The relationship between microscopic hematuria and bladder cancer: are currently available diagnostic methods reliable?

Mikroskopik hematüri ile mesane kanserinin ilişkisi: Bugün için kullanılan tanı yöntemleri güvenilir mi?

Lokman Irkılata[1], Sinan Sözen[2], Hüsnü Tokgöz[3], Yakup Kordan[4], Mustafa Kıraç[5], Metin Onaran[2], Turgut Alkibay[2], İbrahim Bozkırlı[2]

[1]Kastamonu Government Hospital, Department of Urology, Kastamonu, Turkey
[2]Gazi University School of Medicine, Department of Urology, Ankara, Turkey
[3]Karaelmas University School of Medicine, Department of Urology, Zonguldak, Turkey
[4]Uludağ University School of Medicine, Department of Urology, Bursa, Turkey
[5]Yozgat Government Hospital, Department of Urology, Yozgat, Turkey

Abstract

Objective: This study aimed to evaluate the sensitivity and specificity of radiological diagnostic methods, urinary biomarkers and urine cytology for patients with microscopic hematuria in the diagnosis of bladder cancer.

Materials and methods: A total of 150 patients with the initial diagnosis of microscopic hematuria were included in the study. Patients were prospectively evaluated in terms of urinary symptomatology, physical examination, smoking habits, intravenous pyelography (IVP), abdominal ultrasonography (USG), Nuclear Matrix Protein 22 (NMP22), urine cytology and NMP22 BladderChek® test. The cut-off point for NMP22 test was accepted as 10 U/mL.

Results: Mean age of the study population was 49.8±10.79 years with a male to female ratio of 1.08 (78/72). Overall, 10.7% (n=16) of patients had pathological diagnosis of bladder cancer, whereas 89.5% of the study population had no etiology for microhematuria. IVP was considered as the most sensitive and specific test in the diagnosis of bladder cancer. However, voided urine cytology was the least sensitive test, although it has the same specificity with IVP. The best sensitivity and specificity values were demonstrated when NMP22 and abdominal USG were used in double-test combinations.

Conclusion: In microscopic hematuria, the combination of radiological methods with tumor markers such as NMP22 BladderChek® test, provides 100% sensitivity, and protects nearly half of cases from unnecessary cystoscopies.

Key words: Abdominal ultrasonography; bladder cancer; intravenous pyelography; microhematuria; tumor markers.

Özet

Amaç: Bu çalışmada, mikroskobik hematürisi olan hastalarda mesane kanseri tanısında radyolojik tanı metodları, idrar tümör belirleyicileri ve idrar sitolojisinin duyarlılık ve özgüllüğünün değerlendirilmesi amaçlanmıştır.

Gereç ve yöntem: İlk inceleme sonrası mikroskobik hematüri tanısı alan toplam 150 hasta çalışmaya alınmıştır. Hastalar prospektif olarak idrar semptomatolojisi ve sigara alışkanlığı yönünden sorgulanarak fizik muayene, intravenöz pyelografi (IVP), batın ultrasonografisi (USG), Nükleer Matriks protein 22 (NMP22), idrar sitolojisi ve NMP22 BladderChek® testleri ile değerlendirildi. NMP22 testi için eşik değer olarak 10 U/mL alındı.

Bulgular: Yaş ortalaması 49.8±10.79 yıl olup çalışma grubundaki erkek/kadın oranı 1.08 oranında tespit edilmiştir (78/72). Hastaların %10.7 sinde (n=16) alınan biyopsi sonucunda mesane kanseri tespit edilirken %89.5'inde mikrohematüri için bir sebep bulunmamıştır. IVP, mesane kanseri teşhisinde en duyarlı ve özgül test olarak bulunmuştur. Ancak idrar işeme sitolojisi IVP ile aynı özgüllük değerine sahip olmasına rağmen en az duyarlı test olarak izlenmiştir. İkili test kombinasyonlarında, en yüksek duyarlılık ve özgüllük değerleri NMP22 ve batın USG testleriyle sağlanmıştır.

Sonuç: Mikroskobik hematüride radyolojik tanı yöntemlerinin, NMP22 BladderChek® testi gibi tümör belirteçleriyle kombine kullanılması %100 duyarlılık sağlarken hastaların yaklaşık yarısını gereksiz sistoskopiden korumaktadır.

