Turk J Med Sci
36 (2006) 93-96

CLINICAL INVESTIGATION

Determination of Nuclear Matrix Protein 22 Levels in Cystitis, Urothelial Dysplasia and Urothelial Carcinoma

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Received: August 10, 2005

Abstract: Urothelial carcinomas (UC) are the most common cancer type of the urinary system. The early diagnosis and treatment of UC are very important.The aim of this study was to evaluate the importance of the nuclear matrix protein 22 (NMP22) in early diagnosis of UC and to present its variation in cystitis and urothelial dysplasia (UD).

Twenty patients with UC, 20 patients with UD and 20 patients with cystitis diagnosed by urinary cytological examination were enrolled in this study. In addition, 20 healthy people constituted the control group. Urine samples were collected from all patients and control subjects for NMP22 measurement and cytology. Those who were diagnosed with cytological malignancy were also histologically evaluated for accuracy. Additionally, the NMP22 levels of all participants were measured by ELISA.

When compared according to the cytologic results, the NMP22 levels in the UC group were found significantly higher compared to those having UD, cystitis and control group ($P < 0.05$). No statistically significant difference was found between other groups.

It has been concluded that NMP22 can be used in the early diagnosis and determination of recurrences in UC. However, as the NMP22 level may be increased by other factors such as UD, cystitis, bladder stones and non-urothelial carcinomas of bladder, it is suggested urinary cytology that showed be jointly used with NMP22 measuring.

Key Words: Nuclear matrix protein 22, urinary cytology, urothelial carcinoma, urothelial dysplasia, cystitis

Introduction

The bladder urothelial carcinomas (UC) are those which appear in men and women at the 4th and 8th frequency, respectively of all human carcinomas. Approximately 70-80% of these carcinomas are localized in the epithelium or only invasive in the lamina propria. The superficial UC are treated by transurethral resection or fulguration. However, a large portion (50-75%) of them reoccur in a different location within five years. Most of these recurrences take place in the first year (1-4). The existence of recurrences in UC of bladder is controlled by cystoscopy and urine cytology every 3 months during the first two years after diagnosis. In low grade papillary UC, cystoscopy is considerably invasive. More sensitive, especially quantitative and objective non-invasive techniques are required to determine low grade tumors (1,2,5).

Nuclear matrix protein (NMP) was first identified by Berezney and Coffey in the nucleus of rat liver cell (6). Nuclear matrix is composed of insoluble nuclear lamina, pore complexes, residual nucleolus, and internal ribonucleoprotein network (6,7). By determining the three-dimensional structure of the nucleus, the nuclear matrix has an important role in DNA replication and transcription, RNA formation, and gene organization and expression (3,4,6,8,9). Nuclear matrix proteins are a new class of tumor markers which have increased the efficacy of detection and treatment of cancer (10).

NMP22 levels in the urine of patients with bladder cancer are estimated to be at least 25 times higher than the urine of the normal subjects (9). It has been indicated that NMP22, which is spontaneously determined by ELISA method, is an important indicator especially in rapid recurrences following operation (3-5,8).

In this study we aimed to investigate the significant value of NMP22 in UC, urothelial dysplasia (UD) and cystitis, which are diagnosed by urine cytology.

Materials and Methods

A total of 60 patients (46 men- 76.67%, 14 women- 23.33%): 20 UC (15 men- 75%, 5 women- 25%), 20 UD (13 men- 65%, 7 women- 35%) and 20 cystitis (17 men- 85%, 3 women- 15%) who were diagnosed by urine cytology at Fırat University Firat Health Centre Urology Policlinics, and 20 healthy people (14 men- 70%, 6 women- 30%) were enrolled in this study. The patient and control group subjects were informed on the study and written consent was obtained.

Urine samples were collected between 08:00 and 12:00 a.m. and stabilized immediately for NMP22 measurement and cytology. Those diagnosed as malignant by cytology were also proved so histopatologically. Additionally, the NMP22 levels of all participants were measured by ELISA (Matritech NMP22 test kit Newton, MA-USA) method. Preparations were processed by cytospin from urine samples. Some of these samples which were prepared with cytospin were then fixed by alcohol and stained by Papanicolaou and the other slides were dried in air and stained by May Grunwald Giemsa. The samples were then examined using a Leica MMLB (Germany) microscope by the same pathologist.

