Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls *

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Received 16 March 2000; accepted in final form 3 May 2001

Abstract

Urine samples from children in Sierra Leone (134 boys and 110 girls), were collected during the dry season. During the rainy season samples were collected from 97 boys and 93 girls. Analysis of the dry season samples, revealed that, with the exception of one boy, all children had detectable amounts of aflatoxins and/or ochratoxins in their urine. Similarly, with the exception of four children (two from each sex), rainy season urine samples also contained these two mycotoxins. There were significant differences in the frequency of exposure to some mycotoxins: ochratoxin A (OTA), \( p < 0.01 \); 4-hydroxyochratoxin A (4R-OTA), \( p < 0.002 \); aflatoxin M1 (AFM1), \( p < 0.04 \); aflatoxicol (AFL), \( p < 0.03 \); aflatoxin B2 (AFB2), \( p < 0.04 \). There were also significant differences in the levels of aflatoxin B1 (AFB1), \( p < 0.05 \) and AFB2, \( p < 0.02 \) detected in dry season samples. Stratification of these results according to season and sex, has indicated significant differences with respect to 4R-OTA \( p < 0.04 \) and AFB1 \( p < 0.02 \). The results of this study show that in Sierra Leone, children are frequently and constantly exposed to both aflatoxins and ochratoxins.

Key words: aflatoxin, children, dry season, ochratoxin, rainy season, Sierra Leone, urine

Introduction

In Africa, the incidence of Primary Liver Cancer (PLC) among young males has been well-documented [1–3]. The association between aflatoxin ingestion and PLC though controversial has enjoyed considerable support. Several studies to assess the relationship between aflatoxin exposure and liver cancer have been conducted [4–7]. Assay for urinary aflatoxin B1 (AFB1) and its metabolites – aflatoxin P1 (AFP1), aflatoxin M1 (AFM1) and DNA-adduct (AFB1-N7-Gua) showed that subjects with liver cancer were more likely than were the controls, to have detectable concentrations of aflatoxin metabolites [8].

Several studies have been undertaken to detect the differences, if any, in aflatoxin excretory patterns in males and females and the incidence of PLC. It was observed that males had a significant higher rate of exposure to AFB1 than female’s [6] and that PLC mortality rates were higher in males than in female’s [9].

Another toxin that is of great importance but less recognised in Africa is ochratoxin A (OTA). Surveys of indigenous foodstuffs have shown a widespread occurrence of the fungus Aspergillus ochraceus that is responsible for producing this powerful nephrotoxin [10–14]. The only human disease associated with OTA is Balkan Endemic Nephropathy (BEN) and its associated Urinary Tract Tumours [15]. With the exception of Algeria and Tunisia (North Africa) and Senegal (West Africa), very little information on any disease related to OTA ingestion has been documented in other African countries. A very high prevalence of nephropathy has been reported in Tunisia [16] and in Algeria [17]. In Senegal, chronic renal failure accounted for 87.6% of all deaths from urogenital disease [18].

In contrast to aflatoxins, there is virtually no information about the level of exposure of the children in tropical countries to ochratoxins. There is, however, growing information about the prevalence of

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* Published in 2001.
renal diseases in children in the tropics [19] but the role of mycotoxins, especially OTA has never been considered. This study, therefore, seeks to investigate seasonal variation in the detection frequency and levels of both aflatoxins and ochratoxins in urine from boys and girls in Sierra Leone and to identify, if possible, any apparent signs of disease that might be associated with exposure to these mycotoxins.

Material and methods

Study subjects

Primary School children – class one to four (age ranges 5 to 14) were recruited in 1992–1993 for this study. Approval was obtained from the Chairman, Board of Governors and the Headmaster, United Methodist Primary School, Mokonde, Njala, Southern Sierra Leone. These children were chosen because of the ideal location of the school to the Njala University Campus, for future cohort studies planned and also to facilitate sample collection for both the rainy and dry seasons.

