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Evaluation of a New Urinary Bladder Tumor Marker in Comparison with Cytology for Rapid Diagnosis of Bladder TCC

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Abstract: A new Bladder Tumor Marker has been developed to detect early TCC bladder cancer. Objective to evaluate the Bladder Tumor Marker (BTM) as a new developed tumor marker in comparison to cytology. Transurethral specimens and voided urine samples were collected from 30 patients with haematuria selected from inpatients of Theodur Bilharz Institute-Cairo. The samples were diagnosed according to histopathological examination into 25 samples with TCC, 5 samples were served as control since they were complains of no cancerous symptoms and were suffering from other genitourinary pains. Urine samples were tested by BTM and cytology. The results were correlated with the final diagnosis (histopathology) and with tumor grade and stage. Sensitivity of BTM test was 72% and specificity was 60%. The corresponding cytology results were 44 and 100%, respectively. BTM was highly sensitive in grade I and stage I, 86 and 89% in comparison to 57 and 33% in cytology. Cytology appeared to be highly sensitive in grade III compared to BTM, 80 and 60%, respectively. BTM is more sensitive and valuable in detecting low grade and stage compared to cytology for early detection of TCC bladder tumor.

Key words: BTM, bladder cancer, cytology

INTRODUCTION

Bladder cancer is the fourth most common malignant disease in Egypt. It accounts for about 20 to 25% of all cases in both sexes and approximately 38% in males (EL Bolkiny and Chu, 1981; Khaled *et al*., 2003).

Approximately 75 to 85% of these patients present with Transitional Cell Carcinoma (TCC) confined to the superficial mucoca of the bladder (Pagno *et al*., 1991). The risk of recurrence in these patients is 75%. Patients with previous diagnosis of bladder cancer have been routinely followed for recurrence by urine cytology and cytoscopy. Both methods have their limitations.

Early diagnosis of bladder cancer allows the possibility of less invasive surgical treatment and higher 5 year survival rates for superficial tumors (Cheng *et al*., 1999; Sanchez *et al*., 1993). Cytoscopy is considered to be the gold standard for the diagnosis of bladder cancer. The diagnostic workup usnally induces voided or wash-urine cytology. However, urine cytology carries some disadvantages; it is examiner-dependent, it has a relatively low sensitivity for low grade bladder tumors (Ozools, 1995), it is time consuming and its results are not available immediately. To circumvent these pitfalls, several urinary tumor markers were devised and were found to be simple, objective and sensitive alternatives to urine cytology (Leyh *et al*., 1999; Fradet and Cordon-Cardo, 1993; Rozanski and Grossman, 1994; Lokeshwer *et al*., 1997).

Limitations of the currently available techniques of tumor detection has led the investigators to look at the alternative methods to improve the sensitivity and specificity of the test (Moll *et al*., 1987; Van Rhijna *et al*., 2005) explored the possibility of using the intermediate filaments as tumor markers via comparing the pattern of cytokeratin expressed in normal urothelium with that of various forms of transitional cell carcinoma using immunoperoxidase staining of tissue sections.

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Cytokeratin is a normal component of urothelium, it differs according to the grade and cell type (Fussela *et al*., 2004). Other previous studies showed that the simple epithelium type cytokeratin is detected in normal and transitional cell carcinoma, whereas the cytokeratins of the stratified squamous cell carcinoma are detected in the squamous metaplasia or squamous cell carcinoma Baker *et al.* (1988) showed that cytokeratin could be detected in voided urine using an immunoradiometric assay specific for keratin (Martin, 1989).

The present research aimed to study new tumor marker, based upon monoclonal antibody, has been described as a simple, rapid and non-invasive test for early detection of a specific antigen of bladder cancer.

The aim of research is to identify the sensitivity and specificity of the newly developed bladder tumor marker in early diagnosis in comparison to cytology as well as to the gold standard method (Histopathology).

