Original Article

Performance of urinary survivin as a non-invasive molecular marker of bladder carcinoma in a schistosomiasis endemic area

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SUMMARY

Objective: To compare the sensitivity, specificity, positive predictive value, negative predictive value of urinary survivin and that of urine cytology in the diagnosis of bladder carcinoma in a schistosoma endemic area.

Design and setting: This is a 12-month prospective study of patients with features of bladder carcinoma as study group and patients with other urologic conditions and healthy volunteers as control group.

Participants: Patients with features of bladder carcinoma formed the study group, while patients with other urological conditions and healthy volunteers formed the control group.

Results: There were 52 patients in study group and 36 patients in control group. The mean ages of patients in the study and control groups were 47.17 ± 17.00 and 44.19 ± 18.89 years respectively. There were 48 males and 4 females in the study group, giving a male: female ratio of 12:1. Thirty-one (60 %) of the patients were farmers and 44 patients (85%) had history suggestive of schistosomiasis at childhood. The sensitivity of urine cytology and survivin in the study were 29.1% and 100.0% respectively. The specificity of urine cytology and survivin were 100.0% and 100.0% respectively (p= 0.05). The marker was associated with false positive (FP) results in patients with prostate cancer.

Conclusion: Urinary survivin is highly sensitive, specific and predictive of bladder carcinoma in our environment. The marker is associated with false positive results in patients with prostate cancer.

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Keywords: survivin, urinary bladder carcinoma, schistosomiasis, sensitivity, specificity

INTRODUCTION

Bladder cancer is the ninth most common cancer and 13th most common cause of death worldwide.[1] In Sokoto North-Western (NW) Nigeria, bladder cancer is the commonest male malignancy and sixth most common malignancy in women.[2] In the western world, transitional cell carcinoma accounted for 95-97% of bladder cancer.[3] In Africa transitional cell carcinoma and squamous cell carcinoma of bladder accounted for 60-90% and 10-40% of cases respectively.[3] In Sokoto, Nigeria, squamous cell carcinoma and transitional cell carcinoma accounted for 65.1% and 27.9% of bladder cancer due to endemicity of schistosomiasis.[4] Most of the patients present with advanced disease at diagnosis which was also noticed in other African countries where schistosomiasis is endemic.[5] The main stay for screening, diagnosis and surveillance of patients with bladder carcinoma are urine cytology and cystoscopy with or without biopsy.

Urine cytology although specific, is poorly sensitive for low grade tumours and dependent on the experience of pathologist.[6] Cystoscopy with biopsy is the gold standard method for bladder cancer diagnosis but is invasive, costly and cannot reliably identified small or flat lesions (carcinoma in situ) without use of an adjunct such as cytology, narrow band imaging and blue light cystoscopy.[6,7] There is, therefore, need for simple, non-invasive, and effective tool for screening, diagnosis and follow-up of patients after treatment. Urinary molecular markers are currently being investigated and produced encouraging results.[8]Survivin is a multifunctional protein that has been demonstrated to inhibit apoptosis, regulate cell division and promote angiogenesis by binding to and inactivating second mitochondria-derived activator of caspases (SMAC).[9,10]

The objective of the study was to compare the sensitivity, specificity, positive predictive value, negative predictive value of urinary survivin and that of urine cytology in diagnosis of bladder carcinoma in schistosomiasis endemic area.

METHODS

This is a prospective study carried out within 12 months (January- December, 2014) at our hospital. The study was approved by Health Research and Ethics Committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto with a reference number of UDUTH/HERC/2012/No.38. Only patients that gave informed consent were recruited for the study.

Consecutive patients with features of bladder cancer who presented at urology outpatient or Accident and Emergency departments of Usmanu Danfodiyo University Teaching Hospital and met the inclusion criteria were recruited as study group. Patients with other urologic conditions and healthy volunteers formed the control group. Haematuria is defined as presence of ≥ 3 red blood cells/ high power field of urine specimen.

The inclusion criteria included: confirmed cases of bladder cancer before the study, clinical or radiological bladder mass, haematuria in patients with significant history schistosomiasis and or smoking. The exclusion criteria included patients who had previous treatment for bladder cancer such as radical cystectomy, chemotherapy and patients that refused consent.

