Original Article

# Serum bladder tumor antigen levels in subjects with sickle cell anemia: A preliminary report

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## Abstract

**Background:** Sickle cell anemia (SCA) has been linked with the occurrence of some tumors, including that of the urological system; the serum bladder tumor antigen has been shown to be a surrogate marker for bladder carcinoma. **Objective:** The objective of this study was to evaluate serum bladder tumor antigen in SCA subjects in comparison with disease severity and levels in subjects with other hemoglobin phenotypes. **Subjects and Methods:** A total of 50 subjects were randomly recruited which comprised of 20 homozygous SCA subjects in steady state, 20 heterozygous hemoglobin AS (HbAS), and 10 hemoglobin AA (HbAA) subjects. Five milliliters of venous blood was collected from each participant for hemoglobin type confirmation and estimation of bladder tumor antigen levels, using cellulose acetate hemoglobin electrophoresis and enzyme-linked immunosorbent assay, respectively. Disease severity scoring was based on the earlier report of Okocha *et al*. **Results**: The mean serum level of bladder tumor antigen was significantly lower in SCA compared with HbAS and HbAA subjects (23.12 ± 3.75 ng/ml vs. 29.60 ± 3.80 ng/ml and 34.65 ± 4.05 ng/ml, $P < 0.001$, respectively). Correspondingly, the mean serum bladder tumor antigen levels were significantly lower in HbAS compared with HbAA subjects ($P < 0.001$). Serum bladder tumor antigen level was not significantly correlated with disease severity in subjects with SCA ($r = -0.267$, $P = 0.318$). **Conclusion**: The low serum levels of bladder tumor antigen in subjects with SCA may indicate a lower risk of bladder carcinoma.

**Key words:** Bladder tumor, disease severity, sickle cell anemia

## INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder of hemoglobin, characterized by chronic anemia, acute painful episodes, organ infarction with chronic organ damage, and significant reduction in life expectancy.[1,2] It is the most common genetic disorder of hemoglobin in sub-Saharan Africa, where up to 200,000 babies are born with the disease annually.[3-5]

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A point mutation on the 6th codon of the beta-globin gene located on the short arm of chromosome 11 is responsible for the development of the disease. The mutation produces a defective beta-globin chain, which under low oxygen tension polymerizes into long fibers that eventually lead to abnormally deformed (sickled) red cells. The sickled red cells become sticky, adhere to the endothelium, and clump together plugging microvessels and equally causing damage to larger blood vessels.[6] In addition to vasculopathy, sickle red cells have markedly reduced survival in circulation, resulting in a chronic hemolytic anemia state. The interaction of sickled red cells with the vascular

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endothelium leads to episodic microvascular occlusion, ischemia with reperfusion, vascular and inflammatory stress, and ultimately a myriad of potentially life-threatening clinical manifestations.[6,7]

A number of recent reports have shown the utility of a monoclonal antibody-based (mAb-based) assay (BTA-TRAK) for the detection of bladder cancer.[8] The mAb used in the assay targets the bladder tumor antigen (also known as soluble human complement factor H [hCFH] protein) which had entered the urine of patients with bladder cancer from the plasma.[9]

Plasma hCFH is a 150-kDa protein translated from a 4.4 kb mRNA; it is composed of 20 domains called short consensus repeats.[8] The protein is an efficient regulator of the activity of the alternative pathway C3 and C5 convertases in plasma and equally on the surfaces of host cells.[9] It does this by preventing factor B binding to C3b, thereby promoting dissociation of the C3bBb enzyme complex (decay accelerating activity) and equally by acting as a cofactor for factor I-mediated inactivation of C3b. Through its activity against the alternate pathway, the hCFH protein promotes carcinogenesis by binding to tumor cells in the immediate vicinity of the tumor microenvironment and protects these cells from complement-mediated cell lysis, a critical component of normal body tumor surveillance mechanism.[10,11]

Information on the prevalence of malignancies in patients with SCD is sparse; a recent multicenter study reported the prevalence rate of renal medullary carcinoma in Nigerian SCD subjects to be 5.6/100,000.[12] Similarly, a single institution study done among SCD subjects in the United States of America reported a prevalence rate of malignancy of 1.74/1000 patient-years.[13] There has been no study that specifically screened for bladder carcinoma in our population of SCD patients, using surrogate markers of the disease.

In view of some reports of malignancies in subjects with SCD, this pilot study was, therefore, designed to bridge this knowledge gap, by screening for the bladder tumor antigen (hCFH) in subjects with different hemoglobin phenotypes and comparing serum levels with disease severity (in subjects with sickle cell anemia [SCA]). We hope that findings from this pilot may highlight the need for a larger multicenter study on this subject in the future.

## SUBJECTS AND METHODS

### Study area

This was the Nnamdi Azikiwe University Teaching Hospital, (NAUTH), a government owed, tertiary healthcare facility in Nnewi, Anambra State, Nigeria.

