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Evaluation of Serum and Urinary Fibronectin as a Diagnostic Marker of Bladder Cancer

Noor K. Habash[1] *MSc*, **Omar F. Abdul-Rasheed**[2] *PhD*, **Usama S. Al-Nasiri**[3] *FRCS*

[1]Faculty of Dentistry, Dijlah University College ,[2]Dept. of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, [3]Iraqi Board for Medical Specializations

Abstract

Background Accurate and sensitive detection of bladder cancer is important to diagnose this deadly disease at an early stage, estimate prognosis, prediction the response to treatment and for monitoring the recurrence. In past few years, laboratory diagnosis and surveillance of urinary bladder cancer have improved significantly. Although, urine cytology remains the gold standard test, many new urinary biochemical markers have been identified.

Objectives To evaluate the value of fibronectin in serum and urine to detect bladder cancer in different grades and stages.

Methods Thirty five patients diagnosed as bladder cancer with mean age 61.94±11.66 years and thirty five aged-matched healthy volunteers as control group were included in this study. Serum and urinary fibronectin were measured by ELISA technique.

Results The mean±SEM serum and urine levels of fibronectin in patients with bladder cancer (33.11±1.90 µg/ml; 33.08±1.12 ng/ml respectively) were significantly higher than the levels in control group (8.57±1.10 µg/ml; 7.58±1.00 ng/ml, respectively). When using a serum fibronectin concentration of 25.65 µg/ml as a cutoff value for the diagnosis of bladder cancer, sensitivity was 71.4%, specificity 100%, the positive predictive value was 100% and the negative predictive value 77.78%, and the sensitivity and specificity of urine fibronectin were (94.3%, 97.1% respectively); when using a urine fibronectin concentration of 20.00 ng/ml as a cutoff value for the diagnosis of bladder cancer. The positive predictive value was 97.05%, and the negative predictive value was 94.44%.

Conclusion The measurement of fibronectin level in serum and urine is useful in discriminating bladder cancer patients from normal subjects.

Key words Serum and urinary Fibronectin, Bladder cancer.

List of abbreviation: FN= Fibronectin, KD= Kilo Dalton, ECM= Extracellular matrix, BC= Bladder cancer, ROC = Receiver operator characteristic.

Introduction

Bladder cancer (BC) is a major health problem across the world, mainly due to its association with tobacco abuse. The final diagnosis is achieved through cystoscopy and resection of tumors for pathological examination [(1-3)]. It is the fourth most common cancer in men in the USA and the eight most common cancers in women, with an estimated 57,400 cases being diagnosed in 2003, resulting in 25,100 deaths. Commonly accepted risk factors for Transitional cell carcinoma of the bladder include cigarette smoking, occupational exposure to aniline dyes, benzidene compounds, analgesic abuse (phenacetin) and chronic irritation such as indwelling catheters. Most cases of bladder cancer are superficial at the time of diagnosis (stage Ta- T1). The recurrence rate of superficial tumors can be as high as 70 % with 10- 15 %

progressing to muscle invasive disease. The risk of progression is directly related to tumor grade and stage [4].

Bladder malignancies can be treated using different approaches such as transurethral resection for superficial tumors, intravesical chemotherapy and radical cystectomy for non-metastasized tumors, or by systemic chemotherapy for locally advanced or metastasized tumors [5].

Cytological diagnosis is noninvasive and has high specificity but low sensitivity, especially for low-grade tumors. At the same time, it can be a challenging test to perform and highly dependent on the skills and experience of a trained cytopathologist. Because cystoscopy is invasive procedure and because cytology has poor sensitivity, non invasive biomarkers have been sought as alternatives to cystoscopy and cytology for the detection and surveillance of bladder cancer [6].

Fibronectin (FN) is one of the extracellular matrix member that is found in the urine of normal individuals, but found in a higher amount in patients with bladder cancer [7,8]. It is 440-KD glycoproteins as a well characterized extra cellular matrix (ECM) protein playing an important role in the inhibition of cellular attachment and tumor spread. The mechanism of FN action is mediated by specific receptors and growth factor [9-11].

The FN molecule appears to be important in wound healing, is found at sites of inflammation, and functions in normal cell-to-cell cohesiveness [12]. In the urinary tract, FN has been localized to the urothelial basement membrane [13].

Fibronectin is synthesized by many cell types [14]. A large portion of circulating fibronectin is produced by hepatocytes, in which it exists in two forms, termed cellular fibronectin (cFN) and plasma fibronectin (pFN) [14]. Plasma fibronectin is a soluble form produced solely by hepatocytes, whereas cellular fibronectin is an insoluble form produced by a variety of cells and incorporated into tissue extracellular matrix. Both isoforms are generated from a single gene by alternative splicing [15]. In healthy subjects, the human plasma fibronectin level is 300±100 µg/ mL [16], with no differences according to gender or age [17].

The present work was aimed to measure serum and urinary fibronectin in bladder cancer patients.

