THE VALIDITY OF BTA TESTS IN PATIENTS WITH URINARY BLADDER CANCER

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Abstract

This study aimed to evaluate the validity of the BTA STAT and BTA TRAK as biomarker tests in patients with suspected urinary bladder cancer (UBC) using histopathology as a reference method, and to clarify the relationship between the results of these tests and tumor grades.

This is a cross sectional study that was carried out in Basrah, Southern of Iraq, from 1st of October 2010 to the 31st of March 2012. A total of 102 patients with signs and symptoms suggestive of UBC admitted to the urology ward in Basrah General Hospital were enrolled. A voided urine sample was obtained from each patient for analysis of BTA STAT and BTA TRAK prior to cystoscopy.

The sensitivity and specificity of the BTA STAT test were 93.1% and 33.3% while for the BTA TRAK test were 97.7% and 33.3%, respectively. The sensitivity of the two studied urinary bladder cancer biomarkers rose with the increase in the grade of UBC and there was an elevation in the median and mean values of BTA TRAK test with the increase in the grade of UBC, although it was not significant.

In conclusion, the sensitivity of both BTA STAT and BTA TRAK tests was very high; but their specificity was very low with high false positive results, however, they may be helpful in early detection and monitoring of recurrence of the disease in patients suffering from UBC.

Introduction

Bladder cancer is the most common cancer of the urinary system, and is ranked the ninth in worldwide cancer incidence. It is more common in men than in women (it is the seventh in men and the seventeenth in women)[1]. However, there is evidence that the western communities showed a decrease in the burden of UBC (incidence and mortality) over the last decades[2-4]. On the other hand, the incidence of UBC is expected to increase in the developing countries[5]. In Basrah (the largest city in southern of Iraq) UBC is one of major community health problems, it was the first of the top ten primary cancers among males accounting for nearly one out of 10 registered cases in the years 2005-2008[6]. Diagnosis of UBC is generally based on cystoscopy and urinary cytology. Although cystoscopy is considered to be the gold standard method, it can miss certain lesions, in particular small areas of carcinoma in situ (CIS). Moreover, cystoscopy is an invasive procedure for the patient and it is expensive[7]. With respect to urine cytology, it may be useful for detecting high grade UBC, but not low grade cancers and hence its clinical relevance is limited. For these reasons there has been a great interest in other non-invasive tools[8]. More than 20 known urine based biomarkers with high sensitivity and or specificity have been investigated and among these, around 6 are available commercially for the detection and/or monitoring of bladder cancer. However, they are not sensitive and specific enough

to be used as diagnostic tests for UBC as a replacement for cystoscopy[9]. On the other hand, the high sensitivity of these tests and their high negative predictive value lead to the belief that they may be useful in surveillance regimen to increase the interval between cystoscopies[10].

Due to the relatively high incidence of UBC in Basrah, the low sensitivity of urine cytology as screening measure for urinary bladder cancer and the invasiveness of cystoscopy, the initiative to evaluate the validity of the non-invasive urinary bladder markers (BTA STAT and BTA TRAK tests) in voided urine samples of patients with suspected urinary bladder cancer was taken.

Materials and methods

This is a cross sectional study that was carried out in Basrah, Southern of Iraq, from 1st of October 2010 to the 31st of March 2012. The study subjects were all patients admitted to the urology ward in Basrah General Hospital during the period of the study with signs and symptoms suggestive of UBC and who were scheduled for cystoscopy (mainly patients with undiagnosed cause of haematuria). Accordingly, a total of 102 patients fulfilling the criteria of inclusion in the study were included. Patients with haematuria with known cause like renal stones were excluded. The study was approved by the College Council and the ethical committee in Basrah Medical College. The research protocol was also submitted to Basrah Directorate General of Health and research approval was obtained prior to its implementation. An informed consent was also obtained from all participants.

