Urinary tract disorder

Short Communication

A pilot biomonitoring study of bladder tumor antigen (BTA) in aflatoxin exposed Nigerian villagers

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Abstract

Objective: To correlate the levels of bladder tumour antigen (BTA) with aflatoxin M1 (AFM) in a human population in Nigeria.

Subjects and methods: A pilot, observational study was conducted with 22 human subjects randomly recruited from a Nigerian rural community. Serum and first morning urine of participants were analysed for human BTA and AFM1, respectively, using quantitative ELISA assays.

Results: All the subjects were positive to AFM1 (mean = 0.235 ± 0.072 ng/mL) while 19 were positive to BTA (mean = 2.340 ± 1.741 ng/mL). A negative relationship occurred between human BTA and AFM level (r = -0.239; $P = 0.285$). Human BTA (2.86 ± 2.43 ng/mL; $P = 0.306$) and AFM1 (0.258 ± 0.065 ng/mL; $P = 0.643$) were higher in subjects 1–20 years. The two biomarkers were not also associated with sexes of the participants ($P > 0.05$), although they were higher in the female subjects.

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Conclusion: This study showed that there may not be a connection between aflatoxin exposure and human BTA which is one of the biomarkers of bladder cancer.

Introduction

Bladder cancer poses global threat especially in males with an estimated 260,000 new cases occurring each year compared with their female counterparts with estimate totaled 76,000 [1]. Cancer of the urinary bladder is one of the most common urologic malignancies resulting in significant morbidity and mortality [2]. The use of bladder tumor markers for surveillance of at-risk populations will aid rapid identification of recurrence and prevent disease progression [3]. The human bladder tumor antigen (BTA) tests which are either qualitative or quantitative detect human complement factor H-related protein (as well as complement factor H), which is present in the urine of patients with bladder cancer [4].

Aflatoxins, toxic secondary metabolites produced by certain species of *Aspergillus* such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* in agricultural commodities, have been implicated in liver cancer during chronic dietary exposures [5]. Aflatoxin exposure is best monitored using serum and/or urinary biomarkers which reflect chronic and short-term (within 48 h) exposure, respectively. Aflatoxin B1 (AFB), which is the most toxic aflatoxin type, becomes bio-transformed in the liver to AFM1, Q1, B2a, P1 and aflatoxicol [6] and AFM1 is frequently excreted in urine during metabolism. Aflatoxin exposure measured by the urinary AFM1 biomarker has been frequently reported in several countries in Africa including Nigeria [7,8] and had been shown to correlate with AFB1 in diets and serum [9].

Most cancers including aflatoxin-induced hepatocellular carcinoma and the human bladder cancer result from the mutation of the tumor suppressor gene *p53* [10]. However, there is currently no report linking aflatoxin with bladder cancer or bladder-related pathology; a study in this direction which involves the correlation of urinary AFM with human BTA could provide insight on possible involvement of aflatoxin in bladder pathology in aflatoxin-prone areas. The aim of this study was to assess the relationship between urinary AFM and serum BTA levels in a human population that reside in a Nigerian village.

Subjects and methods

The study was carried out in Ilumafon, a village within Ijebu North-East Local Government Areas in Ogun State, Nigeria. Ilumafon is a rural settlement of about 200 dwellers; the households are not formally educated beyond primary school and they solely depend on agriculture, growing and consuming own crops which include cocoa, cassava, maize and oil palm. The settlement lacks good storage and processing facilities for their farm produce, therefore the population are engaged in practices which could easily predispose their produce to fungal invasion and consequent mycotoxin contamination. The settlement has a nearby stream which served as a major source of water supply for bathing and several house chores until six months before this study.

