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The Level of Survivin in The Blood and Urine Samples of Bladder Cancer Patients Using the ELISA Method

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ABSTRACT

Background: Survivin is a member of inhibitors of the apoptosis family (IAP), it is over-expressed in almost all cancers including bladder cancers in which it is excreted in urine.

Objectives: to find the possible suitability of survivin in urine and blood as a novel prognostic and or predictive molecular marker in bladder carcinoma.

Patients with methods: urine and serum samples were taken from five groups: healthy persons, patients who had non-neoplastic urinary tract problems, genitourinary cancers excluding bladder cancer, patients with discovered newly or recurrent superficial urinary bladder carcinoma and patients with muscle-invasive or metastatic bladder cancer. All were attendants of the Urology department at Al-Kadhimiya Teaching Hospital in Baghdad. Survivin levels were analyzed by ELISA test in urine and serum samples of the five groups

Results: The level of survivin in the normal control group was below the cutoff value in serum (71.385 pg/ml) and cutoff value in urine (71.86 pg/ml) while in non-neoplastic urological conditions the level of the survivin was evaluated above the cutoff value in serum and urine in 1/6 of the cases. While it was evaluated in half of the patients with urological malignancies other than bladder cancer and it was increased above the cutoff volume in 73.68% in the serum of patients with superficial urinary carcinoma and 78.9% in the urine of the same group.

Conclusions: specific and sensitive determination of urine and (or) serum survivin provides a simple, non-invasive diagnostic method to complement cytology and (or) other diagnostic markers in persons with new onset or recurrent urinary bladder carcinoma.

Keywords: Survivin, Bladder Cancer, ELISA, Molecular Marker.

INTRODUCTION

Urinary bladder carcinoma is the second most common malignancy that affects the urinary tract. There are limitations to the cytological method and urethrocystoscopy is considered an invasive method for detection of the urinary bladder carcinoma, this generated interest in other non-invasive diagnostic methods including tumor markers [(1)].

Survivin is regarded as a member of the proteins family that regulates mitosis and cell death, named inhibitors of the apoptosis family (IAP) [(2)]. Eight human IAP family members have been identified so far [(3)] and survivin is a unique member of this family with an important role in apoptosis inhibition and the regulation of mitosis [(4)].

But the precise mechanism by which survivin interferes with apoptosis has not been fully known. Survivin counteracts the death of cells by interfering with caspase-9 processing, which is the upstream initiator of the mitochondrial intrinsic pathway of apoptosis [(5)].

Survivin is present ubiquitously and abundantly during fetal development. In adults, although a few normal cells express survivin, for example, thymocytes, CD 34+ stem cells that are derived from bone marrow, and basal colonic epithelial cells, under physiologic conditions the survivin cannot be detected in most of the terminally differentiated normal tissues [(6)].

It has been thought that the survivin gene might be deregulated in transformed cells and this will lead to over-expression at all stages of the cell cycle. This deregulation in survivin may be mediated by oncogenes or may be by the loss of tumor suppressors and these accounts for the expression of survivin in carcinoma [(7)]. Survivin is over-expressed in most cancers including colon, lung, breast, pancreas, liver, stomach, prostate, ovary, and urinary bladder as well as in hematopoietic malignancies and melanoma [(8, 9, 10)].

It is regarded as the fourth most expressed protein in human carcinomatous tissue compared with normal tissues [(11)]. The high expression of survivin in carcinoma carries prognostic and predictive importance since it is always associated with high-grade, advanced disease, abbreviated survival, accelerated recurrences, and resistance to therapy [(11)].

In urinary bladder carcinoma, survivin is expressed in urine and usually, its expression is associated with disease progression, recurrence, stage, and mortality [(12)]. The sensitivity and specificity between 64% to 94% and 93% to 100%, respectively have been seen in some literature [(12, 13)].

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Survivin not consider as biomarker in some of cancer, it is also detected as biomarker in Acne Vulgaris Patients [14] .Because survivin is expressed in carcinomatous but not in normal tissues, we investigated if it is potentially suitable for urine and serum survivin as a new molecular marker for the detection of urinary bladder carcinoma.

