Effectiveness of bladder tumor antigen quantitative test in the diagnosis of bladder carcinoma in a schistosoma endemic area

Abubakar Sadiq Muhammad, Ismaila Arzika Mungadi, Nnaemeka Ndodo Darlington[1], Garba Diffa Kalayi[2]

Department of Surgery, Urology Unit, Usmanu Danfodiyo University Teaching Hospital, Sokoto, [1]Center for Advanced Research and Training, Usmanu Danfodiyo University Sokoto, [2]Department of Surgery, Division of Urology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria

Abstract

Introduction: Bladder carcinoma is the most common male cancer in our environment due to endemicity of schistosomiasis. Squamous-cell carcinoma is the most common histological type and patients present at an advanced stage. The objective of this study is to compare the sensitivity, specificity, and predictive values of the bladder tumor antigen quantitative test (BTA TRAK) and urine cytology in the diagnosis of bladder carcinoma in a schistosoma endemic area.

Materials and Methods: This is a 12-month cross-sectional study of 88 patients, 52 of them with features of bladder carcinoma as study group, and 36 of them with hematuria from other urologic conditions, and benign urologic conditions and healthy volunteers as control group (CG). The mean ages of patients in the study and CGs were 47.17 ± 17.00 and 44.19 ± 18.89 years, respectively ($P = 0.412$). Bladder tumor antigen was assayed using enzyme-linked immunosorbent assay. Data were analyzed using SPSS version 20.0 for Windows.

Results: The sensitivity of urine cytology and BTA TRAK in the study was 29.1% and 98.8%, respectively. The specificity of urine cytology and BTA TRAK was 95.5% and 13.6%, respectively ($P = 0.05$). The positive predictive values of urine cytology and BTA TRAK in the study were 96.2% and 81.7%, respectively. The negative predictive values were 25.0% and 75.0% for urine cytology and BTA TRAK, respectively.

Conclusion: BTA TRAK is more sensitive but poorly specific as compared to that of the urine cytology for bladder cell carcinoma detection in a schistosoma endemic area.

Keywords: Bladder carcinoma, bladder tumor antigen quantitative test, predictive values, schistosomiasis, sensitivity, specificity

INTRODUCTION

Bladder cancer is the 4th most common cancer in men and 9th most common cancer in women and is associated with significant morbidity and mortality.[1] In Sokoto North-Western (NW) Nigeria, West Africa, bladder cancer is the most common cancer in men and 6th most

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common cancer in women and was found in 19.3% and 3.0% of men and women, respectively.[2] In the Western world, transitional cell carcinoma (TCC) accounted for 95%–97% of bladder carcinoma, whereas in Sokoto, Nigeria, TCC and squamous-cell carcinoma (SCC) accounted for 27.9% and 65.1% of the cases, respectively, due to endemicity of schistosomiasis.[3] About 70% of the patients presented with advanced disease at diagnosis which was also noticed in other African countries where schistosomiasis is endemic.[4] Early diagnosis of bladder carcinoma still remains a great challenge due to poor sensitivity and specificity of current diagnostic modalities in detecting early tumors.[5] The main stay for screening, diagnosis, and surveillance of patients with bladder carcinoma is urine cytology and cystoscopy with or without biopsy. Urine cytology though specific, is poorly sensitive for low-grade tumors and is dependent on the experience of pathologist.[6] Cystoscopy and biopsy are the gold standard for bladder cancer diagnosis but is invasive, costly, and cannot reliably detect small and flat tumors (carcinoma *in situ*).[7] Therefore, there is need for simple, noninvasive, affordable, and effective tool for screening, diagnosis, and surveillance of patients after the treatment. Urinary markers currently are being investigated and among the ones with promising results in various studies worldwide, and approved for use in bladder cancer patients by the United States Food and Drug administration includes bladder tumor antigen (BTA).[7] BTA quantitative test (BTA TRAK), measures is human complement factor–H related protein (hCFHrp) which is similar in structure and function to hCFH.[8] The hCFH inhibits alternative complement pathway by interacting with complement factor C3 convertase and serving as cofactor for complement factor 1. This inhibits the formation of membrane attack complex and prevents lysis of cells recognized by host as foreign. Production of hCFHrp by bladder cancer cells may allow the cancer cells to evade the host immunity.[9,10] The antigen is elaborated in to an urine in significant amount in patients with bladder carcinoma.[10] Endogenous hCFHrp can leak in urine in patients with hematuria from other urologic diseases and benign urologic diseases leading low specificity and positive predictive value (PPV) of the marker which manifest clinically as high false positive (FP) rate.[11]

The objective of this study is to compare the sensitivity, specificity, and positive and negative predictive values (NPVs) of BTA TRAK and that of urine cytology in the diagnosis of bladder carcinoma in schistosoma endemic area.