Urine sample collection and processing

Freshly voided urine or babotage sample of 50 ml was collected and divided in to 2 for urine cytology and survivin assay. For urine cytology, the sample was promptly fixed in 50% alcohol within 1 hour of collection and centrifuged at 2000 rpm for 5-10 minutes. The sediment was smeared on albuminised slides, stained with Papanicolaou stain and examined under microscope.[12] Atypical, suspicious and malignant results were considered positive for the presence of cancer. For survivin assay the sample was immediately frozen at -20 0 C till the time of the assay when the samples were brought to room temperature. [13]

Urethrocystoscopy and bimanual palpation

Urethrocystoscopy, biopsy of the suspicious lesion and bimanual palpation under general anaesthesia was done to stage the tumour. The samples were sent for histology in 10% formalin.

Histology

The biopsy samples were grossed, processed and embedded in wax. The tissues were cut in to 5 mm sections and stained with Haematoxylin and Eosin stains. Slides were prepared and examined under microscope for tissue diagnosis, grade and invasion.[14]

Survivin Assay Using Elisa Method

Two (2) ml urine samples were centrifuged at 1000 rpm for 1 minute. The reagents, urine samples and Survivin standards were prepared according to the manufacturer's instruction. Standard wells were prepared from 1-8 by adding 300 µL of human survivin in the first well and diluents in the other 7 wells. The human survivin was serially diluted in the 7 wells containing the diluents, with the 8^{th} well achieving zero concentration of the human survivin serving as negative control. Urine of100 µL was added into the remaining 88 wells which were coated with human anti survivin monoclonal antibody.

The plate containing the wells was covered with paper foil and incubated at 37 ^{0}C for 90 minutes. The plate was washed using automated plate washer for 3 minutes at 500 rpm. The wells were emptied of the remaining wash solution. Biotinylated polyclonal survivin antibody (100 µL) was added to each well, covered with paper foil and incubated at 37 ^{0}C at 500 rpm for 1 hour. The wells were washed 3 times using automated washer and emptied of the remaining wash solution.[15]

The enzyme-conjugate solution (100 µL) was added to each well, covered with paper foil and incubated at 500 rpm at 37 ^{0}C for 30 minutes. The wells were washed 5 times and subsequently emptied. Substrate solution (100 µL) was added to each well and incubated for 30 minutes. Hundred (100) µL of mixture of colour reagent A+B was added to each well and hatched in dark incubator at 37 ^{0}C. One hundred (100) µL of colour reagent C was added to each well and mixed very well. Optical density (OD) was read at 450 nm within 10 minutes.[15]

Data collection

Relevant data was collected through a semi-structured proforma which included clinical features and risk factors for bladder carcinoma, results of relevant investigations, cystoscopy findings, and results of histology, urine cytology, and Survivin assay.

Data analysis

Data analysis was done using Statistical Package for Social Sciences 20.0 version (2011) for windows (IBM, SPSS Inc., Chicago, IL, USA). Categorical data between the study group and control subgroups were compared by chi square and quantitative data were compared by non-parametric tests. Comparisons of the age groups (study and control groups) and urinary markers were done using ANOVA.

Receiver operating characteristic (ROC) curve used to calculate optimal cut off value for survivin with the highest sensitivity and specificity. The level of significance was set as $p < 0.05$ at 95% confidence interval (95% CI).

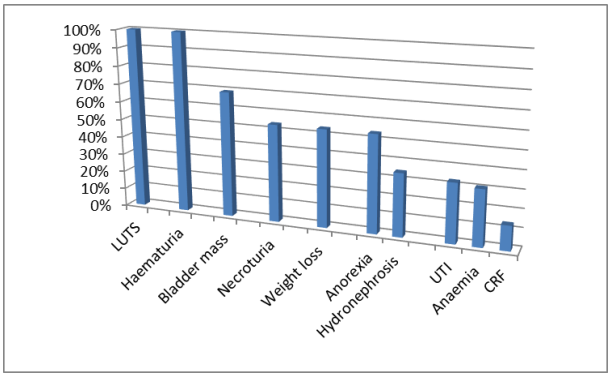
RESULTS

A total number of 88 patients participated in the study with 52 in the study group (SG) and 36 in control group (CG). The socio-demographic characteristics of the patients are shown table 1 below.

