

RESEARCH COMMUNICATION

Utility of Serum and Urine uPAR Levels for Diagnosis of Breast Cancer

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Abstract

Malignant tumors have a capacity to degrade the extracellular matrix by controlled proteolysis. One system involved in these processes is the urokinase-type plasminogen activator (uPA) system. uPAR levels are elevated in tumors from several types of cancer. Our study was planned to investigate serum and urine levels of uPAR in breast cancer patients (n=180) and healthy controls (n=60) by ELISA. Serum (p<0.001) and urine (p<0.001) uPAR values in the patients were both significantly elevated. High serum and urine levels of uPAR can be used as diagnostic tools in lymph node positive patients.

Keywords: Tumor markers - biochemical markers - breast cancer - cancer biomarkers - invasion & metastasis - uPAR

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Introduction

A tumor marker can be defined as a molecule that indicates the likely presence of cancer or that provides information about the likely future behaviour of a cancer (Duffy, 2007). Molecules associated with cancer have been extensively investigated and used in clinical practice for more than 30 years. These serum "tumor markers" play an important role in the management of many malignancies (Yasasever et al., 2007). Generally, tumor markers have low specificity and sensitivity.

Malignant tumors have a capacity to degrade the extracellular matrix (ECM) by controlled proteolysis. The urokinase-type plasminogen activator (uPA) system is involved in these processes and consists of uPA, uPA receptor (uPAR) and uPA inhibitors 1 and 2 (PAI-1 and PAI-2) (Blasi et al., 1999). Binding of uPA to its receptor, uPAR, focuses proteolytic activity on the cell surface and the action of uPA can be localized and intensified resulting in malignant matrix degradation and tumor growth, invasion and metastasis (Andreasen, et al., 1997; Hjertner et al., 2000). Elevated levels of the soluble urokinase plasminogen activator receptor (suPAR) have been reported in several forms of cancer (Duffy et al., 1999; Duffy et al., 2004). High plasma levels of suPAR are observed in patients with colorectal cancer, recurrent metastatic breast cancer and ovarian cancer (Sier et al., 1998; Petersen et al., 1999; Begum et al., 2004). Urine samples from healthy volunteers also contain measurable amounts of suPAR (Sier et al., 1999).

Breast cancer incidence has increased over the last 30-40 years, mortality has remained stable, reflecting the need for earlier diagnosis as well as improved treatment

options (Tabar et al., 1985; De Koning et al., 1995).

The aim of this prospective study was to evaluate the sensitivities and specificities of serum and urine levels of uPAR in breast cancer patients as well as to investigate the clinical utility.

Materials and Methods

180 patients (150 invasive ductal, 30 invasive lobular carcinoma) with pathologically verified breast carcinoma, consecutively admitted to the Istanbul University, Oncology Institute during a ten-month period, January 2007 to November 2007 were investigated.

Staging was performed on a pathological basis according to American Joint Committee on Cancer (AJCC). 110 patients had pathological evidence of lymph node metastasis (node positive), 55 patients had no pathological evidence of lymph node metastasis (node negative) and 15 patients lymph node status was unknown. The median age of patients was 49 (24-71 years) and controls was 43 (28-69 years). Serum and urine samples were obtained on first admission, 10 days within surgery, before initial chemotherapy or hormonal therapy was performed. The protocol was consistent with the Declaration of Helsinki (2000). Informed consent was obtained from all patients.

Blood samples were obtained from patients (n=180) and healthy females (n=60) who were blood donors undergoing regular physical and laboratory examinations by venipuncture and clotted at the room temperature. The sera were collected following centrifugation and frozen immediately at -20°C until analysis.

The 24-hour urine samples were collected with newly

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diagnosed breast cancer patients and healthy individuals. After the volumes were measured, the urine samples were centrifuged and maintained at -20°C pending analysis. Before analysis, frozen urine was thawed overnight in the refrigerator, mixed and prepared to test. Daily produced urine volume was 2.75 liters in patients and 3.5 liters in the healthy controls.

Human uPAR (R & D Systems Inc., Minneapolis, MN, USA) levels were measured by solid-phase enzyme immunoassay (ELISA). The amount of uPAR was quantitated by an automated ELISA reader (Rayto, RT-1904C Chemistry Analyzer). The results were expressed as nanograms per milliliter (ng/mL).

Data analysis was performed using the SPSS 16 software (SPSS, Chicago, IL). The report design was adopted from the standards for reporting diagnostic accuracy (STARD) group (Bossuyt et al., 2004). Nonparametric test (Mann Whitney U) was used to compare the median rank uPAR serum and urine values. p values < 0.05 were considered to be significant. Correlations were calculated using the Spearman's correlation test. The sensitivity and specificity of the tests were calculated by using receiver operating characteristics curves (ROC).

Results

Descriptive statistics and the serum and urine uPAR levels of patients with breast cancer and the control group are shown in Table 1. The median serum uPAR (p<0.001) and urine uPAR (p<0.001) levels were higher in breast cancer patients compared with healthy controls (Table 1). The median, ranges and 95% CI of serum and urine uPAR values in the groups are shown in Figure 1 and Figure 2 (Figure 1, 2).