Biochemical Analysis

A single voided urine sample or urine sample from the catheter was obtained from each patient before undergoing cystoscopy. Both the BTA STAT test the BTA TRAK assay were employed for each urine sample. The BTA STAT test is an in vitro diagnostic immune-chromatographic assay indicated for the qualitative detection of bladder tumor antigen in human urine (The BTA STAT test kit was provided by polymedco Inc. USA; kit No. 661010). While the BTA TRAK assay is an enzyme immunoassay for the quantitative measurement of the bladder tumor antigen in human urine (The BTA TRAK kit was provided by polymedco Inc. USA; kit No. 662160). The bladder tumor antigen levels in the tested samples were quantified by comparing the absorbance of the specimens to those of the calibrators that were run simultaneously. All enrolled patients were also underwent standard urologic evaluation including histopathology for any suspected lesion. Histopathology was used as a reference method for validation of the two tests.

To determine the cutoff point for the BTA TRAK test, bladder tumor antigen levels were also determined quantitatively in urine samples from 50 apparently healthy individuals with different age groups (20-70 years). They were recruited from the primary health care centers in Basrah. In 98% of these individuals bladder tumor antigen concentration was 15µ/ml or less, therefore the cut-off point for a positive result was arbitrarily taken at a value of more than 20 µ/ml. The data were analyzed by SPSS "Statistical Package for Social Sciences programme" version 17. The results were expressed as median, mean $\pm$ SD or percentage (%), as suitable. One way analysis of variance (ANOVA) and t test were used to find out the significant differences between different means. A P value of <0.05 was considered to be significant.

Results

Of the 102 studied patients, 87 were histopathologically confirmed and diagnosed as cases of bladder cancer. Of these, 77 cases (88.5%) had transitional cell carcinoma (TCC), 9 (10.5%) had squamous cell carcinoma (SCC) and only

one case (1%) had adenocarcinoma. With respect to the grade of the tumor, grading was available for only 76 of UBC cases. The tumor of 8 (10.6%) of the cases was well differentiated (grade I), 47 (61.8%) had a moderately differentiated tumor (grade II), and the tumor of the remaining 21 (27.8%) was poorly differentiated (grade III).

BTA STAT test was positive in 81 (93.1%) of the 87 bladder cancer patients and 10 (66.7%) of patients with no bladder cancer. Therefore, the sensitivity and specificity of the BTA STAT test observed in this study were 93.1% and 33.3% respectively. Furthermore, the positive and negative predictive values were 89% and 45.5% respectively; Table (I). A slightly higher sensitivity but similar specificity for the BTA TRAK test was observed (97.7% and 33.3% respectively). The positive and negative predictive values of the BTA TRAK test were 89.5% and 71.4% respectively; Table (I).

Table I: Validity of BTA STAT and BTA TRAK tests as screening tests for urinary bladder cancer.

BTA STAT test	Histopathology				Total
	Bladder cancer		No bladder cancer 10515		
	No.	%	No.	%	
Positive	81	93.1	10	66.7	91
Negative	6	6.9	5	33.3	11
Total	87	100.0	15	100.0	102
BTA TRAK test					
Positive	85	97.3	10	66.7	95
Negative	2	2.7	5	33.3	7
Total	87	100.0	15	100.0	102

The sensitivity of BTA STAT in detecting transitional cell carcinoma (96.1%) was higher than that for other histopathological types of UBC (70%). With respect to BTA TRAK test, although the mean value of the test was significantly higher among cases of TCC compared to the test values for cases with other histopathological types (P<0.001), no difference was observed in its sensitivity between different histopathological types (97.4% and 100% for TCC and for other types respectively); Tables (II & III).

57

Table II: Sensitivity of BTA STAT and BTA TRAK tests according to histopathological types of urinary bladder cancer

BTA test	Histopathological type			
	Transitional cell carcinoma		Others	
	No.	%	No.	%
BTA STAT test				
Positive	74	96.1	7	70.0
Negative	3	3.9	3	30.0
Total	77	100	10	100
BTA TRAK test				
Positive	75	97.4	10	100.0
Negative	2	2.6	0	0.0
Total	77	100	10	100

* The total is not 87 because grading for 11 cases was not available

Table III: The median and mean values of BTA TRAK test according to the histopathological type of urinary bladder cancer

Histopathological type	No.	Median	Mean $\pm$ SD
Transitional cell carcinoma	77	145.91	159.58 ± 73.725
Other types	10	57.27	68.26 ± 46.530
Total	87		

t= 3.623   P<0.0001

Furthermore, the sensitivity of the two studied biomarkers showed an increase with the increase in the grade of UBC. The sensitivity of both tests consistently increased with the increase in the grade of UBC to reach 100% in grade III. However, no significant difference in the mean values of BTA TRAK test for patients with different grades of UBC; Tables (IV &V).

