Urine Concentration of Nuclear Matrix Protein 22 for Diagnosis of Transitional Cell Carcinoma of Bladder

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Introduction: The aim of this study was to determine the diagnostic value of urine nuclear matrix protein 22 (NMP22) level in detection of transitional cell carcinoma (TCC) of the bladder.

Materials and Methods: A total of 76 patients with newly-diagnosed or recurrent TCC and 75 controls without urinary tract disorders participated in this study. A urine sample was obtained for measurement of the NMP22 level using the enzyme-linked immunoabsorbent assay. The resulted values were evaluated in comparison with the results of pathologic examination.

Results: A total of 76 patients with TCC of the bladder and 75 volunteers without TCC were enrolled in the study. The mean level of urine NMP22 had an increasing trend associated with tumor grade ($P = .01$) and tumor stage ($P < .001$). In participant without TCC, the mean urinary NMP22 level was 5.48 ± 6.34 U/mL, while this value was 25.01 ± 35.33 U/mL in patients with TCC of the bladder ($P < .001$). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of urine NMP22 for detection of TCC were 75.5%, 86.7%, 85.1%, 77.4%, and 80.8%, respectively. The sensitivity of NMP22 in detecting stage Ta tumors appeared to be low (31.3%), but for grade 1 tumors, the sensitivity was 66.7%.

Conclusion: Measurement of urine NMP22 is a noninvasive, highly sensitive, and specific method for detecting TCC of the bladder and estimating its grade and stage. Further studies can be helpful to determine whether it can be used in clinical practice.

Keywords: transitional cell carcinoma, bladder neoplasms, diagnosis, nuclear matrix proteins, tumor markers

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INTRODUCTION

Transitional cell carcinoma (TCC) accounts for the majority of bladder tumors.[1] Currently, the standard methods for detecting, staging, and tracking the progress of bladder cancer include urine cytology, cystoscopy, biopsy of the suspected mass, and excretory urography.[2] Urine cytology is easy to perform but not sensitive for detection of grade 1 and grade 2 tumors,[3] while it is very important to diagnose the disease prior to invasion in order to improve the prognosis of the patients. In addition, since TCC of the bladder frequently recurs, close follow-up after the successful treatment of the initial tumor is mandatory.[4]

As referenced above, cytology of urine is not sensitive enough for low-grade tumors, and on the other hand, the gold-standard cystoscopy and biopsy are invasive methods, and they are both costly and uncomfortable for the patient.[5] Therefore, the need for an *in vitro*, noninvasive, diagnostic

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test for providing objective quantitative results to be used in conjunction with the currently accepted diagnostic methods is beyond question. Employing this methodology would significantly improve the urologist's ability to make clinical decisions regarding the status of the disease and effectiveness of the treatment, especially in patients with low-grade tumors.

Nuclear matrix proteins (NMPs) are parts of the internal structural framework of the cell nucleus.[6] They are known to play important roles in DNA replication, transcription and processing of RNA, and regulation of the gene expression.[6] Additionally, it has been demonstrated that the intracellular NMP22 concentration is at least 25 times greater in the bladder cancer tumoral cells than in the normal bladder cells.[7-10] In this study we aimed to determine the diagnostic value of urine NMP22 in detection of transitional cell carcinoma (TCC) of the bladder.

MATERIALS AND METHODS

We selected patients with TCC of the bladder referred to Imam Khomeini Hospital in Tehran, Iran, between December 2005 and April 2007. Assigned as group 1, they were either newly-diagnosed or had recurrence of TCC. The patients were visited in the clinic or admitted to the hospital for follow-up after treatment of the bladder tumor. A control group was selected from among admitted patients who had no evidence of bladder tumor and joined the study voluntarily (group 2). Patients with urinary tract infection, urinary calculi, and any malignancy in other parts of the urinary tract were excluded. For this reason, the health status of the control group was confirmed by medical examination, urinalysis, and sometimes, ultrasonography of the urinary tract system. We obtained informed consent from the participants in both groups.

