

IMMUNOCYTOLOGY OF LEWIS X ANTIGEN, CYTOPATHOLOGY AND ULTRASOUND IN BLADDER TUMORS

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Geliş Tarihi:10.11.2005 Kabul Tarihi: 12.03.2006

ABSTRACT

Lewis X antigen in bladder cancer patients can be found in urine. In order to determine the presence of Lewis X antigen in exfoliated cells from voided urine, also cytopathologic examination and bladder ultrasound were detected in bladder cancer patients. Totally 42 patients were included in the study. Voided urine samples were obtained for immunocytology of Lewis X and cytopathology. Bladder ultrasound and cystoscopy were performed. Bladder tumors were diagnosed in 13 patients. False-negative results were seen in small, superficial and low grade tumors. Both cytopathology and bladder ultrasound increased the sensitivity. Immunocytology of urine sample for the Lewis X antigen is a sensitive method for noninvasive detection of transitional cell tumors.

Key Words: *Lewis X antijeni, Mesane kanseri.*

ÖZET

Mesane Kanserlerinde Lewis X Antijeni İmmünohistolojisi, Sitopatoloji ve Ultrason

Mesane kanserli hastaların idrarlarında Lewis X antijeni bulunabilmektedir. İdrarla atılan hücrelerdeki Lewis X antijenini saptamak, sitopatolojik inceleme yapmak ve mesane ultrasonu için mesane kanseri olan hastalar çalışmaya alındı. Toplam 42 hasta bu çalışmaya dahil edildi. Hastalardan Lewis X antijeni tesbiti ve sitopatoloji için idrar örnekleri alındı. Ayrıca mesane ultrasonu ve sistoskopi yapıldı. Hastalardan 13 ünde mesane kanseri tesbit edildi. Küçük, düşük dereceli ve yüzeysel kanserlerde yanlış negatif sonuçlar izlendi. Sitopatoloji ve mesane ultrasonu ile sensitivite yükseltildi. Lewis X antijeninin idrarda immünohistoloji ile tesbiti, değişici epitelli mesane kanserleri için noninvasif ve sensitif bir yöntemdir.

Anahtar Kelimeler: *Lewis X antijeni, Mesane kanseri.*

INTRODUCTION

The detection and periodic controls of bladder tumors requires invasive procedures such as gold standard cystoscopy and biopsies in cancer follow [1]. Although it is highly reliable in high grade tumors it fails in the detection of about 50 % of low grade bladder tumors [2]. A lot of new antigens can be found in bladder cancer, such as expression of carbohydrate antigens (SSEA-1, sialyl-Lewis X, DU-PAN-2 and CA19-9) and E-selectin in uroepithelial carcinoma (3). Immunostaining of exfoliated uroepithelial cells have a significantly higher sensitivity than conventional cytology. As monoclonal antibodies which is used in these studies are not commercially available, it cannot be used routinely [4-7]. Cordon-Cardo had shown that Lewis X antigen is normally absent in uroepithelial cells. But it is expressed in transitional cell tumors and have a high sensitivity and specificity [8, 9, 11]. Antibodies against Lewis X antigen are commercially available. Sheinfeld used the Lewis X antigen

detection in cells from bladder wash of bladder tumors [10]. As ultrasonography and voided urine examination are noninvasive methods for detection of bladder cancers, we detected the use of Lewis X antigen expression in urine.

MATERIAL and METHOD

42 patients were included the study whose had hematuria or irritative urinary symptoms. Also the patients who had transitional cell tumors and returned for followup were included the study. Voided urine samples were obtained from each patient before cystoscopy or transurethral biopsy. Another urine specimen of 40cc voided urine was obtained from each patient and stored at 4 C for using to prepare cytocentrifuge slides for routine cytological examination [12]. Voided urine specimens for immunocytology were also stored at 4 C and processed with an equal volume of 2 % polyethylene glycol in 70 % ethyl alcohol and processed. Bales modifications were used for

cytopreparations [13]. The voided 80 cc sample of urine was centrifuged at 2,500 rpm for 10 minutes. For preventing cells from clumping, 1 ml. of 2 % polyethylene glycol in 70 % alcohol was added. The cell density was adjusted to about 10^5 cells per ml. Cytospin preparations 6 mm. in diameter, were prepared on poly-L-lysine coated slides. The slides were dried and fixation was done with cold acetone and absolute methanol. Slides were examined for an optimal single cell layer.

Immunostaining of Lewis X antigen was done with BG-7 monoclonal IgM antibody. All slides stained with Mayer's hematoxylin. Microscopic examination was performed without any knowledge of the patients. In each case 100 epithelial cells were evaluated. Typical red-brown staining cells were considered tumor cells. As some of the benign umbrella cells stain positive for the Lewis X antigen, for the diagnosis of the uroepithelial tumor 5 % or more of the cells which were stained were accepted as tumor.

