**Original Article / Orijinal Araştırma**

# Comparison of Nuclear Matrix Protein (NMP22) test with cystoscopy and urine cytology in follow-up of patients with superficial bladder cancer

# Yüzeyel mesane tümörlerinin takibinde sistoskopi ve üriner sitoloji ile NMP22'nin karşılaştırılması

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**ÖZET**

**Amaç:** Mesane tümörü tespitinde NMP22 testini, sistoskopi ve sitoloji sonuçları ile birlikte değerlendirmek. NMP22 değerinin; intravezikal BCG uygulaması, tümör grade ve tümör stage ile ilişkisini karsılastırmak.

**Hastalar ve Metot:** Mesane tümörü nedeniyle sistoskopi takibindeki toplam 78 hasta çalışmaya dahil edildi. Kontrol sistoskopisi yapılan hastaların idrarında NMP22 bakıldı. Mesane yıkantı suyundan sitoloji alındı. NMP22 testinin ve sitolojinin duyarlılığı, seçiciliği, PPD (Pozitif kestirim değeri), NPD (Negatif kestirim değeri) ve toplam tanı değeri hesaplandı.

**Bulgular:** Hastaların ortalama yaşı 56 ± 8 idi. NMP22 'nin duyarlılığı %63,6, seçiciliği %95,4, PPD %70, NPD 93,3, Toplam tanı değeri %90,7 olarak bulunmuştur. NMP22 ve sitoloji karşılaştırıldığında duyarlılık NMP22'de yaklaşık 2 kat fazla bulunmuştur. Toplam tanı değeri bakımından ise aralarında anlamlı fark bulunamamıştır (p> 0,3). Stage ve NMP22 değerleri bakımından anlamlı fark bulunamamıştır (P > 0.4). Ancak grade arttıkca NMP22 değerleri anlamlı olarak artmaktadır (P=0,03). NMP22 sonuçları intrakaviter immünoterapiden etkilenmemiştir.

**Sonuç:** NMP22 üriner sitolojiden daha duyarlı bulunmuştur. NMP22 intrakaviter immünoterapiden etkilenmemiştir. Tümör grade'i arttıkca NMP22 değerleri artmıştır. Ancak tümör stage ile anlamlı ilişki saptanmamıştır. NMP22 testi kontrol sistoskopisinin yerini almada yetersiz kalmaktadır. Ancak daha iyi bir tümör belirleyiciye sahip oluncaya kadar, NMP22 testi kullanılabilir. NMP22 testi negatif gelen hastalarda kontrol sistoskopi aralıkları, açılabilir.

**Anahtar Kelimeler:** NMP22, mesane kanser, sistoskopi ve sitoloji

**ABSTRACT**

Purpose: To evaluate the clinical performance of Nuclear Matrix Protein (NMP22) test and to compare it with cystoscopy and urine cytology in follow-up of patients with superficial bladder cancer. We also investigated in relation of NMP22 value with intravesical BCG ( Bacillus Calmette Guerin) administration, tumor grade and tumor stage.

Patients and Method: A total of 78 patients in cystoscopy follow-up due to superficial bladder cancer were included in the study. NMP22 was scanned in urines of patients whose control cystoscopy was made. Cytology was taken from bladder eluate. Sensitivity, specificity, PPD (Positive Predictive Value), NPD (Negative Predictive Value) and accuracy of NMP22 test were calculated.

Results: The mean age of patients was 56 ± 8. Sensitivity of NMP22 was found %63.6, its specificity was %95.4, PPD was %70, NPD was 93.3, and accuracy was % 90.7. When NMP22 and cytology were compared, sensitivity of NMP22 was found by approximately 2 times more. In terms of accuracy, no significant difference was found between them (p>0.3). In terms of stage and NMP22 values, no significant difference was found (P > 0.4). However, as long as grade increases, NMP22 values significantly increase (P=0.03). NMP22 results were not influenced by intracavitary immunotherapy.