Table 1 Socio-demographic characteristics of the atients

Parameter	Study Group n= 52	Control group n= 36	p value
Mean age + SD	47.0 ± 17.0	44.0 ± 19.0	0.412*
Sex			
Male	48	36	
Female	4	0	0.061**
Occupation			
Farmers	31 (59.6)	20 (55.5%)	
Civil servants	9 (17.3%)	12 (33.3%)	0.186*
Students	3(5.8%)	2 (5.62.7%)	
Others	9 (17.3%)	2 (5.6%)	
Total	52	36	

Key: * = Analysis of variance (ANOVA) **= Fisher's exact test



LUTS- lower urinary tract symptoms, UTI- urinary tract infection, CRF- chronic renal failure

Figure 1 Mode of Presentation of Patients in the Study group (n=52).

The presentations of the patients were shown in Figure 1. There was history suggestive of schistosomiasis in 44 patients (85.0%), while 4 patients (7.7%) smoked cigarette.

At cystoscopy, 7 patients (13.5%) had features suggestive of chronic schistosomiasis, 5 patients (9.6%) had features of cystitis, 2 patients (3.8%) had features of early bladder carcinoma and 38 patients (73.1%) had features of advanced bladder carcinoma. The features of chronic schistosomiasis were sandy patches, tubercles and nodules.

The early bladder carcinoma appeared as red patches, papilloma, papillary or sessile solitary lesions less than 2cm. The advanced carcinoma appeared as huge exophytic bladder masses occupying most or part of the bladder. There were extensive necroturia and ulceration of some of the masses.

Urinary survivin was more sensitive and equaly specific as urine cytology. The comparison of the sensitivity, specificity and predictive values of the markers are shown in table 2 below.

Table 2 Comparison of sensitivity, specificity and predictive values of Survivin and urine cytology

Urine test	Sensitivity	Specificity	PPV	NPV
Survivin	100.0%	100.0%	80.2%	80.0%
Cytology	29.1%	100.0%	96.2%	25.6%

p= 0.05 (ANOVA)

Using receiver operating characteristic (ROC) curve survivin had good and better area under the curve (AUC) of 0.72 when compared to 0.38 of urine cytology (Figure 2) and the difference was statistically significant, p= 0.015. Figure 3 below is the ROC curve of survivin and urine cytology. Using the curve the optimal cut off value for Survivin of 18.81 pg/ml produced sensitivity and specificity of 100.0 % and 100.0% respectively.

The most common histopathological type of bladder carcinoma was SCC, this was found in 25 patients (59.5%) while TCC and adenocarcinoma were found in 16 patients (38.1%) and 1 patient (2.4%) respectively. There was histological finding of schistosoma ova in the biopsy samples of 18 patients (46.6%). Two patients in the study group have premalignant lesions, in form of squamous metaplasia with cystitis cystica and a papilloma.

The mean concentration of urinary markers correlated positively with grade but negatively with stage of bladder carcinoma, (p=0.001). The mean concentrations of the survivin in patients with SCC and adenocarcinoma were significantly higher than that of TCC, p=0.001.

Urine survivin at cut off value of 18.81 pg/ml 100.0% specific in patients with benign urologic conditions (stricture, stone, benign prostatic hyperplasia) and healthy volunteer but false positive in prostate cancer patients.

In sub-group analysis of mean survivin concentration, the lowest values were recorded in the healthy volunteers and those with benign urological conditions, p=0.001. The mean concentrations of the survivin in the various groups and subgroups are shown in Table 3.

