosome 17q25.<sup>(5)</sup> Survivin is expressed during embryonic and fetal periods, but it is undetectable in normal adult tissue.<sup>(5)</sup> It has been shown that survivin is overexpressed in tumoral tissue, reportedly in neuroblastoma, B-cell lymphomas, and the lung, breast, colon, esophagus, and bladder cancers.<sup>(6-12)</sup> Smith and colleague first described urine survivin as a marker of bladder cancer in 2001.<sup>(13)</sup> The subsequent reports, however, have indicated varying diagnostic accuracies.<sup>(14)</sup> In this study, we evaluated the accuracy of urine survivin in diagnosing the bladder cancer.

## MATERIALS AND METHODS

### Patient Selection

In a case-control study, we collected urine samples of the patients with bladder cancer and healthy controls to test for urine survivin. Forty-two patients suspected to have bladder cancer underwent cystoscopy. A papillary tumor in the bladder was detected in 20 patients. None of the tumors had involved the bladder neck. They underwent transurethral resection (TUR) or TUR-biopsy, and pathologic examination confirmed transitional cell carcinoma (TCC) of the bladder.

The exclusion criteria were a positive urine culture for infection; primary involvement of the upper urinary tract or the urethra by the tumor; and a positive history of intravesical chemotherapy, systemic chemotherapy, or pelvic and abdominal radiotherapy. All 20 patients were eligible to be enrolled in the study. A urine specimen was obtained before cystoscopy which was used for detection of survivin. A group of controls was selected from among patients who underwent cystoscopy for other reasons and had not any evidence of malignancy in their physical examination, laboratory studies, and cystoscopic examination.

### Urine Detection of Survivin

Fifty-mL urine specimens were sent to the genetics laboratory of Tarbiat-e-Modarres University in Tehran, to be tested for survivin by reverse

transcription polymerase chain reaction (RT-PCR). Extraction of the total RNA was performed using RNX-Plus solution (Cinnagen, Tehran, Iran) according to the manufacturer's instructions. The extracted RNA was stored in  $-80^{\circ}\text{C}$  for use. Single-stranded complementary DNA (cDNA) was synthesized using 5  $\mu\text{g}$  of RNA and MMLV reverse transcriptase (Gibco BRL, Germany) with oligo (dT)18 priming in a 20  $\mu\text{L}$  reaction as described elsewhere.<sup>(15)</sup> The synthesized cDNA was amplified using specific primers designed for survivin. The designed primers as well as the oligo (dT)18 primer were synthesized by MWG Biotech Company (Ebersberg, Germany) as highly purified salt-free grade. Polymerase chain reaction analysis was performed using 5  $\mu\text{L}$  of synthesized cDNA with 1.25 U of Taq polymerase (Promega, Madison, WI, USA). The amplification was performed for 25 to 35 cycles. The cycling conditions were as follows:  $94^{\circ}\text{C}$  for 30 seconds,  $55^{\circ}\text{C}$  for 30 seconds,  $72^{\circ}\text{C}$  for 1 minute, and a final extension at  $72^{\circ}\text{C}$  for 10 minutes. The PCR products were then separated on a 1.5% agarose gel and visualized by ethidium bromide staining.

### Statistical Analyses

The results of survivin in the 2 groups were compared using the chi-square test and a value of less than .05 for *P* was considered significant.

## RESULTS

A total of 20 patients with papillary bladder tumor (group 1) and 18 controls (group 2) were tested for urine survivin. Demographic and clinical characteristics of the subjects are summarized in Table 1. Urine survivin was positive in the specimens of 18 (90%) and 9 (50%) patients in groups 1 and 2, respectively ( $P = .007$ ).

The results of urine cytology were available in 16 patients of group 1, of which 6 (37.5%) were positive for malignancy. All of the patients with a positive cytology had high-grade tumors. Urine survivin was detectable in 5 out of 6 patients with positive cytology. Furthermore, urine survivin was positive in all 10 patients with negative cytology. Nine patients in this group had low-grade tumors (Table 2). Finally, the 2 patients who were negative for survivin had stage T1 tumors.

**Table 1.** Demographic and Clinical Characteristics of Patients with TCC and Controls\*

Characteristics	Patients with TCC (Group 1)	Controls (Group 2) <sup>†</sup>
Median age, y	60 (17 to 82)	43 (24 to 81)
Male/female	19/1	18/0
Cause of referral		
Obstructive and irritative symptoms	0	12
Removal of stent	0	6
Gross hematuria	18	0
Microscopic hematuria	2	0
Diagnosis		
Papillary TCC	20	0
BPH	0	4
Urethral stricture	0	5
Bladder calculus	0	1
Normal	0	8
Tumor stage		
Ta	6	...
T1	11	...
T2	3	...
Tumor grade		
Low	11	...
High	9	...