MATERIALS AND METHODS

The present study was conducted during (March 2005-April 2006) in our laboratory in the Medicinal Chemistry Department-National Research Center, Dokki, Cairo, Egypt.

Urine samples were collected from 30 patients From Thueodore bilharz, hey were divided into 25 proved histopathologically to be malignant transitional cell carcinoma (TCC) and 5 samples were disease free served as control. The urine samples centrifuged at 2000 rpm for 10 min. The supernatants were collected to BTM test whereas the sediments were prepared to cytological investigations.

Fig. 1: A Bladder Tumor Marker (BTM) with positive and negative control. a: Positive and negative Control, b: Positive samples, c: Negative sample

Cytology

Urine samples were centrifuged at 2000 rpm for 10 min. Two smears were prepared from the sediment of each sample and fixed in 95% ethyl alcohol for a minimum of 20 min. Fixed smears were stained by the modified Papanicolaou stain. The diagnostic interpretation of cellular material was performed by a cytopathologist and it was based on cytopathologic criteria.

BTM Detection Using Dot-ELISA

The BTM was detected in urine according to Attallah *et al.* (1996) using BTM-Fast (ABC diagnostics, New Damietta, Egypt). In brief, 200 µL of urine supernatant were added on the surface of nitrocellulose of device. After washing 50 µL of Mo Ab were added followed by 100 µL of alkaline phosphatase conjugate. One hundred microliter of substrate premixed NBT/BCIP were added to visualize the substrate and take the result (Fig. 1).

Study Analysis

A positive final diagnosis was defined as bladder cancer confirmed by cystoscopy and biopsy. Tumors were graded and staged according to the classification of the International Union Against Cancer Hermanek and Sobin (1987). The primary measures of diagnostic efficacy were sensitivity to bladder cancer, defined as the ratio of the number of true-positive results to the number of positive final diagnosis (based on cystoscopy and biopsy) and specificity for bladder cancer, defined as the ratio of the total number of true-negative results to the total number of negative final diagnosis.

RESULTS

The results of BTM testing with regards to malignant outcomes showed that there was a false negative rate of 2 out of 5 cases, giving a sensitivity of 72%. Seven out of 25 cases were false positive, giving a specificity of 60%. Percent results showed that, positive and negative predictive values are 90 and 30% respectively (Table 1).

However, the results of cytology testing regards to pathological examination showed a sensitivity of 44% while specificity were 100%. Our positive and negative predictive values are 100 and 28%, respectively (Table 2 and 5).

Table 1: Sensitivity and specificity of BTM compared with pathology

Malignancy	Positive	Negative	Total
Positive	18	7	25
Negative	2	3	5
Total	20	10	30

Sensitivity = 72%; Specificity = 60%

Table 2: Sensitivity and specificity of cytology compared with pathology

Cytology malignancy	Positive	Negative	Total
Positive	11	14	25
Negative	0	5	5
Total	11	19	30

Sensitivity = 44%; Specificity = 100%

Table 3: Sensitivity of BTM and cytology in different grades of TCC

	NO		+ ve		- ve		Sensitivity %		
Grade	BTM	CY	BTM	CY	BTM	CY	BTM	CY	P
1	7	7	6	4	1	3	86	57	0.01
II	13	13	11	8	2	5	85	62	ns
III	5	5	3	4	2	1	60	80	ns
Total	25	25	20	16	5	9			

BTM: Bladder Tumor Marker; CY: Cytology; NS: Not Significant

Table 4: Sensitivity of BTM and cytology in different stages of TCC

	NO		+ ve		- ve		Sensitivity %		
Stages	BTM	CY	BTM	CY	BTM	CY	BTM	CY	P
1	9	9	8	7	1	2	89	77	0.01
II	7	7	5	6	2	1	71	85	ns
III	9	9	5	6	4	3	55	57	ns
Total	25	25	18	19	7	6			

BTM: Bladder Tumor Marker; CY: Cytology; NS: Not Significant

Table 5: Sensitivity, specificity, positive and negative predictive values of BTM and Cytology

	Sensitivity (%)	Specificity (%)	+Predictive value (%)	-Predictive value (%)
BTM	72	60	90	30
Cytology	44	100	100	28

The results obtained showed that, there is a significant differences in sensitivities between BTM and cytology in grade and stage I. The sensitivities of BTM were 86 and 89% in grade and stage I, respectively. Whereas cytology recorded a sensitivities of 57 and 77% for grade and stage I, respectively (Table 3 and 4).