A proforma questionnaire was provided to record the name, birth date, anthropometric data and short family history of each child. Urine samples were collected between 10–11 am on each period of collection. Two sets of samples were collected, one in May at the onset of the rainy season and one in March at the height of the dry season.

Urine samples were immediately analyzed using Ames Multi-strips to determine the pH, presence or absence of glucose, proteins and blood. Samples were later stored frozen at $-20^\circ\text{C}$ before dispatch by Air to Liverpool, UK for subsequent extractions and HPLC analysis. A complete description of the methods of extraction and HPLC analysis has been detailed by Jonsyn (1994) [29].

The method to simultaneously determine OTA and aflatoxins in urine samples is as follows: Urine (1 ml) was placed in 10 ml quick fit tubes. A 5 ml 0.05 M HCl plus 0.1M MgCl$_2$ and 2.5 ml chloroform were added. The contents of the tubes were shaken vigorously for 2 min, placed in an ice bath (20 min) and centrifuged for 20 min at 3,000 r.p.m. The lower chloroform layer was carefully removed, transferred to a clean test tube and blown to dryness under a stream of nitrogen. A proforma questionnaire was provided to record the name, birth date, anthropometric data and short family history of each child. Urine samples were collected between 10–11 am on each period of collection. Two sets of samples were collected, one in May at the onset of the rainy season and one in March at the height of the dry season.

Clean up of urine extracts. Five grams of activated silica gel (Sigma Type 1-60-200 mesh) was suspended in 1 ml chloroform and placed in a glass column (1 centimeter in diameter) plugged at the bottom with glass wool. Na$_2$SO$_2$ (1 g) was added. The urine extract was re-dissolved in 1 ml chloroform and transferred to the column. The mycotoxins were eluted with 30 ml chloroform: glacial acetic acid (99 : 1). The first 5 ml was discarded, the next 20 ml collected and the rest discarded. The elute was evaporated to dryness in a stream of nitrogen, stored overnight in a freezer for subsequent HPLC analysis. The sample components were separated, at ambient temperature, on an ODs 5 $\mu$m column, 25 cm $\times$ 5 mm (HPLC Technology, Macclesfield, UK and detected by a fluorescent detector (Fluorescent detector, Kratos, Schoeffel Instruments) fitted with a 365 nm excitation filter and a 418 nm emission filter. The isocratic mobile phase consisted of methanol : water : acetic acid (65 : 35 : 1) at a flow rate of 1 ml/min.

Using the above method, the recovery rate and reproducibility of OTA and the aflatoxins were averaged at 93% and 82%, respectively. The lower limits of detection was between 5 to 50 pg/ml for aflatoxins and 200 pg/ml for OTA.

Statistical analysis. With evidence of skewing, mean concentrations of aflatoxins and ochratoxins were expressed as geometric means, antilog (mean log$c$) and the standard deviations as antilog standard deviation. Comparisons of proportions were explored using $X^2$ tests with one degree of freedom, applying Yates correction. A 5% limit of significance was imposed.

Results

During the dry season, urine samples of 134 males and 110 females were analyzed. Only one sample from a boy was found to be mycotoxin free.

Ochratoxin A (OTA) was detected in 29(21%) males and 34(31%) females. This difference was significant ($p < 0.01$). The metabolic product of OTA-4-hydroxyochratoxin A(4R-OTA) was detected in 37% and 38% of boys and girls respectively. Ochratoxin B (OTB), which often co-occurs with OTA was detected at 47% in both sexes.

With regards to the concentrations of ochratoxins, the highest level (218 ng/ml OTB), was detected in a boy. One girl had 148 ng/ml OTA, while level of 4R-OTA were 29 ng/ml (boy) and 14.7 ng/ml (girl). These differences were however, not significant ($p < 0.13$-OTA; $p < 0.89$-4R-OTA; $p < 0.92$-OTB).