MATERIALS AND METHODS

Study area
Sokoto is the capital city of Sokoto state of Nigeria and is located in the Northwest. The Sokoto state covers a land mass of about 60.33 square kms.[12] Sokoto city has a population of 506,241 while the total population of the state according to 2006 census was 3,696,999.[12] The main occupation of the rural population is farming. The Usmanu Danfodiyo University Teaching Hospital Sokoto serves as a referral center for urology cases from neighboring states of Kebbi, Zamfara, Katsina, Niger, and republics of Benin and Niger.

Sample size determination
The sample size for this study was calculated using Fisher's formula[13] as follows:

$$n = Z^2pq/d^2$$

$n_f = (n/1 + n/N)$
N = 63 (number of bladder cancer cases per year in Sokoto)[2]
n = minimum required sample size in population >10,000
n_f = minimum sample size in population <10,000
Z = standard normal deviate = 1.96
p = prevalence = 0.087
q = 1 − p = 0.913
d = precision or level of significance = 0.05
$n = 1.96^2 × 0.087 × 0.913/0.5^2 = 122$
$n_f = 122/1 + 122/63 = 41.49$

With addition of 20% to cover for attrition, the minimum sample size for this study was 50 patients.

This is a cross-sectional study of 88 patients carried out within 12 months (January–December, 2014) at the Usmanu Danfodiyo University Teaching Hospital and the Center for Advanced Research and Training of Usmanu Danfodiyo University Sokoto, Nigeria, West Africa. The patients were divided into study group (SG) with 52 patients and control group (CG) with 36 patients. The age ranges of SG and CG were 20–78 years and 18–86 years, with mean ages of 47.17 ± 17.00 and 44.19 ± 18.89, respectively, (*P* = 0.412).

The study was approved by the Health Research and Ethics Committee of Usmanu Danfodiyo University Teaching Hospital Sokoto with reference number UDUTH/HERC/2012/No. 38 and was conducted according to the 1964 Helsinki declaration as amended in 2000.[14]

Consecutive patients with symptoms of bladder cancer who gave written consent to participate in the study were

enrolled as SG at urology outpatient or accident and emergency units of our institution. Patients with other urologic conditions with or without hematuria and healthy volunteers (HVs) were enrolled as CG. The patients with hematuria formed the positive control, whereas those without hematuria formed the negative control.

The inclusion criteria included, confirmed cases of bladder cancer before the study, clinical or radiological bladder mass, hematuria in patients with a significant history of schistosomiasis and or smoking. The exclusion criteria included bladder cancer patients after radical cystectomy and urinary diversion or any form of treatment such as immunotherapy and chemotherapy and patients that did not consent.

About 5 mL of urine was collected in a clean container and analyzed within 1 h of collection for urine cytology, whereas 3 mL of the urine for BTA TRAK assay was stored at −20°C between 2 weeks and 2 months when the minimum required number of samples were achieved. Assay of urine BTA was done using enzyme-linked immunosorbent assay.

Bladder tumor antigen assay using enzyme-linked immunosorbent assay

Standard wells (calibrators) were prepared according to the manufacturer's instructions. Standard calibrators' 0, 5, 20, 50, and 100 kilo units/L, high-and low-BTA concentrations provided by the manufacturer were used as the controls.

Urine sample of 25 µL and 175 µL of a diluent was added into each micro well-coated with capture BTA monoclonal antibody (MAb) and allowed to incubate for 1 h at 37°C. Bladder tumor-associated antigen in urine was bound by capture MAb. Unbound urine was washed four times with 300 µL of washing buffer using an automated machine. The alkaline phosphatase labeled reporter MAb was then added into each micro well, and incubated for 1 h at 37°C. The plate was washed four times to remove unbound conjugate. Substrate reagent was added into each well which was subsequently incubated for 30 min at 37°C. Stop reagent was used to stop the reaction. The extent of reaction was determined by measuring absorbance in each well at 405 nm using micro plate reader. The amount of antigen was determined by comparing the absorbance of the sample wells with those of the controls. A cutoff value of 14 units/mL for hCFHrp was used.[9]

Urine cytology

Three milliliter of voided urine was collected and promptly fixed in equal amount of 50% Alcohol centrifuged at 2000 rpm for 5–10 min. The sediment was smeared on albumenized slides and stained with Papanicolaou stain and examined under the microscope.[15] The malignant or suspicious cells were scored positive, whereas benign or acellular smears were scored negative for the presence of bladder carcinoma.