SUBJECTS AND METHODS

Five healthy volunteers, aged from 25 to 60 years were included in this study as the control Group (1). Thirty-eight patients with urinary tract complaints, aged from 25 to 81 years were included in this study. They were attendants of the Urology Department at Al-Kadhimiya Teaching Hospital. They were divided into four groups, Group (2) contained 6 patients with non-neoplastic urinary tract problems or hematuria, Group (3) included four patients with genitourinary (non-bladder) cancer, Group (4) included nineteen patients with first-time or recurrent superficial urinary bladder carcinoma (Ta, T1), and Group (5) included nine patients with invasive bladder cancer (T2 and above). From all patients and controls, 3 ml of venous blood and midstream urine sample were collected. Blood samples were centrifuged, and sera were separated and stored in a freezer with the urine samples.

100 µL of the samples (serum, urine, and standards) were added to a microtiter plate coated with anti-survivin monoclonal antibody and incubated at room temperature for one hour, then the wells were washed to remove excess materials, then 100 µL of rabbit polyclonal antibody to survivin were added and incubated at room temperature for another hour, then washed again to remove excess materials. 100 µL of goat anti-rabbit immunoglobulin G attached to Horseradish peroxidase were added and incubated at room temperature for thirty minutes then washed again to remove excess materials, and 100 µL of the substrate was added and incubated at room temperature for thirty minutes, then 100 µL of Stop Solution were added and the optical density was read at 450-nanometer wavelength using ELISA reader.

The results were calculated using the MMT computer program, which plots the optical density (O.D.) for each standard versus the survivin concentration in each standard. The concentration of survivin in the unknowns (serum, urine samples, and controls) was determined by computerized interpolation using the same program.

Ethical considerations:

The research was sanctioned by the Urology Department at Al-Kadhimiya Teaching Hospital's Ethics Committee and College of Medicine, Kirkuk University, A consent document was signed by all those involved. The World Medical Association's Declaration of Helsinki was strictly adhered to in all human subjects' studies.

RESULTS

All serum and urine samples were assessed for survivin using a monoclonal antibody against survivin by ELISA method, the obtained levels were statistically analyzed. **(Table 1)**

Table (1): Age, sex distribution, mean survivin level in serum and urine of the five studied groups

Groups	Control (Group1)	Non-neoplastic urological conditions (Group2)	Urological malignancies other than bladder cancer (Group3)	Superficial bladder cancer (Group4)	Muscle-invasive bladder cancer (Group5)
No. of patients	5	6	4	19	9
Mean age (yr)	50.0	54.50	58.33	65.63	66.88
M: F ratio	1.5:1	1:1	2:1	2.8:1	2:1
Mean Survivin serum pg/ml	62.80	69.87	72.46	76.59	69.68
Mean survivin urine pg/ml	71.34	71.20	80.96	81.39	83.29

A cutoff value of 71.385 pg/ml was calculated for serum survivin which revealed a sensitivity of 71.4% and a specificity of 71.4%. A cutoff value of 71.86 pg/ml was calculated for urine survivin which showed a sensitivity of 82.1% and a specificity of 78.6%. Serum and urine levels in the control group (group 1) were below the cut-off value.

Survivin was elevated in the serum and urine of a 60-year-old female patient in group 2 who had attacks of painless hematuria. Serum and urine survivin levels in the remaining patients in group 2 were normal. Survivin was elevated in the serum of 2 patients in group 3 both were suffering from advanced renal cell carcinoma. Also, survivin was elevated in the urine of the other 2 patients in this group both of them had carcinoma of the prostate, and one of them had T4 stage in which the tumor was found extending to the bladder.

Survivin was elevated in the serum of 14 patients out of 19 patients (73.68%) with first onset or recurrent superficial urinary bladder carcinoma and in urine samples of 15 patients out of 19 patients (78.9%) in the same category (Group 4), furthermore, 12 patients out of those 19 patients (63.1%) had an elevation in both serum and urine survivin simultaneously.

Survivin was elevated in the serum of 2 patients out of 9 patients (22.2%) with a history of invasive urinary bladder carcinoma and it was elevated in the urine samples of 6 patients out of 9 patients (66.6%) in the same category (Group 5).