### Research design

A total of 50 subjects were randomly recruited from our weekly hematology outpatient clinic and from among consenting members of the hospital community. These included 20 confirmed homozygous SCA patients in steady state, 20 heterozygous hemoglobin AS (HbAS) subjects, and 10 hemoglobin AA (HbAA) individuals who served as the control group. The selection criteria for SCA subjects in the steady-state group were based on the absence of any form of crisis for at least 3 weeks and no blood transfusion, 4 weeks before recruitment. The information utilized for severity scoring was obtained from questionnaires administered to the subjects, as adapted from the earlier report of Okocha *et al*.[14] The parameters used for calculating severity score included the hemoglobin concentration, total white cell count, number of vaso-occlusive crisis, lifetime blood transfusion rate, and sickle-related complications. Subjects with score ≤3 were classified as mild, those with score of >3–≤7 were classified as moderate, while those with score >7 were classified as severe disease.

### Ethical consideration

The ethical approval for this research was sought from the NAUTH Ethics Committee, and each subject gave written informed consent at the point of recruitment.

### Sample collection and processing

About 5 ml of venous blood was collected from each participant following the standard protocol for venesection; 2 ml was dispensed into ethylene diamine tetra acetic acid (EDTA) specimen container for full blood count (FBC) and hemoglobin phenotype determination, while the remaining 3 ml was collected into plain container and spurn at 3000 rpm for 5 min. Serum was extracted and used for the determination of bladder tumor antigen levels. FBC and hemoglobin phenotype of each participant were done using automated hematology analyzer (Mythic 22, Switzerland®) and cellulose acetate paper electrophoresis (Helena Biosciences, UK®), respectively. Estimation of bladder tumor antigen levels was based on immunometric double-antibody "sandwich" technique, using factor H human enzyme-linked immunosorbent assay test kits procured from Abcam diagnostics® Inc., USA. The test protocol involved the addition of standards and samples on antibody-coated plates followed by incubation. Horseradish peroxide-labeled factor H monoclonal antibody was subsequently added after the plates were properly rinsed. A "sandwich" was formed by the two antibodies, followed by the addition of a chromogenic substrate and stop solution. The reaction mix was gently mixed for 30 s and read at 450 nm using a microplate reader.

### Statistical analysis

Data analysis was done using the Statistical Package for the Social Sciences, version 20 computer software

(SPSS Inc., Chicago, IL, USA) and results of serum bladder tumor antigen were presented as means ± standard deviation. Comparison of means of bladder tumor antigen among the various hemoglobin phenotypes was done using the Student's *t*-test and analysis of variance as appropriate, while the relationship between antigen level and objective scores of disease severity (in SCA only) was determined by the Pearson's linear regression for bivariate correlation; $P < 0.05$ was accepted as statistically significant.

## RESULTS

The means of serum bladder tumor antigen were significantly lower in subjects with SCA compared with those with HbAS hemoglobin type [23.86 ± 8.47 ng/ml vs. 29.60 ± 3.80 ng/ml respectively, Table 1]. Correspondingly, serum bladder tumor antigen level was significantly higher in subjects with HbAA compared with those with SCA and HbAS [34.65 ± 4.05 ng/ml vs. 23.86 ± 8.47 ng/ml and 29.60 ± 3.80 ng/ml, respectively, Table 1].

Serum bladder tumor antigen levels were negatively correlated with objective score of disease severity in subjects with homozygous hemoglobin SS ($r = -0.267$); this was, however, not statistically significant ($P = 0.318$) [Figure 1].

There was no statistically significant difference in the serum levels of bladder tumor antigen in SCA subjects with mild, moderate, and severe disease [23.34 ± 4.43 ng/ml, 22.03 ± 1.61 ng/ml, and 18.90 ± 0.99 ng/ml, respectively, $P = 0.24$, Figure 2].

## DISCUSSION

Historically, the development of malignancy in persons living with SCD has been documented over the past 50–60 years.[15-18] These reports were at best scattered and neither provided complete data on the types of cancer nor attempted to define the epidemiology of specific cancers that affect subjects with SCD. On the basis of a single institution study, the cancer incidence among patients with SCD has been reported to be 1.74 cases/1000 patient-years by Dawkins *et al.*, at the Howard University Hospital, USA.[13] Similarly, Schultz and Ware identified 52 cases of cancer among 16,613 patients with SCD followed at 52 institutions in the United States of America and reported a preponderance of carcinomas among adult study subjects.[19] While the above studies emphasized the general prevalence of all cancers in SCD subjects, the multicenter study of Anazoeze *et al.* reported the prevalence of 5.6/100,000 for renal medullary carcinoma among adult Nigerian SCD population; this was, however, not higher than the general population prevalence.[12] Some factors have been hypothesized to potentially contribute to the occurrence of cancer in SCD subjects, ranging from transfusion transmissible viruses (which may be acquired through recurrent blood transfusions) to the chronic inflammation of SCD and other comorbid variables such as smoking and use of hydroxyurea.[19] To date, there is insufficient comprehensive compilation of malignancy in patients with SCD, using data from multiple institutions with a large patient-year denominator to establish definitive associations.