Urethrocystoscopy and bimanual palpation

Urethrocystoscopy was done for all the patients in the SG, biopsy was taken from the suspicious lesion for histological confirmation, and bimanual palpation was done to classify the stage of the tumor.

Data collection

Relevant data were collected through a structured pro forma which included clinical features, risk factors for bladder carcinoma, results of relevant investigations, cystoscopy findings, and the results of histology, urine cytology, and BTA TRAK.

Data analysis

Data analysis was performed using the Statistical Package for Social Sciences 20.0 version (2011) for windows (IBM, SPSS Inc., Chicago, IL, USA). Sensitivity, specificity, PPV and NPV for BTA TRAK, and urine cytology in the study were assessed. False positivity of the BTA TRAK was assessed in the sub-CGs. Categorical data between the SG and CG and sub-groups were compared by the Chi-square test, and quantitative data were compared by the nonparametric tests. Comparisons of the age groups (study and CGs) and urinary markers were made using ANOVA. The level of statistical significance was set as $P < 0.05$ at 95% confidence interval (95% CI).

RESULTS

There were 48 men and four women with male-to-female ratio of 12:1 in the SG, but all the patients in the CG were males. The most predominant occupation of the patients in SG and CG was farming, in 31 patients (60.0%) and 20 patients (56.0%), respectively.

The findings on cystoscopy and bimanual examination are shown in Figure 1.

The most common histopathological type of bladder carcinoma was SCC, this was found in 25 patients (59.5%), whereas TCC and adenocarcinoma occurred in 16 patients (38.1%) and one patient (2.4%), respectively. There was histological finding of schistosoma ova in the biopsy samples of 18 patients (46.6%). Two patients in the SG have premalignant lesions, in the form of squamous metaplasia with cystitis cystica and a papilloma.
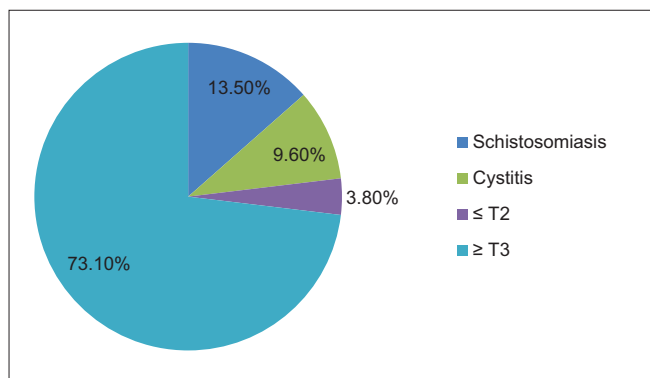
The comparison of the sensitivity, specificity, and predictive values of BTA TRAK and urine cytology is shown in Table 1. The BTA TRAK has higher sensitivity, but lower specificity than urine cytology in the diagnosis of bladder cancer.

In receiver operating characteristic (ROC) Curve, BTA TRAK had good area under the curve (AUC) of 0.751 as compared to that of the 0.355 of urine cytology [Figure 2] and the differrence was statistically significant, $P = 0.015$.

The use of BTA TRAK in the diagnosis of bladder carcinoma at cutoff value for bladder tumor antigen (BTA) of 14 units/mL was associated with 100% FP results in patients with hematuria (10/10) from other conditions, benign urologic conditions (7/7), cancer of the prostate (3/3), and 81.3% of HVs (13/16).

Adenocarcinoma had the highest concentration BTA (338.0 units/mL) in a single patient while the TCC has the lowest value of the marker. The mean concentration of BTA in patients with SCC (276.5 units/mL) was significantly higher than that of the TCC (258.4 u/mL), $P = 0.0001$.