Conclusion: NMP22 was found more sensitive than urinary cytology. NMP22 were not influenced by intracavitary immunotherapy. As long as tumor grade increased, NMP22 values increased. But no significant relation with tumor stage was detected. The NMP22 test remains incapable for the supplanting of control cystoscopy. Until a better tumor marker was acquired, NMP22 test can be used. In patients with negative NMP22 test, control cystoscopy intervals may prolonged.

**Key words:** NMP22, bladder cancer, cystoscopy and urine cytology

## Introduction

Bladder cancer represents the fourth most common cancer in men and the eighth most common cancer in women. It is an important cause of morbidity and mortality (1–4). Cystoscopy and voided urine cytology are effective methods for diagnosis and follow-up of superficial bladder cancer. But, Cystoscopy is an invasive and expensive procedure tolerated hardly by patients. Urine cytology also has several drawbacks such as the need for a cytopathologist to be trained to make evaluations , inadequate sensitivity, especially for low-grade tumors (5,6). So, A wide range of alternative urinary cytological techniques and new tumor markers have been proposed and studied for the detection of recurrent bladder tumors. One of these tumor markers is also nuclear matrix protein (NMP22).

NMP22 is released at the time of cell death and is found 10-25 times more in uroepithelial cancer cells than normal cells (7–9). NMP22 can be identified by Mab 302-22 and Mab 302-18 monoclonal antibodies produced from rats immunised with NMPs obtained from cancer cells (8,10). NMP22 in tumor cell nucleus is released through apoptosis during cell death and is detected by both monoclonal antibodies mentioned above, so, is able to be titrated (11).

The purpose of this trial was to evaluate sensitivity, specificity, positive and negative predictive values of NMP22 in urine samples of the patients performed TUR (Transurethral resection) and intracavitary BCG (Bacillus Calmette Guerin) immunotherapy due to superficial bladder cancer and to compare the NMP22 test with voided urine cytology and cystoscopy for the detection of recurrent bladder cancer. We also evaluated to association of NMP22 value with intravesical BCG application, tumor grade, and tumor stage.

## Patients and Methods

A total of 78 patients with previously diagnosed superficial bladder cancer (TNM stages Ta–T1, G1–G3, N0, M0) were prospectively enrolled in the study. All of the patients were on follow-up for 3 months cystoscopic control protocol for 3 months after performed transurethral resection (TUR). To research effects of intravesical immunotherapy on NMP22 values, patients were divided two groups. The mean age of the patients in group I was 55 years. The mean age of the patients in group II was 57 years. The Group I included 38 patients on follow-up protocol after performed TUR due to superficial bladder tumor, 38 patients on follow-up protocol by administration of intravesical immunotherapy (BCG) also were added into Group II. Patients with urinary tract infections, open tumor resection were previously performed, urinary system calculus, a history of bladder interposition and other malignancies were excluded from the study.

A single voided urine sample was collected just prior to cystoscopy. Two aliquots were divided from this sample, one of the NMP22 test and the other for complete urine analysis. The NMP2 assay was performed according to the instructions provided in the NMP22 BladderChek test kit (Matritech Inc. Cambridge. Mass. U.S.A.). After then the patient was accessed by rigid cystoscopy shaft, bladder eluate was taken for cytologic examination by irrigation of the patient's bladder 5 times at minimum, using 50 cc normal saline with a glass injector for bladder washing. Urine taken for NMP22 test kit was removed from its sludge by centrifuging in cooling centrifuge at 500-1000 x G for 15 minutes at 10-15 centigrade degree and was refrigerated in a plastic tube at -80 centigrade degree to study later.