**Table 1: Comparison of serum bladder tumor antigen in subjects with different hemoglobin phenotypes**

| Hemoglobin phenotype | Bladder tumor antigen levels (ng/ml) | *P* |
|---|---|---|
| HbSS (*n*=20) | 23.12±3.75 | <0.001* |
| HbAS (*n*=20) | 29.60±3.80 | |
| HbSS (*n*=20) | 23.12±3.75 | <0.001* |
| HbAA (*n*=10) | 34.65±4.05 | |
| HbAS (*n*=20) | 29.60±3.80 | <0.001* |
| HbAA (*n*=10) | 34.65±4.05 | |

*Significant *P* values. HbSS: Homozygous hemoglobin SS, HbAS: Heterozygous hemoglobin AS, HbAA: Hemoglobin AA

In this study, serum bladder tumor antigen (which is equally a component of the alternate complement pathway protein) was significantly lower in SCA compared with HbAS and HbAA subjects [Table 1]. Other studies had emphasized lower serum levels of alternate complement pathway proteins in SCA subjects.[20] The lower serum levels of these proteins are thought to significantly increase the infectious risk in subjects with SCA and are believed to result from excess consumption due to the activation of the alternate pathway.[20] The complement system is a central part of the immune system that has developed as a first line of defense against nonself cells, including tumor cells.[21] It can be activated by one of the three pathways, the classical, lectin, and alternative pathways.[22] Low levels of serum complement proteins (especially those of the alternate pathway) have equally been documented in some malignant conditions, and this significantly increases disease-related morbidity due to high infection risk.[23-25] Infarct, low complement levels (with suboptimal complement activation) have been shown to allow cancer cells to escape complement-mediated elimination and equally hamper the clinical efficacy of monoclonal antibody-based cancer immunotherapies.[21] In view of the established role of bladder tumor antigen in creating enabling environment for tumor growth (through inhibition of complement-mediated tumor surveillance) vis-a-vis the low serum levels observed in our subjects, we hypothesize that bladder carcinoma may not be common in our SCA population. Although our finding is in keeping

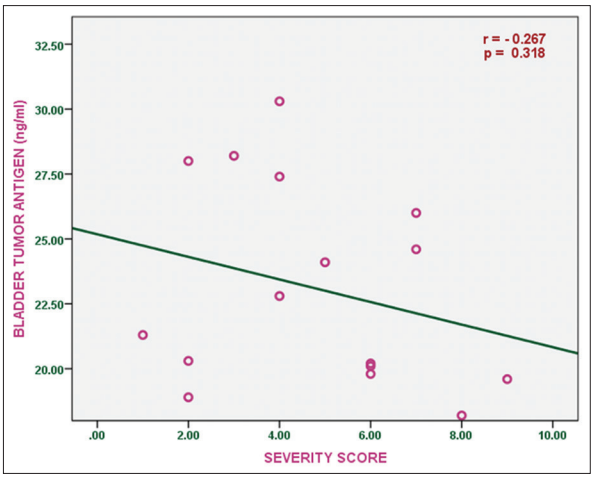


**Figure 1:** Correlation of serum bladder antigen levels with disease severity score in subjects with sickle cell anemia

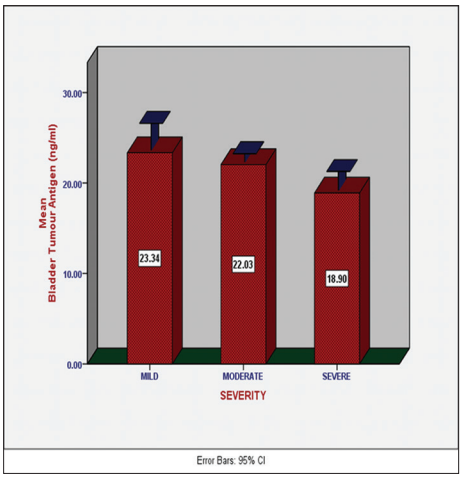


**Figure 2:** Serum levels of bladder tumor antigen in sickle cell anemia subjects with different degrees of disease severity

with the report of Anazoeze *et al.*, it contrasts other studies which appear to suggest that SCD subjects have increased tendency to develop malignant conditions.[12,15-19,26]

Interestingly, serum levels of bladder tumor antigen were not significantly correlated with disease severity in subjects with SCA ($r = -0.267$, $P = 0.318$). We did not find this surprising as an intact complement system (devoid of the inhibitory effects of bladder tumor antigen) could significantly reduce infection risk and engender a less severe disease phenotype. Infection (including malaria, in endemic parts of the world) is a significant contributor to morbidity in SCA subjects.[27,28]

## CONCLUSION

In contrast to a number of earlier reports which suggested higher risk of malignancy in subjects with SCA, the low serum level bladder tumor antigen (a surrogate marker for bladder carcinoma which is believed to encourage tumor growth through inhibition of complement-mediated tumor surveillance) observed in this study could suggest that bladder carcinoma may not be common in our population. We recommend larger multicenter studies to confirm this finding.

### Strength of the study

This is the first study that evaluated the risk of bladder carcinoma among African steady-state SCA subjects, in comparison with disease severity.

### Limitation of the study

Due to the small sample size, these results are preliminary and will need to be confirmed by larger (possibly multicenter) studies.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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