The mean concentration of the BTA was highest in the SG, positive control subgroup (controls with hematuria) and BPH patients but lowest in the HVs. This difference was statistically significant, $P = 0.0001$. The mean concentration of the BTA in the various groups and subgroups is shown in Table 2.
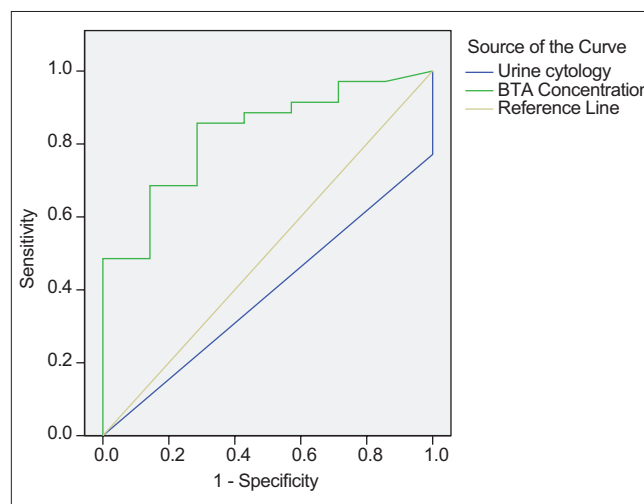
DISCUSSION

Bladder carcinoma is the most common male cancer and the 6th most common female cancer in Sokoto, NW Nigeria.[2] The mean age of 47 years in bladder carcinoma patients in this study is comparable to 46 years found in previous studies in schistosoma endemic areas.[3,4] The exposure to schistosomiasis occur in childhood and hence the development of bladder carcinoma at young age. In the Western world, occupational exposure to aromatic hydrocarbons and smoking occurred in adulthood thus the malignancy occurred in older age group at the 7th decade of life.[16,17] The Male: Female ratio of 12:1 is comparable to the previous study done by Mungadi and Malami[3] where the ratio was 11:1. All the patients in CG were males. The predominance of males is not surprising as they formed the main agricultural and fishing workforce in this environment.[3] The predominant occupation of the patients in both study and CGs was farming, this was found in about 60% and 56% of patients, respectively. Bladder carcinoma in African countries was associated with schistosomiasis and described in rural farming communities dwelling in the riverine areas.[3,4]

The most common histopathological type of bladder carcinoma in this study was SCC which was found in about 60% of the patients. This is comparable to the previous finding of SCC of 65.1% in this environment.[3]

Figure 1: Findings on cystoscopy and examination under anesthesia ($n = 52$). Schistosomiasis = vesical schistosomiasis, T2 = Stage 2 bladder tumor, T3 = Stage 3 bladder tumor

Figure 2: Receiver operating characteristic curve for bladder tumor antigen quantitative test and urine cytology

Table 1: Comparison of sensitivity, specificity, and predictive values of bladder tumor antigen quantitative test and urine cytology

Urine test	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
BTA TRAK (%)	98.8 (97.6-100)	13.6 (9.4-17.4)	81.7 (83.4-100)	75.0 (70-80)
Cytology (%)	29.1 (17.1-41.1)	95.5 (91-100)	96.2 (92.4-100)	25.6 (20.6-30.6)
BTA TRAK + cytology (%)	98.8 (97.6-100)	95.5 (91-100)	96.2 (92.4-100)	75 (70-80)

CI: 95% confidence interval, BTA TRAK: Bladder tumor antigen quantitative test, NPV: Negative predictive values, PPV: Positive predictive values

Table 2: comparison of mean values of bladder tumor antigen among study group, control group and sub-groups

Urinary marker	Group	Mean value±SD	P
BTA TRAK	SG	242.03±114.70	0.0001**
	CG	179.86±138.69	
	CG+	281.40±80.22	
	BPH	304.35±48.01	0.0001*
	Other benign diseases	209.59±163.92	
	CAP	231.30±150.18	
	HV	54.14±84.12	

*Kruskal-Wallis test, **Friedman's test, SG: Study group, CG: Control group, CG+: Patients in control group with hematuria, BPH: Benign prostatic hyperplasia, CAP: Prostate cancer, HV: Healthy volunteers, BTA TRAK: Bladder tumor antigen quantitative test, SD: Standard deviation