Anahtar sözcükler: Abdominal ultrasonografi; intravenöz pyelografi; mesane kanseri; mikrohematüri; tümör belirteçleri.

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Bladder cancer is the sixth most frequent malignant disease in the world.[1] Among the bladder cancer cases, 90% take the pathological diagnosis of transitional cell carcinoma. Of these, most is presented as superficial transitional cell carcinoma initially.[2] Asymptomatic microscopic hematuria is described approximately in 2.5-20% of the cases without urologic symptomatology.[3,4]

Carson et al.[5] demonstrated urological malignancy in 3 to 13% of patients who were evaluated for microscopic hematuria. Thus, we need rapid, highly sensitive and specific methods to prevent expensive and unnecessary diagnostic procedures in patients undergoing first clinical evaluation for microscopic hematuria.

During routine clinical procedures, urine culture, voided urine cytology, abdominal ultrasonography (USG), intravenous pyelography (IVP) and computerized tomography are used in the initial diagnosis and follow-up. In recent years, specific urine biomarkers are being investigated, and many reports started to be published with acceptable sensitivity and specificity values, namely Nuclear Matrix Protein 22 (NMP22), BTA, M344 antigen, and a relatively new test NMP22 BladderChek®.

In this prospective study, we aimed to evaluate the sensitivity, specificity and accuracy of radiological diagnostic methods like IVP and abdominal USG with the urinary biomarkers; NMP22 and NMP22 BladderChek® in combination with voided urine cytology for patients with microscopic hematuria in the diagnosis of bladder cancer.

Materials and methods

A total of 150 patients with the initial diagnosis of microscopic hematuria were included in the study. All patients were referred from the Nephrology Department after exclusion of urolithiasis, urinary tract infections, and renal parenchymal disease in order to form a homogenous study population to prevent false positive and false negative results. Financial support for this study was given by Gazi University, Scientific Projects Research Unit.

Patients were prospectively evaluated in terms of urinary symptomatology, physical examination, smoking habits, IVP, abdominopelvic USG, NMP22, voided urine cytology, and NMP22 BladderChek® test.

The patients having at least two consecutive hematuria (>3 red blood cells per high power field under 40x light microscope) were included in the study. The cut-off point for NMP22 test was accepted as 10 U/mL. All patients underwent both flexible and rigid cystoscopic examination under general anesthesia at the end of the study period in order to evaluate the diagnostic value of flexible cystoscopy and to compare the outcomes with the gold standard technique, which is rigid cystoscopy. Cold cup biopsies were taken from bladder mucosa if there is any suspicion for premalignant lesion. If atypical cells were seen in the cytologic evaluation of the voided urine sample, random cold cup biopsies were also taken.

Voided urine samples were collected and divided into three parts; the first one was transported to the cytopathology laboratory for cytologic evaluation and the second one to the urology laboratory for the NMP22 BladderChek® test. For the third one, a urine collection kit of urine stabilizers containing protein stabilizers, protease inhibitors, and buffers was used for the NMP22 test. The samples were frozen at –20°C after collection for NMP22 measurement. The endoscopist, pathologist, cytologist, and physician performing the NMP22 BladderChek® and NMP22 tests were all blinded to the results of the other tests.

NMP22 test kit using monoclonal antibodies 302-18 and 302-22 (Matritech, Cambridge, MA, USA) were used for the measurement of urinary NMP22.

The Matritech NMP22 BladderChek® test technology uses a lateral flow immunochromatographic strip encased in a plastic cartridge to detect nuclear matrix protein qualitatively in the patient's urine sample. The antibodies in the lateral flow immunochromatogaphic strip are monoclonal antibodies (MAbs) raised against (nuclear mitotic apparatus protein (NuMA) which is a component of the nuclear matrix extracted from a cervical cancer cell line by the method of Fey and Penman.[6] Two different MAbs are used, one as a capture antibody and one as a reporter antibody. Four drops of voided urine is added to the sample well of the cartridge and allowed to react for 30 min. There are no other procedural steps. If the antigen is present in the urine, it will interact with the colloidal gold conjugated particles to form an immune complex. The reaction mixture flows through the membrane, which contains zones of immobilized antibodies. In the test zone antigen-conjugate complexes are trapped by the capture antibody, forming a visible

line if the concentration of antigen in the urine is elevated. The procedural control zone contains an immobilized goat anti-mouse IgG-specific antibody that will capture the colloidal gold conjugated antibody, thereby producing a visible line in the control window. This procedural control assures the operator that each device is working properly, independently of the presence or absence of the antigen in the urine sample. The result is the visible line of any intensity if the test is positive.