Measurements were made by computerized method, using linear regression analysis. For NMP22 measurements, the test produced by, where Mab 302-18 and Mab302-22 was used as a monoclonal antibody, was used. Calibrators, controls and NMP22s in stabilizing patient's urine samples react with antibodies in a condition covered with microplate cells. NMP22 antigens involved after washing react with secondary antibodies marked with digoxigenin (DIG). After a second washing, antibodies marked with DIG are fixed with anti-digoxigenin antibody coupled by Horseradish Peroxidase (HRP-SAD), using O-phenylenediamine (OPD). The reaction is completed by the addition of 2M sulfuric acid (2M H2SO4). The concentration of antigen in urine is in direct proportion to emergent density color and the real concentration was specified in standard curve. NMP22 results were calculated by a computer-assisted method. Pathologies of patients where tumor was identified after cytoscopic examination were detected applying TUR. All test results were

recorded with cystoscopy and cytology results. The cut-off value was accepted as 10U/ml.

Statistical analysis was performed using the Fisher-Exact Test chi-square test, with $P < 0.05$ being considered.

**Results**

Of the 78 patients, 62 of patients were males, 16 were females. The mean age of patients was 56 (range 26-77 years). Distributions of patients with grades and stages were as follows: 7 grade-1 patients (9.2% ), 58 grade-2 patients (76.3%) and 11 grade-3 patients (14.4%), 50 PTa patients (65.7%), 26 PT1 patients (34.2%).

In cystoscopic controls of 78 patients received for the study, tumor was detected in 11 patients. NMP22 value was over the cut-off value in 10 patients. In 7 of these patients, the tumor was also cystoscopically identified. In conclusion, sensitivity of NMP22 was found as 63.6%, specificity as 95.4%, PPD as 70%, NPD as 93.9%, accuracy as 90.7% (Table-I, Figure-I).

In cytologic examination of patients, positivity was detected in 6 patients. In 4 of these patients, the tumor was also cystoscopically observed. We carried out a punch biopsy in 2 patients who have cytology positivity but we did not identify a tumor in cystoscopy. In one of the patients, while punch biopsy was resulted as erosive cystitis, it was resulted as normal mucosa in another patient. In conclusion, sensitivity of cytology was found as 36.4%, specificity as 96.6%, PPD as 66.7%, NPD as 90%, accuracy as 88.2% (Table-I, Figure-I).When NMP22 and cytology were compared, sensitivity of NMP22 was statistically found significantly higher ($p=0.01$). In terms of accuracy , no significant difference was found between them ($p> 0.3$).

Relation to NMP22 values with histopathologic grade and stage was researched. As long as grade increases, NMP22 values significantly increase ($P > 0.03$). However, no significant difference was found between PTa and PT1 in terms of NMP22 values ($P > 0.4$).

Effects of intravesical immunotherapy on NMP22 values were researched and statistically, no difference was found in terms of accuracy and sensitivity ($p > 0.4$ and $p > 0.3$) (Table-II).

**Discussion**

Bladder cancer is a common disease that causes significant morbidity and mortality throughout the world. Cystoscopy is ihe gold standart in follow-up of the patients with bladder cancer at 3 monthly intervals for 2 years after the initial diagnosis. Although cystoscopy is the gold standard for detecting bladder cancer, it is invasive and relatively expensive (12). Voided urine cytology also is the standard non-invasive method for diagnosis in the detection of bladder carcinoma (13,14). One of the greatest successes of urine cytology is to detect urothelial carsinoma at preclinic phase before cystoscopic and radiologic diagnoses (15).

**TABLE I**: Values of sensitivity, specificity, PPD, NPD and accuracy of cytology and NMP22 test

| | NMP22 test (%) | Cytology (%) |
|---|---|---|
| Sensitivity | 63.6 | 36.4 |
| Specificity | 95.4 | 96.6 |
| PPD | 70.0 | 66.7 |
| NPD | 93.9 | 90.0 |
| Accuracy | 90.7 | 88.2 |

Before cellular changes such as dysplasia in the bladder during follow-up, atypia are cystoscopically observed, they can be specified at the rate of 15-20% by urine cytology (16). Before high-grade sessile neoplasms become visible by endoscopy, they can be described by urine cytology. Despite of this success of urine cytology, its most important disadvantage is that sensitivity, particularly in the lower stage and grade tumors, is placed at a very wide range like 26-100% (17). The sensitivity is closely related to histopathologic grade; as long as grade increases, sensitivity also increases. In cases of carcinoma in situ, the sensitivity elevates to 100%. In our study, the sensitivity of cytology was found as 36.4%, specificity as 96.6%, PPD as 66.7%, NPD as 90%.