Adenocarcinoma was found in 2.4% of patients which was lower than 4.7% found in the previous report in our environment but comparable to 2.0% reported worldwide.[3,17] However, the finding of TCC of 38.1% was higher than the 27.9% reported by the previous study in our environment, which might be attributable to more awareness and campaigns about schistosomiasis in which population at risk were treated with Praziquantel in mass chemotherapy. This tends to lead to progressive increase in the rate of TCC which may reverse the ratio of SCC and TCC with time as it was observed in Egypt.[18] In this study, schistosoma ova was found in biopsy specimens of 47% of the patients with bladder carcinoma, this is comparable to 50% and 46% reported in our environment and Tanzania schistosoma endemic areas, respectively.[4] The finding of schistosoma ova in biopsy tissues of the bladder carcinoma patients in this study was not only restricted to SCC but also TCC and adenocarcinoma, which is in keeping with was reported in the literature, that schistosomiasis can predispose to other histopathological types of bladder carcinoma in pure or mixed forms.[16]

The high sensitivity of BTA TRAK in this study was similar in the previous studies.[18,19] The specificity of BTA TRAK in this study of 13.6% was much lower than that of 48%–92.5% reported by the previous studies.[7,18,19] As reported by the previous studies, BTA TRAK has higher sensitivity but lower specificity than the urine cytology. The lower specificity of BTA TRAK recorded in this study, may be related to the fact that the most common predisposition to the development of bladder carcinoma in this study was chronic schistosomiasis which itself as an inflammatory process can lead to FP results in the absence of bladder carcinoma. Using ROC curve, BTA TRAK performed better than urine cytology with higher AUC of 0.751 as compared to that of the AUC of cytology of 0.355, and the difference was statistically significant ($P = 0.015$). In the current study, there are comparable sensitivity and

specificity of 97.4%–90.0% at ROC curve cutoff value of 13.7 units/mL when compared with the sensitivity and specificity of 100.0%-92.0% reported by Abd El Gawad *et al.*[19] in Egypt at a higher cutoff value of 78 units/mL. The authors also proposed the use of combination of markers (BTA TRAK and cytology) to increase the both sensitivity and specificity, more than using a single marker alone. This study is in line with their finding as combination of BTA TRAK and urine cytology will produce sensitivity and specificity of 98.8% and 95.5%, respectively, better that recorded by the individual markers, taking advantages of the high sensitivity and specificity of BTA TRAK and urine cytology, respectively, recorded by this study. The use of BTA TRAK alone in the diagnosis of bladder cancer in our environment may lead to unnecessary invasive interventions and more financial burden while use of urine cytology alone will missed many bladder tumor leading to more morbidity and mortality from the advanced malignancy. However, the combination of the two tests will minimize the unnecessary interventions, morbidity, and mortality of advanced malignancy due to improved sensitivity and specificity.

The finding of high false-positive (100%) in patients with hematuria from other urologic conditions and in patients with benign urologic conditions was similar to the previous studies.[18,20,21] The 81% FP in HVs recorded in this study is contrary to what was reported by the previous studies, in which HVs tested negative for BTA. This might be explained by the reference cutoff value of 14 μ/mL for BTA used in the current study as compared to that of the cutoff value of 78 μ/mL used by Abd El Gawad *et al.*[19] Subgroup analysis of BTA concentration among various groups, HVs had significantly lowest BTA mean concentration of 54 μ/mL ($P = 0.00$). At this cutoff value, the BTA TRAK will test negative in the HVs. Exclusion of patients with hematuria from urologic conditions, benign and malignant urologic conditions other than bladder cancer will increase the specificity of the marker. Therefore, the establishment of reference values for BTA in our population for various disease groups and subgroups such as HVs is necessary.

No previous study assesses the detection rate of BTA by histopathological types of bladder carcinoma. In the current study, the highest BTA concentrations were found in patients with adenocarcinoma and SCC, whereas the lowest concentrations were found in patients with TCC and this difference was statistically significant $P = 0.001$. This finding might be explained by the de novo invasive and aggressive nature of adenocarcinoma and SCC of the bladder.

CONCLUSION

BTA TRAK is highly sensitive but poorly specific for bladder carcinoma diagnosis in our environment, which is endemic for schistosomiasis. The marker correlates positively with the stage of bladder carcinoma. There were high FP results (100%) in patients with hematuria from other urologic conditions and patients with benign prostatic hyperplasia. Review of cutoff value of BTA of 54 μ/mL is suggested in our community, this will increase the specificity of the marker for the diagnosis of bladder carcinoma in our environment.

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Conflicts of interest

There are no conflicts of interest.

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