Kruskal-Wallis test and Mann-Whitney U test were used for statistical analysis and a threshold value as $P < 0.05$ was considered to be statistically significant.

Results

Mean age of the cases were found to be 56 ± 14 years in UC, 50 ± 13 years in UD, 50 ± 15 in cystitis groups, and 49 ± 11 in healthy person.

It was found that NMP22 levels of patients, who were cytologically diagnosed as malignant, were significantly higher than those patients with UD and urinary system infection, and control group ($P < 0.05$). No significant differences were seen between the other groups ($p> 0.05$). The mean ages and the values of NMP22 levels of all participants are given in the Table.

We found that the sensitivity and specificity of this test were 44% and 56%, respectively. The cut off value was 2.60 U/mL.These results were given in Figure.

Table. Urinary NMP22 values (Units/mL).

Groups	n	Age (years; Mean ± SD)	NMP22 (Mean) (U/mL)	SD	SE	Max	Min
Controls	20	49 ± 11	5.02	5.28	1.32	16.90	0.50
Cystitis	20	50 ± 15	22.17	33.90	6.41	120.30	0.20
UD	20	50 ± 13	39.58	104.70	31.58	354.12	0.90
UC	20	56 ± 14	111.50*	142.90	41.27	392.10	1.50
Total	80	51 ± 14	36.90	83.01	10.14	392.10	0.20

*: $P < 0.05$

UD: urothelial dysplasia, UC: urothelial carcinoma, SD: standard deviation, SE: standard error, Max: maximum, Min: minimum

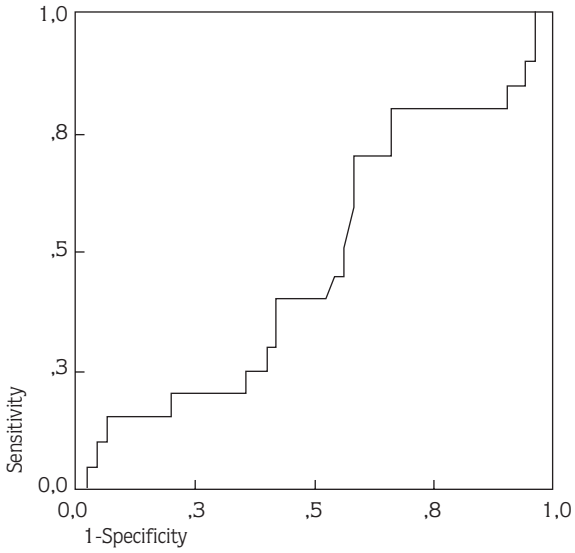

Figure. Sensitivity and specificity calculation according to ROC curve.

Discussions

In order to follows recurrency of tumor, in patients with UC cystoscopy, barbotage of bladder and urine catheterization can be use for getting urine samples. However, these investigation are rather invasive and less sensitive investigations compared to histopathological diagnosis methods. It is necessary to repeat these tests at certain periods after treatment as there is a strong possibility that the superficial carcinomas may recur after operation. Especially in low grade tumors, the diseases require effective treatment and non-invasive and objective diagnosis of tests (2,4). The spontaneous urine cytology is a non-invasive method. However, its sensitivity is about 60% in diagnosing especially low grade UC (11).

Various urinary indicators have been investigated in order to diagnose and follow up UC. Some of those are NMP, bladder tumor antigens, receptor of autocrine motility factor, and fibrinogen destruction factor. M344 and Lewis X, which are urinary cytologic biomarkers, are known as additional indicators. It has been pointed out that these indicators can be determined at the initial stage of tumor or in recurrences (2,3,5).

In previous studies, it has been shown that urine NMP22 measurements can be used as a non-invasive, sensitive, objective and qualitative marker, in the determination of postoperation recurrences of UC (2-4,8). However, the NMP22 levels are affected by inflammation, benign urological diseases, age, race, gender and smoking. It has been determined that NMP22 increases in prostate, colon, breast, kidney, bone, and other tissues or in occult extravesical neoplasm. However, the increase of NMP22 in these conditions is lower compared to the increase in UC. It may also increase following systemic chemotherapy used for other malignancies (5,7,12).

In our study, the NMP22 levels measured in UC patients were statistically significantly higher compared to levels measured in UD and cystitis patients, and in control group. However, the levels measured in some UC patients were similar to those of other groups. Due to grey zones between malignant tumors and other groups, it was considered that the measurement of NMP22 was not a good enough criterion to decide the malignancy. The sensitivity and specificity of this test is not high enough for the diagnosis of UC.