Table IV: Sensitivity of BTA STAT and BTA TRAK tests according to grade of urinary bladder cancer

BTA test	Grade I		Grade II		Grade III	
	No.	%	No.	%	No.	%
BTA STAT test						
Positive	7	87.5	45	95.7	21	100
Negative	1	12.5	2	4.3	0	0.0
Total	8*	100	47*	100	21*	100
BTA TRAK test						
Positive	7	87.5	47	100	21	100
Negative	1	12.5	0	0.0	0	0.0
Total	8*	100	47*	100	21*	100

* The total is not 87 because grading for 11 cases was not available

58

Table V: The median and mean values of BTA TRAK test according to grade of urinary bladder cancer

Grade of UBC	No.	Median	Mean + SD
Grade I	8	136.4	122.70 +72.93
Grade II	47	145.63	154.64 + 74.92
Grade III	21	154.94	165.75 + 68.48
Total	76*		

The grade of 11 UBC cases was not available

F= 1.00   P= 0.37

Discussion

The BTA STAT and BTA TRAK tests are designed to detect UBC associated antigen in voided urine. This antigen which is a human complement factor H-related protein interacts with the complement factor C3b and interrupts the complement cascade, potentially providing a selective growth advantage to tumor cells. The complement factor H-related protein production by tumor cells has been suggested that it may help tumor cells to avoid attack by the host's immune defenses and therefore may prevent lyses by immune system[11].

The results of this study showed that both BTA STAT and BTA TRAK tests have high sensitivity and low specificity. The BTA STAT showed sensitivity of 93.1% and specificity of 33.33%, while BTA TRAK showed sensitivity of 97.7% and specificity of 33.33%. Similar results were reported by other studies[12,13]. In these studies, similar to the present study, the calculation of the specificity was based on the ability of BTA tests to differentiate between UBC from other nonmalignant urinary tract diseases. However, a higher specificity was reported when subjects with non urinary tract conditions or healthy subjects were used as controls[14-17]. A specificity greater than 90% was reported for these tests when healthy subjects were employed as controls[12,15].

Benign condition of the urinary tract, urinary stone, nephritis, cystitis, benign prostatic hypertrophy, proteinuria, hematuria due to other causes may lead to false positive result for BTA tests and hence low specificity[12,18]. The low

specificity of these tests may be partly due to their ability to detect both complement factor H-related protein and complement factor H (complement factor H is present in human serum at high concentration). This may result in falsely positive BTA tests in benign hematuria causing conditions[19]. Therefore, these tests cannot be used instead of cystoscopy in the diagnosis of bladder cancer and treatment decisions cannot be based on a positive test result. It is generally recommended to be used for monitoring of UBC patients in combination with cystoscopy thus aid in the early diagnosis and monitoring of recurrence of the UBC[20].

With respect to histopathological type, the present work reported a sensitivity of BTA STAT for TCC and non TCC of 96.1% and 70% respectively, while for BTA TRAK it was 97.4% and 100% respectively. Similar results were obtained by Ibrahaim et al[21], whereas Abd El-Gawad et al[22] stated that sensitivity of the BTA test does not differ between the TCC and SCC of the bladder because bladder tumor antigen released by either tumor cell line.

The result of this study also revealed that the sensitivity of both BTA STAT and BTA TRAK test correlate with bladder tumor grade, being lower in grade I and higher in grade III. Published data also demonstrated that the sensitivity of BTA tests was improved with increasing histological grade with higher sensitivity in advanced grade tumor[11,23-26].

In conclusion the sensitivity of both BTA STAT and BTA TRAK tests was very

high; but their specificity was very low with high false positive results i.e. these tests could not correctly exclude UBC in patients suffering from other urinary tract problems. Thus, these tests are not suitable alternative to cystoscopy for the provisional diagnosis of UBC. However, they may be helpful in early detection and monitoring of recurrence of the disease in patients suffering from UBC.

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