The study was a pilot, observational study. A total of 22 participants (range: 4–62 years; mean age = 27 ± 16.8 years) were randomly selected based on their willingness to participate in the study. About 20 mL of first morning urine sample was collected from each subject prior to consumption of water or food for the day. Blood sample (3 mL) was also collected in a plain tube from each participant. The urine and blood samples were immediately centrifuged at 5000 rpm for 5 min to obtain a clear supernatant and serum, respectively, and then stored at $-20\,^{\circ}C$ until further analysis.

Participation was voluntarily and written informed consent was obtained from adult participants. For children, consent was obtained from their parents or guardians. The study was approved by Babcock University Health Research Ethics Committee. Only the permanent residents of the study area were included. Individuals who had undergone surgical treatment or instrumentation within 14 days of testing were excluded from the study.

A quantitative ELISA kit assay (Helica Biosystems, Inc. Cat. No. 991 AFLMO1U) was used for detection of AFM in urine according to manufacturer instruction. Briefly, all reagents and urine samples were brought to room temperature. An aliquot of both the urine standards and samples was diluted 1:20 with distilled water. 200 μL of the assay buffer was dispensed into each mixing well. Then 100 μL of each diluted standard and sample was added to the appropriate mixing well containing the assay buffer and was mixed by priming pipettor at least 3 times. Using a new pipette tip for each, 100 μL of contents from each mixing well was transferred to a corresponding antibody coated microtiter well and the mixture was incubated at room temperature for 1 h. The content of each microwell was discarded and the microwells were washed with PBS-Tween wash buffer 3 times. The microwells were dried and 100 μL of conjugate was added to each antibody coated well and incubated at ambient temperature for 15 min. Substrate (100 μL) was added and incubated at room temperature for 15 min. Then 100 μL Stop solution was added and the optical density (OD) was read within 2 min at 450 nm using a Rayto (RT-2100C) Microplate reader (Rayto Life and Analytical Sciences Co. Ltd, Shenzhen, China). The corresponding aflatoxin concentration in each well was estimated from standard curves using AFM1 standard solution (0–4 ng/mL).

Human BTA (Bladder Tumor Antigen) ELISA Kit (Cat. No: E-EL-H0579) was purchased from Elabscience, China. This ELISA kit uses Sandwich-ELISA principle. The test was performed in a

blinded fashion by an impartial observer on all subjects' serum in accordance with the manufacturer instructions. Briefly, 100 μL standard or sample was added to each well and incubated for 90 min at 37 °C. Supernatant was removed and 100 μL of biotinylated detection antibody was added and then incubated for 1 h at 37 °C. The well content was aspirated and washed 3 times. 100 μL of Avidin-Horseradish Peroxidase (HRP) Conjugate was added and incubated for 30 min at 37 °C. The well content was aspirated and washed 5 times. Substrate reagent (90 μL) was added followed by incubation at 37 °C for 15 min. Stop solution (50 μL) was added and the plate was read immediately at 450 nm wavelength. The concentration of BTA was estimated from a standard curve.

Data generated was analysed using GraphPad Prism 5 (GraphPad Software, Inc., LaJolla, CA, USA). One way ANOVA and Student's t-test were used to determine significant differences in aflatoxin and BTA levels. Pearson correlation analysis was used to determine the relationships between AFM and BTA. The same was used to determine the relationship between AFM1/BTA with age of the participants. P-values less than 0.05 were considered statistically significant.