The hypothesis test for two proportions from the independent groups was used to compare discordance of results between NMP22 versus cytology, NMP22 versus USG, NMP22 versus IVP, NMP22 versus NMP22 BladderChek®, cytology versus USG, cytology versus IVP, cytology versus NMP22 BladderChek®, USG versus IVP, USG versus NMP22 BladderChek® and IVP versus NMP22 BladderChek®.

Results

Mean age of the study population was 49.8±10.79 years. Male to female ratio of the study group was 1.08 (78/72). Overall, 10.7% (n=16) of patients revealed the pathological diagnosis of bladder cancer; whereas 89.5% of the study population had no etiology for microhematuria. Twelve out of 16 patients (77.7%) in bladder cancer group had a history of smoking and/or voiding symptoms.

In all bladder cancer cases, the histopathological diagnoses were the transitional cell carcinoma. Cystoscopic evaluation was accepted as the gold standard diagnostic tool for the initial diagnosis. However, the diagnostic value of the flexible and rigid technique did not differ in our study group. Because all bladder cancer cases were catched both with the flexible and rigid cystoscopic evaluation. Although both rigid and flexible cystoscopic examinations were normal, 8 patients revealed atypical cells in the cytological evaluation of their urine. We took random cold cup biopsies from their bladder mucosa. The histopathologic diagnoses were reported as chronic cystitis in 3 and normal bladder mucosa in 5 cases.

The sensitivity, specificity, and accuracy of each diagnostic test alone and the best 3 of double test combinations were represented in Table 1 and Table 2, respectively.

IVP was considered as the most sensitive (87.5%) and specific test (94%) in the diagnosis of bladder cancer. However, voided urine cytology was the least sensitive test (31.2%), although it shared the same specificity with IVP (94%) (Table 1). The best sensitivity and specificity values were demonstrated when NMP22 and abdominal USG were used in combination (100% and 58.9%, respectively) (Table 2).

Discussion

The most important etiologic factor for microscopic hematuria is the tumor of the urinary tract. Howard et al.[7] diagnosed bladder cancer in 16 patients among 246 patients who had asymptomatic microscopic hematuria. In the literature, asymptomatic microscopic hematuria has an incidence of malignancy ranging from 2% to 20%.[8,9] Close screening of those patients is essential for the initial diagnosis of bladder cancer, thus diagnostic methods with high sensitivity and specificity values are nedeed to provide easy and early diagnosis.

Stampfer et al.[10] reported 66 patients with bladder cancer and the analysis for NMP22 results confirmed that a cut-off value of 6.4 U/mL would give the best results. But in the same study, when 10 U/mL was used as a reference value, sensitivity would decrease to 48.5% while specificity would increase to 91.8%.[10] In our study group, we reached 88.8% sensitivity and 59.2% specificity values with a cut off value of 10 U/mL. Ramakumar et al.[11] confirmed NMP22 test as a faster and less expensive test over urine cytologic evaluation with high sensitivity and specificity valu-

Table 1. The sensitivity, specificity and accuracy values of each test alone

	Sensitivity (%)	Specificity (%)	Accuracy (%)
IVP	87.5	94.0	93.3
USG	75.0	100	97.3
Cytology	31.3	94.0	87.3
NMP22	81.3	59.7	62.6
NMP22 BladderChek®	75.0	58.9	60.6

IVP: Intravenous pyelography, USG: Ultrasonography.

Table 2. The sensitivity, specificity and accuracy values of the best three of double test combinations

	Sensitivity (%)	Specificity (%)	Accuracy (%)
NMP22+USG	100	58.9	52.7
NMP22+IVP	100	55.2	49.3
NMP22 BladderChek®+IVP	100	55.2	49.3

IVP: Intravenous pyelography, USG: Ultrasonography.

es. Our study supports this idea revealing the lowest sensitivity value for urine cytologic evaluation.

