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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.

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

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