Table (2) the number and percentage of individuals at or above the cut-off value for the 5 groups

Groups	**Above the cutoff value n (%) Serum**	**Above the cutoff value n (%) Urine**
Group1 (control healthy volunteers)	0	0
Group 2 (non-neoplastic urinary tract problems)	1 (16.6%)	1 (16.6%)
Group 3(genitourinary cancers except bladder)	2 (50%)	2 (50%)
Group 4 (superficial bladder cancer)	14 (73.7%)	15(78.9%)
Group 5 (muscle-invasive bladder cancer)	2 (22.2%)	6 (66.6%)

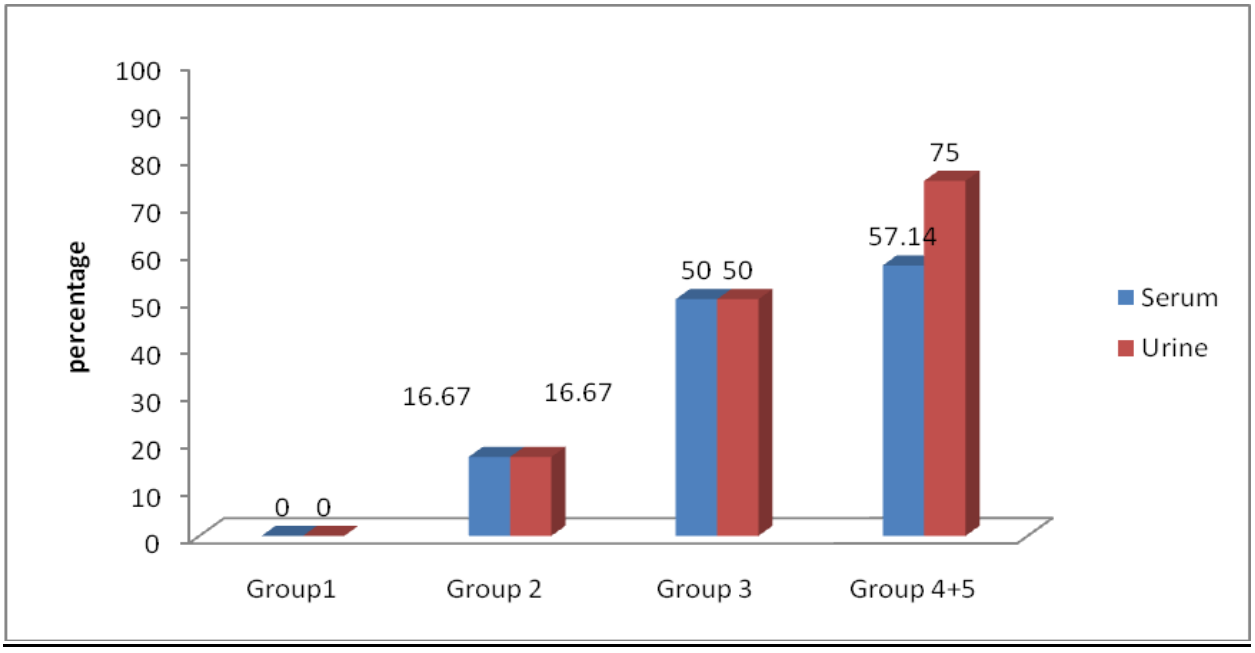

Figure (1): percentage of survivin positive cases in the serum and urine of the 5 groups

DISCUSSION

In the current study, we described an easy, antibody-based method to detect the apoptosis inhibitor survivin in the urine and serum of patients with urinary bladder carcinoma. In Group 1 (controls) serum and urine survivin levels were all found to be below the cut-off value. In Group 2 (patients with some non-neoplastic urinary tract contditions) survivin was found to be elevated in the serum and urine of a 60 year old female patient who had attacks of painless hematuria for which an ultrasound study was done and showed no abnormalities and diagnostic cystoscopy with subsequent biopsy revealed evidence of cystitis only ,serum and urine survivin levels in the remainder patients in Group 2 were found to be normal, since survivin detection in the current study showed a reliable specificity and sensitivity for detection of bladder cancer and since diagnosing bladder cancer is based on cystoscopic findings using a white-light cystoscope which has an imperfect sensitivity especially for very small growths and some cases of carcinoma in situ (CIS) the patient that showed positive serum and urine survivin levels should be closely followed up by cytology and cystoscopy (preferably with the aid of a photosensitizer like 5-aminoleuvulinic acid in conjunction with blue light cystoscopy which can detect bladder lesions not detectable with the oridinary white light cystoscopy) if needed because she may subsequently develop bladder cancer especially since her age and symptoms (intermittent painless hematuria) put her at high risk for developing bladder cancer .