All of the microscopic examination was done by the same pathologist (H.Y.). The slides were labeled in four categories. 1- Negative, 2- Atypical, 3-Suspicious or 4- Positive for tumor cells.

All patients were underwent bladder ultrasound examination with full bladder. Results were determined according to the standard statistical methods.

RESULTS

Totally 42 patients were evaluated. Fourteen of them were the first time because of hematuria

and 28 patients had a history of bladder tumor. Cystoscopy and biopsy revealed for bladder tumors in 13 cases, 8 of them were newly diagnosed tumors and 5 of these were recurrent tumors. Seven of these were low grade transitional cell tumors (grades 1 to 2), 5 patients were high grade transitional cell carcinoma (grades 3 to 4) or squamous cell carcinoma and 1 patient was carcinoma in situ only. Preparations for immunocytology 90% were in good quality and 6 (11.3 %) contained less than 100 epithelial cells (range 15 to 91) but they could be evaluated without any difficulty.

In immunocytology examination, positive result was found in 10 of 13 patients with bladder tumor, resulting in a sensitivity of 79.8 % and specificity of 86.4%. Immunocytology examination missed 3 tumors. Cytopathological examination of voided urine have a sensitivity of 47.6 % and when correlated with the tumor grade, have a sensitivity of 25.0% for the low grade tumors and 80.0% for high grade transitional cell carcinoma. Specificity of urinary cytology was 97.7%. The detection rate of bladder tumors by ultrasound was 66.7%. Specificity of bladder ultrasound was 97.2 %. The combination and individual results of the various tests are shown in **Table 1** and **Table 2**.

The combination of bladder ultrasound and immunocytology resulted in the detection of 94% of the tumors, and the combination of ultrasound, cytopathology and immunocytology for Lewis X antigen had a sensitivity of 95.2% in bladder tumors.

Table 1. Sensitivity and specificity of immunocytology, cytology and bladder ultrasound in the detection of bladder tumors.

	No. Pts.	% Sensitivity	% Specificity
Immunocytology	42	79.8	86.4
Cytopathology	42	47.6	97.7
Bladder ultrasound	42	66.7	97.2

Table 2. Sensitivity and specificity of various combinations of tests for noninvasive detection of bladder tumors.

	No. Pts.	% Sensitivity	% Specificity
Immunocytology + cytology	42	88.1	85.2
Immunocytology + ultrasound	42	94.0	83.0
Immunocytology + cytology + ultrasound	42	95.2	82.4

DISCUSSION

Urine cytology is a diagnostic method for transitional cell carcinoma. Since it has less sensitivity, especially in low grade tumors, it cannot be a substitute for cystoscopy. Flow cytometry is also a technic for measuring the deoxyribonucleic acid (DNA) in urine for diagnosis of bladder tumors, but flow cytometry requires a minimum of 5,000 cells, so it can be obtained only by bladder lavage, an invasive procedure [14]. Some novel biomarkers of bladder tumors have been identified. The BTA test detects antigens in voided urine specimens but BTA test has a negative result in 60% of patients who had tumors [15]. Another test, NMP22 measures the concentration of nuclear matrix proteins in voided urine. Had a sensitivity of 70% and a specificity of 79% in bladder cancer [16]. Berlac and Holm used ultrasound and urine cytology as an alternative to cystoscopy for bladder tumors [17]. In our study we used ultrasound for the detection of primary bladder tumors and for surveillance after transurethral resection of bladder tumors. All of the tumors missed by ultrasound were small papillary tumors or carcinoma in situ. The specificity of ultrasound was 97.2% in our study but ultrasound cannot be substitute for cystoscopy.

Some antibodies are expressed in high grade or invasive tumors. The expression of T43 and

T138 antigens occurs in 15% of superficial tumors but more than 60% of invasive bladder cancers [18]. Also M344 and 19A211 are expressed on 70% of superficial bladder tumors [19]. Sagerman used combinations of antibodies against Lewis X antigen, M344 and 19A211 for the detection of bladder tumor [20]. Friedrich found false positive results in 9.5 % for Lewis X detection [21]. The combination of cytopathology and immunocytology had a sensitivity of 83.7% and specificity of 83.3%. In Cordon-Cardo's study they had shown that the Lewis X antigen may be expressed on normal uroepithelial umbrella cells [9]. So we tried to determine the percentage of transitional cells in the urine of optimal threshold for the diagnosis of a bladder tumor. In our study we found that for the diagnosis of a transitional cell tumor, 5% or more of the cells must have to express the Lewis X antigen. These result are same as Sheinfeld's study [10].

As a result immunostaining of cells from voided urine for the Lewis X antigen detection is more sensitive than cytopathology, but this method may miss small, superficial or low grade tumors. And cystoscopy is still the gold standard for evaluation and following of bladder cancers until the new methods have to need more studies to improve these technics.

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