Results

All the subjects tested positive to AFM (mean = 0.235 ± 0.072 ng/mL) while 19 were positive to BTA (mean = 2.340 ± 1.741 ng/mL). A negative relationship occurred between human BTA and AFM1 level (r = −0.239; P = 0.285) (Fig. 1). Levels of BTA (2.86 ± 2.43 ng/mL; P = 0.306) and AFM (0.258 ± 0.065 ng/mL; P = 0.643) were higher in subjects 1–20 years than in subjects within other age groups (Fig. 2). The mean AFM level (0.25 ± 0.061 ng/mL) in female subjects was not significantly higher than the level recorded in male subjects (0.228 ± 0.061 ng/mL) (P = 0.517) (Fig. 3). Human BTA was also higher in female (2.598 ± 2.025 ng/mL) than in male subjects (2.151 ± 0.718 ng/mL) and the variation was also not gender dependent (P = 0.654) (Fig. 3). AFM1 (r = −0.135, P = 0.580) and BTA (r = 0.275, P = 0.256) showed negative relationship with age of participants.
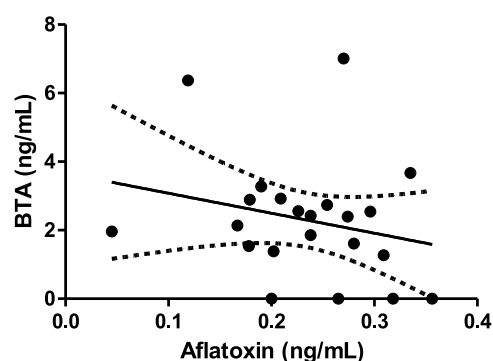

Figure 1   Relationship between urine AFM1 and serum BTA.

Discussion

Aflatoxin exposure is a global public health challenge; especially in high risk regions such as sub-Saharan Africa (SSA) and some parts of Asia [11]. Human exposures to aflatoxins usually result from the ingestion of contaminated staple crops (e.g. groundnuts, maize and melon) which have been reported to contain high levels of AFB [12]. Although bladder cancer is the second most common urological cancer type after prostate cancer in Nigeria [13], the use of BTA as a biomarker in population at risk of bladder cancer is yet to gain wide advocacy especially in resource-scarce regions such as SSA. This study is the first to relate aflatoxin levels in humans with BTA levels with the aim of unravelling the possibility of linking the aetiology of bladder cancer to aflatoxin by measuring serum BTA level in aflatoxin exposed individuals.

The studied village is a high risk zone for aflatoxin exposure having all the randomly selected subjects positive to AFM1. Their exposure could be linked to heavy reliance on aflatoxin-prone crops (maize, cassava, cocoa and palm), poor storage and processing of the crops. Cassava flour, cocoa, maize grains and palm kernel nuts are often sun-dried in close proximity to soil, thus could enhance easy contamination with fungal spores.
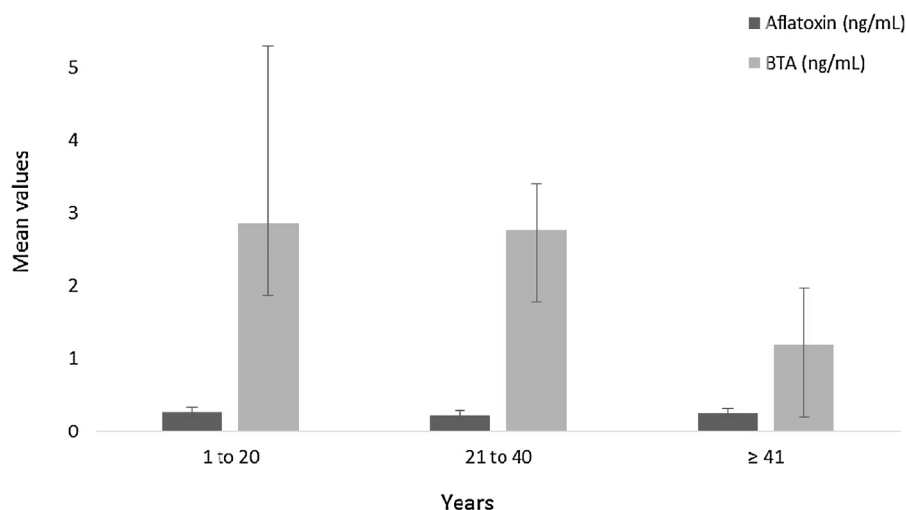

