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AFLATOXINS IN BODY FLUIDS AND FOOD OF NIGERIAN CHILDREN WITH PROTEIN- ENERGY MALNUTRITION

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ABSTRACT

Aflatoxins are natural contaminants of food crops implicated in the pathogenesis of various human diseases. This study aimed to determine the associations between aflatoxins and protein- energy malnutrition (PEM) by measurements of aflatoxins in serum, urine and food on plate of Nigerian children with PEM. A cross- sectional study was undertaken in 3 agro- ecological regions of Nigeria (Guinea savannah, Sudan savannah and Rain forest), where aflatoxins B1, B2, G1, G2, M1, and M2 were measured in sera, urine and food on plate of 79 children with PEM (kwashiorkor n=36, marasmic kwashiorkor n=29 and marasmus n=13) and 33 healthy controls, matched for age and sex. Among healthy controls, aflatoxin detection rates were higher in the Guinea Savannah (72.2%) than in the Sudan Savannah (53.8%), albeit statistically insignificant. In relation to nutritional groups, the rates of detection of aflatoxins were higher in marasmic kwashiorkor (93.1%) and kwashiorkor patients (88.9%), compared to marasmus (76.9%) and controls (63.6%, p=0.013). The rates of detection of B1 aflatoxin followed a similar trend viz. marasmic kwashiorkor (82.4%), kwashiorkor (69.4%), marasmus (53.8%) and controls (42.4%, p=0.007). Of all types of aflatoxins detected in serum, M2 had the highest rates of detection in all patient groups and controls. The median concentrations of aflatoxins detected in sera of each PEM group were significantly higher than those of controls, but comparisons between PEM groups were not statistically significant. The frequency and concentration of aflatoxins detected in urine and food of PEM groups and controls were not statistically different. However, controls had the lowest serum /urine aflatoxin ratio as well as lowest median aflatoxins concentrations in their food as compared to PEM patients. In conclusion, aflatoxins are commonly detected in the body fluids and food of Nigerian children, but more frequently and at higher concentrations in children with PEM, possibly due to decreased excretion or increased exposure. Future prospective studies are desirable to determine if aflatoxins contribute to the pathogenesis of all types of PEM and not necessarily kwashiorkor alone.

Key words: Aflatoxin, Malnutrition, PEM, Kwashiorkor, Nigeria

INTRODUCTION

Aflatoxins are naturally occurring toxic metabolites of ubiquitous fungi, namely *Aspergillus flavus* and *Aspergillus parasiticus* [1, 2]. There are at least 16 different types of aflatoxins produced in nature but the most common types are aflatoxins B1, B2, G1 and G2 [1, 2]. Aflatoxin M1 and M2 are hydroxylated metabolites of B1 and B2, frequently detected in milk, meat, eggs and their products. Of all aflatoxins, B1 is most toxic as well as the most commonly detected in nature [2].

Aflatoxins are natural contaminants of food crops such as cereals, oilseeds, spices, and tree nuts as well as milk, meat, and dried fruit. Most of these food crops constitute the major staple diet of people in the developing world, and in view of challenges of food insufficiency combined with sub-standard food production and food storage methods, it is estimated that about 4.5 billion people in the developing world are exposed to aflatoxins through their diet [3].

Acute and chronic exposures to aflatoxins have been linked to various deleterious health effects such as hepatotoxicity, liver cancers, infertility, malnutrition, growth retardation and immunosuppression [1-3]. Although, the evidence in support for these health effects are variable, aflatoxins (particularly B1) have been identified as class I carcinogens in man due to the incontrovertible causal role in primary liver cell carcinoma [4].

Aflatoxins have been implicated in the pathogenesis of protein energy malnutrition (PEM), a condition affecting more than 118million (32% of) children in the developing world [5]. In the spectrum of PEM including kwashiorkor, marasmus and marasmic kwashiorkor, some investigators have associated aflatoxins specifically with the development of kwashiorkor alone [6-9]. This was based on the detection of aflatoxins in higher frequencies and concentrations in kwashiorkor patients than in other nutritional groups, attributed to a possible decrease in metabolism of aflatoxins in patients with kwashiorkor as compared to other groups [7]. However, other studies have also implicated aflatoxins in the pathogenesis of other types of malnutrition such as wasting and stunting [10, 11] and in experimental animal studies aflatoxins resulted in micronutrient deficiencies including vitamins A and D as well as zinc and selenium deficiencies [3].