Low grade tumors are only detected in 30% to 40% with urine cytology.[11,12] Lahme et al.[13] also compared urine cytology and NMP22 test at different cut-off values and suggested 10 u/ml as the most suitable value. They reached 100% sensitivity with NMP22. Sensitivity was 66.7% with urine cytology in grade 3 transitional cell tumors.[13] Similar study from our clinic demonstrated NMP22 test as the most accurate test over cytology and Bladder Tumor Antigen (BTA) stat test at the cut-off value of 12 U/mL.[14] However, false positive results are not uncommon with NMP22. Stones, urinary tract infections, urological instrumentation, and genitourinary tumors may cause false positivity.[15] Therefore, we excluded cases having above disorders in order to prevent false positive results with NMP22 and false negative results with cytologic evaluation.

Khadra et al.[16] diagnosed 230 cases with bladder cancer among 1,930 patients who were admitted both for microscopic and macroscopic hematuria. IVP demonstrated a filling defect in 60 cases (26%). However, in 19 cases IVP was positive but subsequent cystoscopy did not confirm bladder cancer. This data concluded a 24% (19/79) false positive result when IVP was used as a diagnostic tool alone.[16] We reached better sensitivity and specificity values with IVP (87% and 94%, respectively).

Medical companies investigate new tumor biomarkers or test kit that will help easier and less expensive diagnostic methods for bladder cancer detection from urine specimen. One of them is the NMP22 BladderChek® test. The test kit has some advantages over already used NMP22 test kit. First of all, easy application even in office standards gives superiority to other biomarkers. The major concern before we start this prospective study was that if we would reach acceptable sensitivity and specificity values. Our sensitivity and specificity values for this test were 75 and 59%, respectively. Recently, an interesting study was published to evaluate whether screening high risk asymptomatic individuals with NMP22 BladderChek® test could lead to earlier detection of the disease. In this study, the low prevalence of bladder cancer in that population did not permit assessment of intervention efficacy. But, it was shown that screening of asymptomatic, high risk population with NMP22 BladderChek® could catch noninvasive tumors.[17] In contrast, last year, Steiner et al.[18] reported first results of an early bladder-cancer detection programme, and evaluated the detection rate and diagnostic value of the tests-the urinary dipstick, NMP22 BladderChek®, cytology and molecular cytology test (UroVysion). In their series the most efficient screening tool was the combination of UroVysion, cytology, and urinary dipstick testing.[18]

The contribution of the NMP22 BladderChek® test with IVP improved the sensitivity to 100% but reduced the specificity to 55.2%. Nevertheless, the comparison of accuracy values for NMP22 BladderChek® test plus IVP with the other double test combinations: NMP22 test plus USG and NMP22 test plus IVP, did not demonstrate any significant difference (60%, 63%, and 60%, respectively) ($p>0.05$). So, as an easier and more simple diagnostic tool, NMP22 BladderChek® could reach the accuracy levels that NMP22 provided.

Considering the combinations of NMP22 BladderChek® test with cytology and USG, sensitivity values were reached to 77.7% and 88.8%, respectively. As seen in Table 2, probably the most practical combinations of two tests which would protect more than half of the patients from unnecessary cystoscopies were the NMP22 BladderChek® test plus IVP or NMP22 plus USG evaluation.

In 2007, Tritschler et al.[19] from Germany, collected voided urine samples from 100 patients with suspicion of bladder cancer in order to perform the NMP22 BladderChek® test and voided urinary cytology. The NMP22 levels were measured by a lateral flow immunochromatographic qualitative assay, using 10 U/mL as the cut-off value. Subsequently patients underwent photodynamic diagnosis using 5-aminolevulinic acid or hexyl-aminolevulinate. The sensitivity was 65% for the NMP22 BladderChek® test, 44% for voided cytology, and 93% for photodynamic diagnosis. Specificity rates were 40%, 78%, and 43%, respectively.[19] Results were comparable with our data. Hopefully, screening with photodynamic diagnosis was significantly superior.