Additionally, the NMP22 levels of the patients with different grades of UD initial malignancy, were lower than those of patients with cystitis. No significant difference was observed in the NMP22 levels of patients with dysplasia and cystitis, and control groups. These results show that determination of NMP22 levels is not a very useful criterion in diagnosing bladder precancerous lesions.

It has been reported that NMP22 shows more than 80% (13) or 37-41% (14) false-positive results in benign inflammatory or infectious diseases, in the presence of bladder stones, in cases of urinary system infections, or other genitourinary malignancies.

The conventional method for evaluating initial stage bladder cancers in the postoperative period is cystoscopic evaluation or cytologic examination of spontaneous and catheterized urine or bladder washing water. Urinary cytology has low sensitivity in low grade papillary and flat lesions. It also has the risk of not specifying the diagnosis of invasive lesions. The evaluation of different the same or pathologists at different times may also be different (11). Intravesical chemotherapy, radiotherapy and repetitive catheterization may lead to wrong evaluation of UC diagnosis by causing changes in exfoliative cells in urinary infections and kidney stones (2,5).

Soloway et al (5) have reported that the risk of malignant disease is low if the NMP22 level is lower than 10.0 U/mL, while it is high if the level is higher than 20.0 U/mL. In the last case, cystoscopic examination and

pathologic evaluation have been suggested. On the other hand, Stampfer et al (2) reported that the lowest value for NMP22 is 6.4 U/mL and they suggested that immediate cystoscopic examination will be necessary if the levels rise above this critical value.

In conclusion, urine NMP22 levels are significantly higher in UC, and its measurement can be used in examining the recurrences. However, as it can be increased by other factors, it is better to combine it with cytologic examinations.

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References

1. Wingo PA, Tong T, Bolden S. Cancer statistics 1995. Cancer J Clin; 45: 8-30, 1995.
2. Stampfer DS, Carpinito GA, Rodriguez-Villanueva et al. Evaluation of NMP22 in the detection of transitional cell carcinoma of the bladder. J Urology 159: 394-8, 1998.
3. Carpinito GA, Stadler WM, Briggman JV et al. Urinary nuclear matrix protein as a marker for transitional cell carcinoma of the urinary tract. J Urology 156: 1280-5,1996.
4. Chen YT, Hayden CL, Marchand KJ et al. Comparison of urine collection methods for evaluating urinary nuclear matrix protein, NMP22, as a tumour marker. J Urology 158: 1899-1901,1997.
5. Soloway MS, Briggman JV, Carpinito GA et al. Use of a new tumour marker, urinary NMP22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. J Urology 156: 363-7, 1996.
6. Berezney R, Coffey DS. Identification of a nuclear matrix protein. Biochem Biophys Res Commun 60: 1410-17, 1991.
7. Lakshmanan Y, Subong ENP, Partin AW. Differential nuclear matrix protein expression in prostate cancers: Correlation with pathologic state. J Urology 159: 1354-58, 1997.
8. Partin AW, Briggman JV, Subong ENP et al. Preliminary immunohistochemical characterization of a monoclonal antibody (pro: 4-216) prepared from human prostate cancer nuclear matrix proteins. Urology 50:800-8, 1997.
9. Keesee SK, Briggman JV, Thil G et al. Utilization of nuclear matrix proteins for cancer diagnosis. Crit Rev Eukary Gene Exp 6: 189-214,1996.
10. Carpitino GA, Stadler WM, Briggman JV et al. Urinary nuclear marix protein as a marker for transitional cell carcinoma of the urinary tract. J Urol 156:1280-1285, 1996.
11. Curry JL, Wojcik EM. The effects of the current World Health Organisation/ International Society of Urologic Pathologists bladder neoplasm classification system on urine cytology results. Cancer Cytopathol 96: 140-5, 2002.
12. Huang S, Rhee E, Patel H et al. Urinary NMP22 and renal cell carcinoma. Urology 55:227-30, 2000.
13. Sharma S, Zippe CD, Pandrangi L et al. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. J Urol 162:53-7, 1999.
14. Friedrich MG, Hellstern A, Toma MI et al. Are false positive urine markers for the detection of bladder carcinoma really wrong or do they predict tumour recurrence? Eur Urol 43:146-51, 2003.