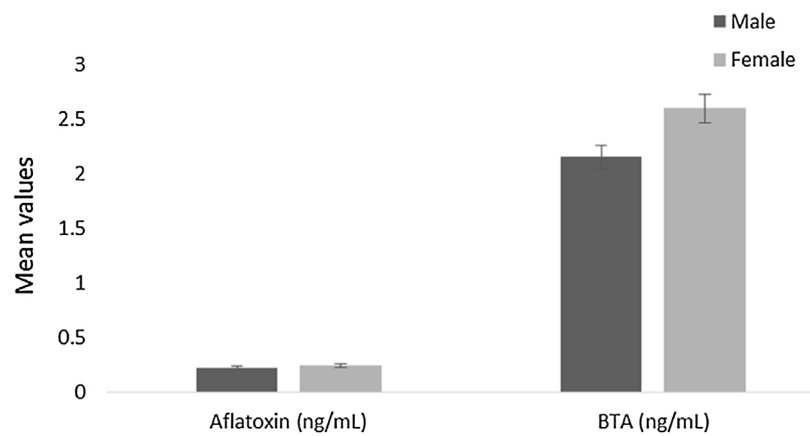**Figure 2**   Variations in AFM1 and BTA with age.

Figure 3 Variations of AFM1 and BTA with sex.

The negative relationship between BTA and AFM in this study may suggest noninvolvement of AFB in cancer-associated bladder pathology. However, this cannot be explicitly concluded due to the small sample size of the population studied and perhaps the application of urinary AFM1 for aflatoxin exposure determination which reflects short-term rather than long-term exposure. Immunoassays for urinary AFM1 biomarker analysis are readily available, less expensive and requires less expertise compared to serum aflatoxin albumin (AF-alb) biomarker analysis. The higher BTA and AFM1 level recorded in the lower age group may be linked to different aetiologies. Higher aflatoxin exposures are expected in children compared to adults [8,14], mostly due to higher risky feeding habits (complete dependence on family meal options), higher food consumption rates *vs* small body weight, and less developed body system which is highly susceptible to adverse effects of natural toxic chemical. The higher BTA levels in the same age group could however be linked to higher *Schistosoma haematobium* infection in the age range. A baseline epidemiological study on schistosomiasis showed high exposure to *S. haematobium* in the group (data not shown). With evidences of *S. haematobium* involvement in bladder cancer pathology [15], it is reasonable to suggest that higher BTA in the group could be a result of higher schistosomiasis infection in the group. Higher BTA in the female subjects also corroborates with higher burden of schistosomiasis in our preliminary epidemiological study on schistosomiasis in the study area. The co-occurrence of these significant public health challenges (aflatoxin and schistosomiasis exposure) among residents of this community, demands prompt attention as the villagers may be at high risks of hepatocellular carcinoma and bladder cancer.

Conclusion

This study showed that there may not be a connection between aflatoxin exposure and human BTA which is one of the biomarkers of bladder cancer. A related study with larger sample size and possibly aflatoxin exposure analysis by serum AF-alb rather than urinary AFM1 or even a combination of both biomarkers will help to validate this observation. More importantly, study on the role of schistosomiasis in bladder cancer through biomonitoring of human BTA is highly recommended. Community education of rural agrarian households on best practices to mitigate mycotoxin exposure in farm produce is also recommended.

Ethical Committee Approval

This study was approved by Babcock University Health Research Ethics Committee (BUHREC154/17).

Consent from the Patients

Each of the participants gave a written consent.

Source of Funding

Authors' contribution

O Oyeyemi conceived the study. O Oyeyemi, C Ezekiel designed the study. O Oyeyemi, C Ezekiel, K Ayeni, W Nabofa, I Oyeyemi, O Oyedele, A Adefalujo, C Nwozichi, A Dada carried field and laboratory studies. O Oyeyemi wrote the first manuscript draft. All authors revised and approved the final manuscript.

Conflict of interests

None declared.

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