In conclusion, NMP22 BladderChek® test was a sensitive and rapid test for bladder cancer screening, and it could easily be performed even in office standards with acceptable costs. But combination of NMP22 BladderChek® test with radiological diagnostic tools such as IVP/USG had statistically insignificant lower sensitivity and specificity rates when

compared with NMP22 plus IVP/USG. However, the value of the test was limited by its low specificity, presumably due to frequent positive reaction in benign conditions. Therefore, selection of patients is essential to avoid unnecessary further invasive procedures. But for today cystoscopy still remains the gold standard technique in the diagnosis and follow-up of bladder cancer.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Zaak D, Karl A, Knüchel R, Stepp H, Hartmann A, Reich O, et al. Diagnosis of urothelial carcinoma of the bladder using fluorescence endoscopy. BJU Int 2005;96:217-22.
2. Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 2001;51:15-36.
3. Grossfeld GD, Carroll PR. Evaluation of asymptomatic microscopic hematuria Urol Clin North Am 1998;25:661-76.
4. Golin AL, Howard RS. Asymptomatic microscopic hematuria. J Urol 1980;124:389-91.
5. Carson CC, Segura JW, Greenee LF. Clinical importance of microhematuria. JAMA 1979;241:149-50.
6. Fey EG, Penman S. Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci USA 1998;85:121-5.
7. Howard RS, Golin AL. Long term follow up of asymptomatic microhematuria. J Urol 1991;145:335-6.
8. Britton JP, Dowell AC, Whelan P, Harris CM. A community study of bladder cancer screening by the detection of occult urinary bleeding. J Urol 1992;148:788-90.
9. Ritchie CD, Bevan EA, Collier SJ. Importance of occult hematuria found at screening. Br Med J 1986;292:681-3.
10. Stampfer DS, Carpinito GA, Rodriguez-Villanueva J, Willsey LW, Diney C, Grossman HB, et al. Evaluation of NMP22 in the detection of transitional cell carcinoma of the bladder. J Urol 1998;159:394-8.
11. Ramakumar S, Bhuiyan J, Besse JA, Roberts SG, Wollan PC, Blute ML, et al. Comparison of screening methods in the detection of bladder cancer J Urol 1999;161:388-94.
12. Del-Nero A, Esposito N, Curro A, Biasoni D, Montanari E, Mangiarotti B, et al. Evaluation of urinary level of NMP22 as a diagnostic marker for stage pTa-pT1 bladder cancer:Comparison with urinary cytology and BTA test. Eur Urol 1999;35:93-7.
13. Lahme S, Bichler KH, Feil G, Krause S. Comparison of cytology and NMP22 for the detection and follow up of bladder cancer. Urol Int 2001;66:72-7.
14. Sözen S, Biri H, Sınık Z, Küpeli B, Alkibay T, Bozkırlı İ. Comparison of the NMP22 with voided urine cytology and BTA stat test in the diagnosis of transitional cell carcinoma of the bladder. Eur Urol 1999;36:225-9.
15. Sharma S, Zippe C, Pandrangi L, Nelson D, Agarwal A. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. J Urol 1999;162:53-7.
16. Khadra MH, Pickard RS, Charlton M, Powell PH, Neal DE. A prospective analysis of 1930 patients with hematuria to evaluate current diagnostic practice. J Urol 2000;163:524-7.
17. Lotan Y, Elias K, Svatek RS, Bagrodia A, Nuss G, Moran B, et al. Bladder cancer screening in a high risk asymptomatic population using a point of care urine based protein tumor marker. J Urol 2009;182:52-7.
18. Steiner H, Bergmeister M, Verdorfer I, Granig T, Mikuz G, Bartsch G, et al. Early results of bladder-cancer screening in a high-risk population of heavy smokers. BJU Int 2008;102:291-6.
19. Tritschler S, Scharf S, Karl A, Tilki D, Knuechel R, Hartmann A, et al. Validation of the diagnostic value of NMP22 BladderChek test as a marker for bladder cancer by photodynamic diagnosis. Eur Urol 2007;51:403-7.

Correspondence (Yazışma): Yard. Doç. Dr. Hüsnü Tokgöz.
Zonguldak Karaelmas Üniversitesi Tıp Fakültesi, Üroloji Anabilim Dalı, 67600 Zonguldak, Turkey.
Phone: +90 372 261 30 75 e-mail: h_tokgoz@hotmail.com