Serum uPAR levels were significantly higher in the patients with nodal involvement compared with node negatives (p=0.043).

To determine the cut-off values and sensitivity and specificity of serum uPAR and urine uPAR tests in

Table 1. Group Characteristics

	Patient (n=180)	Control (n=60)	Statistical
	x±sd/Med/min-max	x±sd/Med/min-max	Significance
Age	58.4±8.69/49/24-71	45.05±14.98/43/28-69	p<0.001
Serum uPAR (ng/mL)	5.55±3.44/4.35/16.5-148.5	2.73±1.62/2.1/11.5 -57.9	p<0.001
Urine uPAR (ng/mL)	9.85±11.70/4.49/2.4-391.5	2.42±2.98/1.27/4.5-102.4	p<0.001

*Median

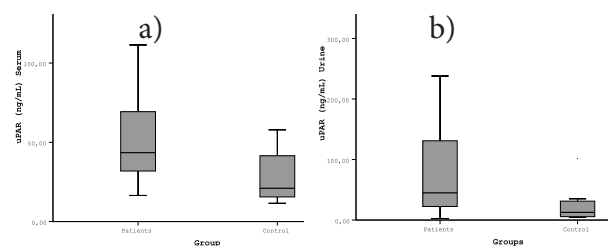


Figure 1. a) Median Serum uPAR Values in the Groups, b) Median Urine uPAR Values in the Groups

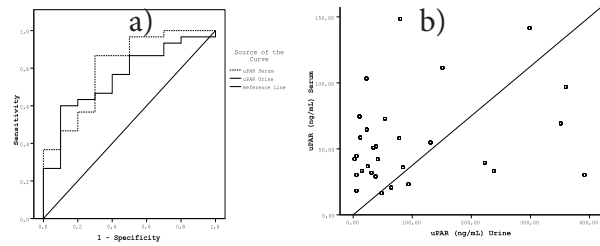


Figure 2. a) The Sensitivities and Specificities of Serum and Urine uPAR, b) The Correlation between Serum and Urine uPAR Levels.

the breast cancer patients, we used receiver operating characteristic (ROC) curves. The cut-off values were chosen according to the ROC curve coordinate points and cut-off point for serum uPAR was equal to its mean value. The cut-off levels were 2.73 ng/mL for serum uPAR and 2.87 ng/mL for urine uPAR, respectively. The sensitivities and specificities determined from the ROC curves were 86.7% and 70% for serum uPAR and 66.7% and 70% for urine uPAR, respectively. The accuracies were calculated as 82.5% and 67.5% for serum and urine uPAR, respectively. The sensitivities and specificities according to this cut-off values are shown in Figure 3.

We observed a significant correlation between serum and urine uPAR tests (r=0.165, p=0.27) in this study (Figure 4).

Discussion

The primary aim of this study was to evaluate the serum and urine uPAR concentrations together in breast cancer patients. Despite several studies in cancer on serum uPAR in the literature we couldn't find any study investigating both serum and urine uPAR combination performed by ELISA in breast cancer patients.

uPAR plays a key role in pericellular proteolysis, cancer invasion and progression (Gong et al., 2000). Increased tissue uPAR concentrations have been reported in both initial tumor invasion and during metastasis to other organs (Abendstein et al., 2000; Fisher et al., 2000). However, soluble urokinase plasminogen activator receptor concentrations in the sera of breast cancer patients has not been extensively studied and its diagnostic potential in the sera is less clear than its role in the tissue. In a primary study, significantly increased mean soluble uPAR levels have been observed in breast cancer patients which correlated with the severity of the disease (Mabrouk and Ali-Labib,2003).

In the current study the most striking finding was the demonstration that uPAR levels were significantly higher in patients with breast cancer than in the healthy controls. The serum and urine uPAR levels showed a high diagnostic value with higher sensitivity, specificity and accuracy. Our results confirm primary observations indicating that the serum uPAR could be a useful marker in the clinic. Serum uPAR showed the highest sensitivity, specificity and accuracy in breast cancer.

uPAR can be useful as prognostic factor for lymph node negative breast cancer patients, who may not receive adjuvant chemotherapy. Increased levels of uPAR have

been reported to be associated with poor prognosis in patients with breast cancer (Han et al., 2005). Serum uPAR levels were significantly higher in the patients with nodal involvement compared with node negatives ($p=0.043$) as like as in literature.

There are some studies investigating urine uPAR levels in bladder cancer and various types of cancer (Casella et al., 2002; Ecke et al., 2005). However, we could not find any report on the measurement of urine uPAR levels in breast cancer. According to the our results, a significant difference was observed between the patients and the control group. The sensitivity, specificity and accuracy were high according to the ROC curves and it was found that there is a significant correlation between serum and urine uPAR ($r=0.165$, $p=0.27$) in this study. Since collection and measurement of the urine samples are easy, this test may be easily applied in the routine.

In conclusion, high serum and urine levels of uPAR in breast cancer are correlated with increased tumor growth and can be used as diagnostic parameters for initial diagnosis as well as to determine the right therapy, especially in lymph node positive patients.

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