A total of 151 urine samples were collected. In group 1, a single-voided urine sample from each participant was collected just prior to cystoscopy. Biopsy was taken from any visible tumor or suspected lesion. The biopsies were evaluated using the TNM staging system and the World Health Organization's grading. A urine collection kit, containing urine stabilizers, was used for urine sample collection for the NMP22 test, and the urine was immediately stored at under -20°C. Demographic information including date of birth, sex, and pathology report of the biopsy were recorded. For both groups, the urine samples were sent to the laboratory with an identification number without any detailed demographic information.

The NMP22 was determined by 2-step-sandwich enzyme-linked immunoabsorbent assay (Matritech, Newton, USA). All samples were processed according to the written instructions provided by the kit manufacturer. Diluted urine samples were added to microplates which were antibody coated. After washing the captured NMP22 antigen, we allowed the antigen to react to a second antibody which was labeled by digoxigenin. The excessive digoxigenin was washed and a new antidigoxigenin antibody, which was coupled with horseradish peroxidase, was added. The remaining antibody was also washed and the sandwich was detected using o-phenylenediamine substrate. For stopping the reaction, 2 mol/L of sulfuric acid was added. The NMP22 concentration was proportional to the developed color intensity, and its level was calculated from the standard curve. The reference cutoff level of NMP22 had been set to be 10 U/mL by the manufacturer.

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA). The chi-square test and the *t* test were used for comparisons between the two groups. Diagnostic value of the NMP22 was tested by calculation of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy. In addition, the receiver operating characteristic curve was used for obtaining threshold values. Correlations between the level of the NMP22 and tumor grade and stage were evaluated by the Kendall's tau-*b* test. A *P* value less than .05 was considered significant.

RESULTS

A total of 76 patients with TCC of the bladder and 75 volunteers without TCC were enrolled in the study. The patients with TCC (group 1)

and the volunteers (group 2) were comparable in terms of age and sex distribution (Table 1). Histopathological data of group 1 is shown in Table 2. The patients in group 1 had a significantly higher mean urinary NMP22 level than those in group 2 (25.01 ± 35.33 U/mL versus 5.48 ± 6.34 U/mL, $P < .001$). The data obtained on the mean urinary NMP22 levels of each study individual was analyzed with the Kruskal-Wallis nonparametric test as shown in Table 2, which revealed an increasing trend in urine NMP22 values associated with tumor grade ($P = .01$) and tumor stage ($P < .001$). Also, using the Kendalls' tau-*b* test, it was shown that the urine NMP22 values correlated significantly with the tumor stages (r = 0.37, $P < .001$).

The diagnostic profile of NMP22 was evaluated using the receiver operating characteristic curve analysis. The optimal combination, defined by the largest area under the ROC curve (0.88; 95% confidence interval, 0.83 to 0.95), obtained a sensitivity of 75.0% and a specificity of 86.7%, taking a threshold value of 10.1 U/mL for NMP22 level in urine which is nearly the same as the manufacturer's recommendation (10 U/mL). Based on the cutoff point of 10.1 U/mL, the NMP22 was positive in 5 (31.3%), 27 (90.0%), 6 (66.7%), and 18 (90.0%) of the patients with Ta, T1, T2, and T3 bladder tumors, respectively ($P < .001$). Overall, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of NMP22 for detection of TCC were 75.0%, 86.7%, 85.1%, 77.4%, and 80.8%, respectively. As shown in Table 3, the

Table1. Age and Sex Distribution of Patients with Transitional Cell Carcinoma (Group 1) and Participants Without Bladder Cancer (Group 2)*

Characteristic	Group 1 (n = 76)	Group 2 (n = 75)	*P*
Mean age, y	66.5 ± 11.3	65.7 ± 10.5	.84
Sex			
Male	61 (80.3)	62 (82.7)	
Female	15 (19.7)	13 (17.3)	.85

*Values in parentheses are percents.

Table 2. Grade and Stage of Transitional Cell Carcinoma and Urine Nuclear Matrix Protein 22 (NMP22) Levels in Each Tumor Grade and Stage

Tumor Class	Patients (%)	Mean NMP22, U/mL	NMP22 Range, U/mL
Stage			
CIS	1 (1.3)	11.00	…
Ta	16 (21.1)	8.17 ± 3.87	2.5 to 17.0
T1	30 (39.5)	17.81 ± 16.91	4.5 to 76.5
T2	9 (11.8)	39.39 ± 38.37	5.5 to 105.0
T3	20 (26.3)	43.50 ± 55.39	3.7 to 232.0
Grade			
1	39 (51.3)	14.01 ± 15.25	2.5 to 76.5
2	11 (14.5)	34.50 ± 33.37	5.0 to 105.0
3	26 (34.2)	37.48 ± 50.54	2.5 to 232.0

CIS indicates carcinoma in situ.