Methods

Thirty five patients with bladder cancer with mean age 61.94±11.66 years and 35 age-matched healthy subjects (controls) with mean age of 59.54±10.18 years were studied. Blood and urine samples from all 70 subjects were collected from Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq, between September 2012 and August 2013. The approval of the Al-Nahrain University/ college of Medicine Research Ethics Committee and written consent of every patient included in the study were obtained. All control subjects were with different non malignant urological disorders (i.e., hydrocele testis, ureteropelvic junction obstruction, stone disease, urinary incontinence). Patients with other malignancies in their medical history were excluded.

Diagnosis of bladder cancer was based on clinical assessment; cystoscopy was done for all patients as the reference standard for identification of bladder cancer. All tumors and suspicious lesions found were either resected or biopsied. The final diagnosis of bladder cancer based on histopathological examination. Fresh voided urine samples and blood were collected from 35 patients with newly diagnosed bladder cancer before they underwent transurethral resection of bladder tumor (TURB). Additionally urine and blood samples were collected from 35 healthy volunteers (controls). Blood samples were centrifuged at 3000 rpm and serum was stored at -40 ^{o}C, urine samples were centrifuged at 3000 rpm and the supernatant was pipette and stored at -40^{o}C. Urothelial cancer grading and staging were performed according to the World Health Organization criteria [18]. Serum and urine fibronectin levels were measured by monoclonal antibody Enzyme Linked Immuno Sorbent Assay (ELISA) technique.

The values of laboratory tests are presented as mean ± standard deviation and mean ± standard error for mean (SEM). The comparison of means between the different groups was performed using the Student's t test.

Receiver Operator Characteristic (ROC) curves was constructed to plot sensitivity against specificity of high serum, urine fibronectin levels as diagnostic tests for BC. The areas under the ROC curves (AUC) were calculated and compared with the AUC (0.5) of the non-diagnostic test (the line with the slope). To determine the cut-off values of significant sensitivity and specificity (>70%); contingency tables (cross-tabs) were constructed for the calculation of positive and negative predictive value were calculated.

All other analyses were performed using SPSS version 16 computer software (Statistical Package for Social Sciences). A *P* value less than the 0.05 level of significance was considered statistically significant.

Results

The concentration of serum, urine fibronectin of the studied subjects is summarized in Table 1. Serum fibronectin levels were significantly higher in the patients group with BC compared with the controls ($P < 0.001$).

Table 1. Serum and urine fibronectin levels of the subjects studied.

Parameter		Controls N = 35 mean±SEM	BC N = 35 mean±SEM
FN	Serum µg/ml	8.57±1.10	33.11±1.90*
	Urine ng/ml	7.58±1.00	33.08±1.12*

BC = bladder cancer, * $P = 0.001$

The ROC curves demonstrated a significant discriminatory ability of increase serum FN levels for the diagnosis of bladder cancer. The AUC for serum FN was 0.966 (95% CI: 0.931-1.001). A significant difference was found in the BC patients group ($P < 0.001$) as seen in fig. 1 and table 2.

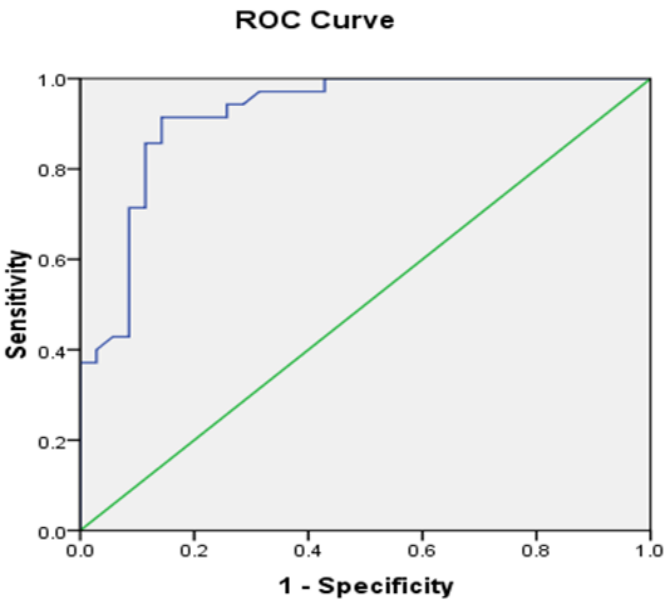

Fig. 1. Receiver Operator Characteristic (ROC) curve of high serum FN levels as a diagnostic test for bladder cancer

Table 2. Area under the curve for ROC analysis of parameters with testing for statistical differences

Fibronectin	AUC±SEM	95% CI
Serum	0.966±0.018	0.931-1.001
Urine	0.976±0.020	0.937-1.016

$P = < 0.001$

When the serum FN concentration of 25.65 µg/ml was used as a cutoff value for the diagnosis of bladder cancer in the control group; the sensitivity was 71.4%, specificity was 100%, and the positive predictive value was 100%, while the negative predictive value 77.78% (Table 3).