**TABLE II:** Statistical assessment of effects of intracavitary immunotherapy on NMP22 values

| | NMP22 test (Group I) % | NMP22 (Group II) % |
|---|---|---|
| Sensitivity | 62.5 | 66.7 |
| Specificity | 96.7 | 94.3 |
| PPD | 83.3 | 50.0 |
| NPD | 90.6 | 97.1 |
| Accuracy | 89.4 | 92.1 |

Non-invasive urine markers can offer an alternative to the standard mode of detecting bladder cancer or they can be used as an adjunct to cystoscopy (18). In the previous many clinical studies, it was found that

patients with bladder cancer may have had urinary levels of NMP22 (19),(20). The level of NMP22 in urine is influenced by several factors. The primaries of these are various inflammatory diseases (urinary system infection, urinary system calculus disease etc.), malignant diseases of urinary system and other organs (19). Also in our study, patients with an additional factor to may effect on the level of NMP22 in urine were excluded from the study.

Soloway *et al* (18)-(21) found that the mean of NMP22 values of patients as 5.45 U/ml to urinary NMP22 values were measured during postoperative follow-ups of patients with bladder tumor. This value was found as 20.81U/ml in cases where the tumor was detected. In this study, NMP22 values of patients, where intravesical chemotherapy was administered, were found significantly higher in proportion to patients, where no intravesical chemotherapy was administered. In our study, while the mean of NMP22 values of 65 patients, where no tumor was detected, was found as 6.14U/ml, this value was found as averagely 24.04U/ml in 11 patients, where the tumor was detected. These values show a correlation with the study of Soloway. We also found that there was no significant difference between groups of patient receiving and not receiving intravesical chemotherapy according to sensitivity and specificity of NPD. But, PPD was found significantly higher between two groups. However, in terms of total diagnostic value, no difference was detected between these two groups. In this study of Soloway, NMP22 reference value to be used for discrimination in cases with tumor from those who without tumor was specified as 10U/ml . In cases with tumor by this reference value, sensitivity was found 69.7%, specificity as 78.5%, PPD as 57.5%, NPD as 86.1%. In our study, these values were detected as follows: sensitivity 63.6%, specificity 95.4%, PPD 70%, NPD 93.9%.

The sensitivities of NMP22 were found as 56% and 80% in stages of Ta and T1 by Sinan S. *et al* (21) As long as grade increases, NMP22 values increases, but, while no statistically difference was observed between mean values of NMP22 in grade-1 and grade-2 tumors in conclusion of statistical analysis, the mean NMP22 value found for grade-3 tumor was statistically found significantly higher in proportion to other two grades. As a result of this study, NMP22 test was reported to be more sensitive than cytology. Also in other studies, NMP22 was reported to be more sensitive than cytology (6,22). In our study too, while sensitivity of cytology was 36.4%, the sensitivity of NMP22 was found as 63.6% and so, statistically, NMP22 was detected to be more sensitive than cytology. Also, in our study, as long as the grade increased, positivity of NMP22 was observed to increase. However, in PTa and PT1 tumors, no statistical difference was detected to be between mean NMP22 values.

**Conclusion**

NMP22 was found more sensitive than urinary cytology. Results of NMP22 were not influenced by intracavitary immunotherapy. As long as tumor grade increased, an increase in values of NMP22 was detected. No statistical difference was detected between PTa and PT1 in terms of NMP22 values. It is clear that the NMP22 remains incapable for the supplanting of control cystoscopy. However, until a tumor marker with 100% sensitivity and specificity is acquired, the necessity to be benefited from present opportunities is a non-negligible truth. In patients whose NMP22 tests are negative, intervals of control cystoscopy may be prolonged. But, cystoscopy should be definitely performed for all patients whose NMP22 values are found positive.

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