Table 3. Diagnostic Value of Urine Nuclear Matrix Protein 22 Level in Transitional Cell Carcinoma (TCC) of Bladder

Tumor Class	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %	Accuracy, %
Stage					
Ta	31.3	86.7	33.3	85.5	76.9
T1	90.0	86.7	73.0	95.6	76.6
T2	66.7	86.7	37.5	95.6	84.5
T3	90.0	86.7	64.3	97.0	87.4
Grade					
1	66.7	86.7	72.2	83.3	79.8
2	81.8	86.7	37.5	97.0	86.0
3	84.6	86.6	68.7	94.2	86.1
Superficial TCC	70.2	86.7	76.7	82.3	80.3
TCC (overall)	75.0	86.7	85.1	77.4	80.8

sensitivity of urine NMP22 to detect stage Ta tumors appeared to be only 31.3%. On the other hand, the sensitivity for grade 1 tumors was 66.7%.

DISCUSSION

Although cystoscopy is the "gold standard" method for detecting bladder cancer, it is invasive and expensive.[11] On the other hand, urine cytology is not sensitive enough for the low-grade disease; therefore, a noninvasive tool is necessary to be introduced to help the urologist in diagnosis and treatment planning in patients with bladder tumor. Several tumor markers have been employed for this purpose with different accuracy levels, including bladder tumor antigen, urinary bladder cancer antigen, telomerase, hyaluronic acid, and hyaluronidase.[12-14] However, none of these markers are sensitive enough to be recommended for daily practice.[14]

The use of NMP22 as a marker in urine for diagnosis of TCC has been proposed by a few studies.[15-20] Our study revealed that the mean level of urine NMP22 in the patients with active bladder tumor was 5 times higher than that in individuals with an intact urinary tract. A significant relationship was also found between the level of urine NMP22 and stage and grade of the tumor. In terms of diagnostic accuracy, we found acceptable sensitivity and specificity. Table 4 highlights the results of the diagnostic value of urine NMP22 in several studies. Soloway and colleagues[15] found a sensitivity of 100% for invasive disease and 70% overall and a negative predictive value of 86% by urine NMP22. Shariat and associates[16] studied NMP22 in 209 patients and controls and determined a sensitivity of 50% and a positive predictive value of 81%. Zippe and colleagues[17] studied 18 patients with biopsy confirmed bladder cancer and 312 with benign conditions of the bladder and found the highest sensitivity of 100% and specificity of 85%. However, the positive predictive value was low (29%) in this study. There are also some other published studies on urine NMP22 with various reports of sensitivity (48.5% to 85%).[18-20] The current study yielded diagnostic test values above 75%; however, it was also shown that the urine NMP22 test had significantly low sensitivity for detection of the tumors with Ta stage.

CONCLUSION

We found that measurement of urine NMP22 is a noninvasive, highly sensitive, and specific method for detecting TCC of the bladder and evaluating its grade and stage. However, this test cannot be trusted in detection of superficial bladder cancer, especially stage Ta cancer. The promising results for this tumor marker make its further evaluation for clinical usage beneficial.

CONFLICT OF INTEREST

None declared.

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Table 4. Reported Diagnostic Value of Urine Nuclear Matrix Protein 22 Level in Literature

Study	Participants	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %
Current study	151	75.0	86.7	85.1	77.4
Soloway et al[15]	90	78.5	75.9	57.5	86.1
Shariat et al[16]	209	50.0	50.0	81.0	57.0
Zippe et al[17]	330	100	85.0	29.0	100
Atsu et al[18]	202	78.1	66.0	59.5	82.5
Eissa et al[19]	168	85.0	91.3	89.5	87.5
Stampfer et al[20]	231	48.5	91.8	65.3	84.9

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