Table 3. Validity Indications of serum and urine fibronectin levels in prediction of bladder cancer

Parameter	Fibronectin	
	Serum	Urine
Cutoff value	25.65µg/ml	20.00ng/ml
Sensitivity	71.4%	94.3%
Specificity	100%	97.1%
PPV	100%	97.05%
NPV	77.78%	94.44%
Accuracy	85.71%	95.71%

Urine FN levels were significantly higher in patients with BC compared to the control group (p< 0.001) as shown in table 1. The receiver operating characteristic analyses showed that urine FN values can be used for the diagnosis of BC from the control group, with the areas under the curve being 0.976 (95% CI: 0.937-1.016). A significant difference was found in BC ($P < 0.001$) as noticed in Fig. 2 and table 2.

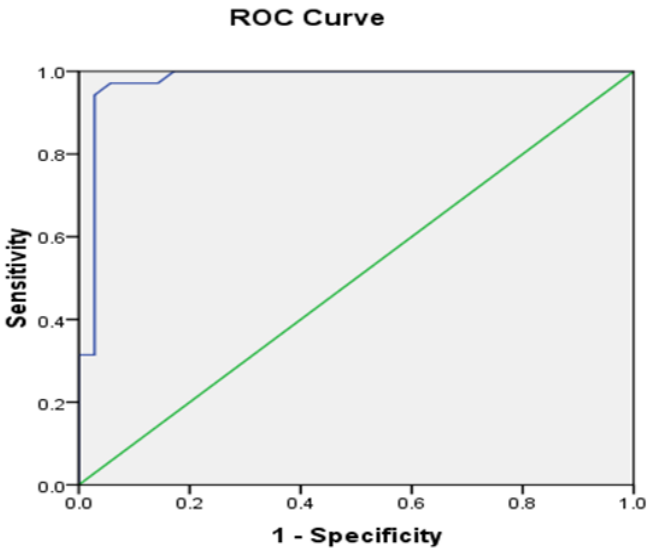

Fig. 2. Receiver Operator Characteristic curves of high urine fibronectin levels as diagnostic tests for bladder cancer from control group.

When using urine FN concentration of 20.00 ng/ml as a cutoff value for the diagnosis of bladder cancer from control group, sensitivity was 94.3%, specificity 97.1%, the positive predictive value was 97.05% and the negative predictive value 94.44% (Table 3).

Discussion

Development of new methods for bladder cancer detection is required because cystoscopy is invasive, and voided urine cytology has low sensitivity. The goal of the present study was to evaluate the clinical suitability of promising bladder tumor marker, named fibronectin in serum and urine. The high serum FN level in bladder cancer was explained by the formation of metastases and local progressions of tumors presuppose degradation of extracellular matrix (ECM) components. These essential steps are possibly mediated by hydrolytic enzymes of the tumor itself and induced by the tumor in the stromal cells [9,19]. Products of this various ECM components degradation are released in the circulation and determination of these components can be helpful for early detection of several malignancies [20].

The results of the current study are similar with the study by Hegele *et al*. who found that patients suffering from bladder cancer showed significantly higher serum FN levels. It was found that the mean serum fibronectin in the cancer group was significantly higher compared to the control group ($P < 0.001$); this result is in agreement with the results obtained by (Kirkali *et al*., Who found a significant elevation of fibronectin level in tissue of bladder cancer patients [21].

It is very interesting that, by means of ROC curve analysis (Fig.1), the measurement of serum FN level was found to be a reliable test for discriminating bladder cancer from normal subjects, when the positive predictive value (PPV) was interestingly high (100%) and the negative predictive value (NPV) for the diagnosis capacity in the exclusion of bladder cancer versus normal subjects was accepted (77.78%). The sensitivity and specificity were (71.4% and 100% respectively).

From these findings, it can be stated that the test is quite specific to differentiate the normal and bladder cancer subjects.

Urine fibronectin mainly originates from the basement membrane of the bladder, not from the kidney, since renal glomeruli cannot filter this large protein to the urine. Turnover of the suburothelial matrix can be responsible for the low but measurable urine FN level in healthy individuals [22,23].We evaluate the use of urinary fibronectin as a tumor marker of bladder cancer.

In this study, it was found that urine fibronectin level to be significantly higher in bladder cancer group ($P < 0.001$) than in normal group.

So it was concluded that the urine fibronectin measurement is useful to differentiate normal subjects from subjects with bladder cancer. This

result is consistent with the results of other studies [8,23-25]. Although each author found its own cutoff value, most of them conclude that urine Fibronectin measurement is important to discriminate bladder cancer subjects from normal subjects.

Criteria for the ideal tumor marker, has been described by Huben [26]. Urinary fibronectin fulfills most of these criteria. It is easy to do, relatively inexpensive, found in body fluid that iseasily collected, and not affected by other variables like systemic diseases [27].

In conclusion, measurement of serum fibronectin may be of value in the early diagnosis of bladder cancer. Urine FN test has a very good accuracy (95.71%) by the test of ROC analysis, when used to differentiate between bladder cancer and normal subjects.

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Author contribution

Dr. Abdul-Rasheed suggests the study and co-writes the manuscript; Dr. Al-Nasiri makes the diagnosis of patients and Miss. Habash writes the paper and analyzed the results statistically.

Conflict of interest

There was no conflict of interest.

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Correspondence to Omar F. Abdul-Rasheed
E-mail: omar.rasheed@colmed-alnahrain.edu.iq
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