\*TCC indicates transitional cell carcinoma.

<sup>†</sup>Ellipses indicate not applicable.**Table 2.** Pathology and Cytology Results in Patients with Transitional Cell Carcinoma

Cytology and Pathology	Survivin Positives	Survivin Negatives
Urine cytology		
Positive	5	1
Negative	10	0
Tumor stage		
Ta	6	0
T1	9	2
T2	3	0
Tumor grade		
Low	10	1
High	8	1

## DISCUSSION

We performed the present study to assess the value of urine survivin in the diagnosis of bladder cancer. As a member of apoptosis inhibitor proteins, survivin is overexpressed in undifferentiated cells associated with a high potential of cell proliferation.<sup>(5)</sup> Survivin is also reported to be highly expressed in cancerous cells.<sup>(6-12)</sup> Following the introduction of survivin by Ambrosini in 1997,<sup>(5)</sup> the first study on bladder cancer that revealed survivin overexpression was done by Swana and colleagues in 1999.<sup>(12)</sup> They found that

survivin expression could be documented in 78% of bladder tumor specimens; 65%, 90%, and 100% of grade 1, grade 2, and grade 3 tumors were positive for survivin expression. Recurrence of TCC was more frequent among patients with detectable survivin. They also showed that survivin was not expressed in normal transitional cells. However, survivin expression was reported in normal bladder mucosa by Lehner and associates in 2002.<sup>(16)</sup> Later on, low rates of survivin expression (13% to 30%) in tumoral cells were demonstrated.<sup>(17,18)</sup>

Our previous experience with survivin was indicative of a positive survivin expression in only 50% of tumors.<sup>(19)</sup> We also studied survivin- $\Delta$ Ex3—a variant product of survivin gene which was reported in renal cell carcinoma.<sup>(20)</sup> It was more strongly associated with bladder cancer than survivin.

Detection of survivin in urine specimens of patients with bladder cancer was first studied by Smith and colleagues in 2001.<sup>(13)</sup> They evaluated healthy subjects and patients with benign urogenital disorders; prostatic, renal, vaginal, or cervical tumors; newly diagnosed bladder cancer; and treated bladder tumors. The sensitivity and specificity of urine survivin for the diagnosis of bladder cancer were 100% and 95%, respectively. They used the Bio-Dot microfiltration detection system for all and the RT-PCR analysis for 20 samples of each group of patients. Shariat and coworkers compared 117 patients with bladder cancer and 92 controls and reported that higher levels of urine survivin are associated with bladder cancer and high-grade tumors.<sup>(14)</sup> We performed a study with 2 phases; the expression of survivin in tumoral cells of bladder cancer was confirmed in the first phase.<sup>(19)</sup> In the present study (phase 2), we assessed urine survivin. The sensitivity and specificity of urine survivin were lower than those reported by Smith and colleagues (90% and 50%, respectively). It may be due to the limited sample sizes and unequal groups in both studies (31 and 16 patients in the subject and control groups of Smith and colleagues' study). Furthermore, we excluded any factor that might lead to false-positive results for urine survivin (urinary tract infection, primary upper urinary tract disorders, chemotherapy, etc).

We compared the results of urine survivin and urine cytology and their relation with tumor grade. Although the number of the patients is too small for

a precise conclusion, we can note that urine survivin seems to be more sensitive for diagnosis of bladder cancer, especially high-grade tumors. This test, itself or in combination with cytology, may be useful in the workup of the patients and provide a high sensitivity. For clinical application of survivin, a quantitative test would be more useful. New techniques have been studied recently to determine the levels of survivin in urine,<sup>(14)</sup> and we have planned a third phase for our study to test the level of survivin in patients with suspected bladder cancer and in patients with treated cancer. Also, a comprehensive comparison of urine survivin and urine cytology in different stages and grades is necessary.

## CONCLUSION

The preliminary results of the studies on the expression of survivin have shown that it can be a valuable marker for bladder cancer. We found that urine survivin provides a higher sensitivity compared to urine cytology in low-grade tumors. More studies are required for better clarifying the diagnostic value of urine survivin in bladder cancer.

## CONFLICT OF INTEREST

None Declared.

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