In Group 3 (patients with genitourinary malignancies other than bladder cancer) survivin was found elevated in the serum of 2 patients both were suffering from advanced renal cell carcinoma, this finding correlates with another study [15] which showed that survivin was elevated in patients with renal cell carcinoma and that higher levels of survivin were significantly associated with poorly differentiated, advanced stage and more aggressive RCCs [15]. The survivin was elevated in the urine of other 2 patients in the same group (group 3) both of them had carcinoma of the prostate and one of them had T4 stage in which the tumor was found extending to the bladder, again this finding comes in agreement with other studies which showed that survivin overexpression was seen in patients with prostate cancer and that this overexpression was an independent predictor of distant metastasis [16, 17]. In Group 4 (patients who had superficial urinary bladder carcinoma) the survivin was elevated in the serum of 14 patients out of 19 patients (73.68%) with first onset or recurrent superficial urinary bladder carcinoma and in the urine of 15 patients out of 19 patients (78.9%). Twelve of those 19 patients (63.1%) have an elevation in both serum and urine survivin simultaneously.

In Group 5 (patients with muscle invasion) survivin was elevated in the serum of 2 patients out of 9 patients (22.2%) with a history of invasive bladder cancer and it was elevated in the urine of 6 out of 9 patients (66.6%) in the same category. The sensitivity of urine survivin was 82.1%, and its specificity was 78.6%. Nearly comparable results were obtained with serum survivin which showed a specificity and sensitivity of 71.4% and 71.4% respectively, this finding is in line with other studies which stated that sensitivity and specificity of survivin range between 64% to 94% and 93% to 100%, respectively [2, 13, 14]. However, the observed difference in specificity from our study might be attributed to the small sample size, in addition, the whole specificity of the test may vary depending on which patient group is the focus of clinical interest since a screening method for group number 1 individuals will have a false positive result of zero, but patients with symptoms in Groups 2 and 3 will have a combined false positive of 30% for urine survivin and a false positive of 30% for serum survivin.

When comparing survivin levels in the urine and serum of patients with superficial and more advanced bladder cancers (muscle invasive and above),i.e. comparing survivin levels between Groups 4 and 5, no significant differences were noticed between these 2 groups (p-value 1.185 for serum survivin and p value 0.766 for urine survivin), this finding was also noticed by other studies [14] which concluded that higher levels of survivin were found with an increased risk of urinary bladder carcinoma and with a higher tumor grade, but not associated with an invasive stage [14,18] which studied the expression of the survivin in histopathological samples of patients with transitional cell carcinoma of the bladder and found no significant difference between superficial and muscle-invasive cancers regarding survivin expression [18].

Because survivin is expressed in carcinomatous but not in normal tissues [19] and because of its unfavorable prognostic and predictive significance in various cancers [20, 21, 22, 23], survivin may become a useful molecular marker in malignancy. This may be relevant in urinary bladder carcinoma [24, 25], in which non-invasive and simple diagnostic methods to monitor response to treatment and to simplify follow-up protocols, are needed. Urine cytology has low sensitivity (30-40%) in bladder cancer, although regarded as the standard criterion [26], but fails to detect low-grade, superficial lesions. So several urine markers including urinary bladder tumor antigen, telomerase activity, nuclear matrix protein, fibrin degradation, and hyaluronic acid/hyaluronidase products have been characterized for their potential predictive/ diagnostic value in urinary bladder carcinoma [27, 28]. Recently, it has been proposed that PCR and other forms of

molecular technology be used in the diagnosis of hereditary diseases and other medical conditions, ex: CML [29], Adenocarcinoma [30,31], and SARS-Cov-2 [32].

CONCLUSION

Because of its high sensitivity and specificity, survivin testing in serum or urine may be useful to complement cytology and/or other diagnostic markers to better monitor urinary bladder carcinoma patients and identify early recurrences or de novo tumors. Other possible advantages of the survivin marker include its suitability and simplicity as a point-of-service procedure, and its cost-effectiveness, using one-step detection with one antibody to survivin that now became commercially available. Analysis of more patients may give a better idea about the general suitability of survivin for monitoring the response to treatment and follow-up protocols in urinary bladder carcinoma.

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