Also, there is no significant differences in sensitivities between BTM and cytology in grades and stages II, III. The sensitivities in grades II and III in BTM were 85 and 60% while in cytology were 62 and 80% respectively. In addition, in the stages II and III the sensitivities of BTM and cytology were 71, 55% and 85, 57%, respectively (Table 3 and 4).

DISCUSSION

Cystoscopy is considered the gold standard for identifying bladder cancer, but it is invasive technique. Urine cytology is noninvasive and quite accurate in detecting high-grade bladder cancer and carcinoma in situ, but its ability to detect low-grade cancer is limited. Therefore, urine-based marker tests are being developed to fill some of the remaining needs. These new tests are noninvasive and accurate in detecting low-grade bladder cancer, so they are especially useful in monitoring for recurrence. Therefore, efforts are under way to develop tests that are easy to administer and interpret, objective and noninvasive and that have high sensitivity as well as high specificity (Landman *et al*., 1998).

Many tests were used to detect bladder tumor antigen in urine such as, BTA stat and BTA track. BTA stat and track tests detect a human complement factor H-related protein in the urine (Pode *et al*., 1999).

Considerable progress has been made in the early detection of bladder carcinoma with the advent of urinary cytology. The present markers was based on the production of a mouse Mo Ab (CK, Kio) specific to cytokeratin from grade 1 squamous cell carcinoma. The primary goal of this study was to better define the potential roles of the BTM assay and cytology in the diagnosis and management of bladder cancer. While several possible markers for urinary bladder cancer are still under investigations, the clinical usage of the BTM test as such a marker was already evaluated in previous studies (Attalla *et al*., 1996).

The results of BTM (Dot ELISA) at the present study were proved to be high sensitive 72% in comparison to 44% in cytology with a low specificity of 60% compared to 100% in cytology. These results were in full agreement with those of (Wald *et al*., 2002) who reported that the BTA state test have a higher sensitivity but lower specificity when compared with urine cytology. The high specificity of cytology(100%) was clearly superior to that of the BTM assay in this patient population. The low specificity of the BTM test tested in the present test was suggested to be related to certain urinary tract pathologic conditions not associated with urothelial cancer. Thus, the positive BTM values in the patients with benign urologic disorders would not be surprising.

In grades I, II and III, the sensitivities were 86, 85 and 60% for BTM, while it were 57, 62 and 80% for cytology, respectively.

In stages I, II and III, the sensitivities are 89, 71 and 55% for BTM and 77, 85 and 57% for cytology, respectively.

For surprising BTM results were highly sensitive in grade and stage I compared to urine cytology which seem to be on line with results of Thomas *et al*. (1999). who found that BTA TRACK assay was more significantly sensitive than cytology for tumor grades I and II ($p<0.05$) and for stage I, while the specificity still low. Our results seemed to be on line with those of Khaled *et al*. (2001) who reported that specificity of BTA stat or track was so low 67% but they are extremely sensitive in the detection of bladder cancer in the Egyptian population.

In conclusion, when choosing a test, we should keep in mind that none of the currently available tests is 100% accurate However, the new urine based tests are more sensitive than urine cytology and hence more reliable in detecting low-grade bladder cancer. They are useful tools in patients with urinary symptoms or microscopic hematuria or as office-based adjuncts to diagnostic procedures. So the results of the present study evaluate the BTM test as a fast, simple, cheep and its relative high sensitivity and can be used as a complement cytology as an adjunct to cytoscopy in the diagnosis and follow-up of most patients with bladder cancer.

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