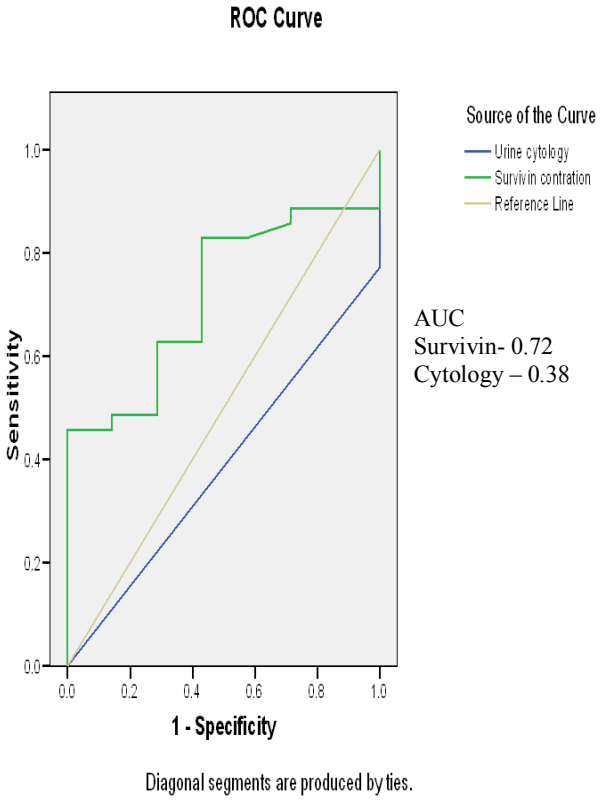


Figure 2 ROC curve for Survivin ELISA and Urine cytology

Table 3 Comparison of Mean values of Survivin among SG and CG

Urinary marker	Group	Mean value ± SD	p value
Survivin	SG	243.01 ± 374.5	0.0001 **
	CG	187.88 ± 558.71	
	Benign diseases (stricture, stone, BPH)	4.46± 0.00	
	CAP	280.04 ± 739.51	0.0001*
	HV	17.42 ± 14.9	

Key:* = Kruskal- Wallis test, ****= Friedman's test, BPH= benign prostatic hyperplasia

DISCUSSION

The finding of young age group of 47 years in bladder carcinoma patients in this study is comparable to 46years found in previous studies done in schistosomiasis endemic areas.[4,5] The exposure to schistosomiasis occur in childhood and hence development of bladder carcinoma at young age. The Male: Female ratio of 12: 1 is comparable to the previous study done by Mungadi and Malami[4] where the ratio was 11:1. All the patients in control group were males. The predominance of males is not surprising as they formed the main agricultural and fishing work force in this environment.[4] most of our patients are farmers as reported in other schistosomal endemic areas.[4,5]

The commonest risk factor (85%) of bladder carcinoma in the present study was schistosomiasis as observed by previous study in this area. [5]The high sensitivity and specificity of Survivin of 100.0% is comparable to the 94.0-100.0% observed by previous studies.[16- 17] Urine survivin had PPV and NPV of 100.0% and 80.2% respectively in this study, this is similar to the 100.0% and 86.0% respectively reported by a previous study.[18]

In the present study survivin cut off value of 18.81 pg/ml was used, this was higher than 12 pg/ml value used by most of the studies, [13-16] but comparable to 17.7 pg/ml used by one of the studies. [17]Urine cytology has lower sensitivity but similar specificity as compared to the urine survivin in the present as reported by a previous study. [13,17] The sensitivity of urine survivin and urine cytology were 100.0% and 29.1% respectively, p= 0.05. Using ROC curve the optimal cut off value of 18.81 pg/ml for survivin that yielded 100% sensitivity and specificity is comparable to 17.7pg/ ml suggested by Srivastava et al[17], which gave sensitivity and specificity of 82.9% and 81.1% respectively.

The commonest histopathological type of bladder carcinoma in this study was SCC as reported by the previous study.[4] The finding of schistosoma ova in biopsy tissues of the bladder carcinoma patients in this study was similar to what were reported by the previous studies.[4,5]

Patients with benign urological conditions and HVs were negative for survivin as observed by a previous study. [14] But in contrary to the previous study, the patients with prostate cancer in current study yielded false positive results.[19] The finding of high concentration of survivin in patients with bladder carcinoma compared with HVs and patients with benign urologic diseases is similar to what were observed by previous studies.[5,17] There is correlation of mean survivin concentration with grade of bladder carcinoma as reported by the previous studies, (p=0.001).[10,13]

The concentration of the Survivin in this study correlated negatively with the stage of the bladder carcinoma which was contrary to what was observed by the previous studies.[19,17] This may be explained by fewer number

of patients with early disease (2) as compared to those with advanced disease (38).In the present study, there is significant correlation between the mean survivin concentration and histological sub-types. The mean survivin concentrations in the patients with adenocarcinoma and SCC were significantly higher than that of the patients with TCC (p=0.001).

CONCLUSION

Urinary survivin at a cut off value 18.8 pg/ ml of is 100% sensitive, specific and predictive of bladder carcinoma in our environment, which is endemic for schistosomiasis. The marker correlated positively with the grade of bladder carcinoma and is associated with false positive results in patients with prostate cancer.

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