In Nigeria, close to 30% of children below 5 years are malnourished [12] but studies suggesting a possible role of aflatoxin in PEM were based only on detection of high concentration of aflatoxins in autopsy lung, kidney and liver specimens from children who died from kwashiorkor [13-15]. Aflatoxins have been previously detected aflatoxins in sera of healthy first time Nigerian blood donors as well as autopsy tissues of cases of primary liver cell carcinoma [16, 17]. To our knowledge, aflatoxins have not been measured in the food and body fluids of Nigerian children with PEM.

This study aims to provide further information on the associations between aflatoxins and PEM by measurements of aflatoxins in serum, urine and food on plate of children in Nigeria.

PATIENTS AND METHODS

A total of 111 study subjects were enrolled from 3 agro- ecological regions of Nigeria including Guinea savannah (Zaria and Jos) and Sudan savannah (Malumfashi and Funtua) in the north and Rain forest (Afikpo and Owerri) from the south (Table 1). However, majority of the study subjects were enrolled in Ahmadu Bello University Teaching Hospital, Zaria (Guinea Savannah), where all analyses were done.

Before commencement of the study, study subjects' parents and caregivers gave written consent for the study and ethical approval was obtained from the various regional hospitals where children were enrolled.

Sample collection

Sera were obtained from 78 children (49 males, 29 females) with PEM (Wellcome classification) including kwashiorkor (n=36), Marasmic kwashiorkor (n=29) and Marasmus (n=13), as well as 33 healthy children (22 males, 11 females) without evidence of PEM. Study subjects were aged 7-60 months (median of 24months) and they were matched for age.

Early morning urine samples (15-25mls) were also obtained from 44 children (26males 18 females) with PEM and 11 healthy children (8 males 3 females), and if not treated immediately were stored at -20°C until analysed.

Composite cooked meals (food on plate) from children's homes were collected from 13 PEM patients and 4 controls. Examples of diet obtained for analysis included rice, beans, vegetables, maize-derived local delicacies named 'Akamu' and 'Kunu' as well as cow-milk derived local delicacy named 'fura'.

Aflatoxin extraction and analysis

The total aflatoxins were quantified by the minicolumn method using the Velasco flurotoxin meter (Neotec Instruments Inc, Maryland USA) as previously described in an earlier publication [16,17]. Extraction and analysis of aflatoxins from serum, urine and food samples were undertaken according to the Velasco Flurotoxin meter operation manual.

Briefly, 1ml of each serum was treated with 9ml of 10% trichloroacetic acid to precipitate out the serum proteins. 0.5ml of the filtrate was added to a mini-column in which a special layer of florisil traps the aflatoxin and the aflatoxin concentration is then determined by using a Velasco Flurotoxin meter. Microcolumn standard preparations of aflatoxins B1, B2, G1, G2, M1 and M2 were used to calibrate the flourometer. Concentrations of aflatoxins were expressed in parts per billion -ppb (equivalent to ug/L for sera and urine and ug/kg for food).

STATISTICAL ANALYSIS

Aflatoxin data were not normally distributed; thus, data were expressed in median and ranges (minimum and maximum values) and differences between variables were analysed by non-parametric tests, as well as by chi square as appropriate. To determine possible differences in excretion of aflatoxins in urine, the ratio of serum and urinary aflatoxin concentrations were compared in simultaneous samples from patients and controls. Statistical package of social sciences (SPSS) version 17 was used for all analyses. $P<0.05$ was considered significant.

RESULTS

The detection rates and concentrations of total aflatoxins (total of B1, B2, G1, G2, M1, M2) in serum, urine and food on plate of study subjects are shown in table 2 and represented in figure 1, 2 and 3. There was no significant difference in rates of detection and concentrations of aflatoxins in relation to sex (not shown).

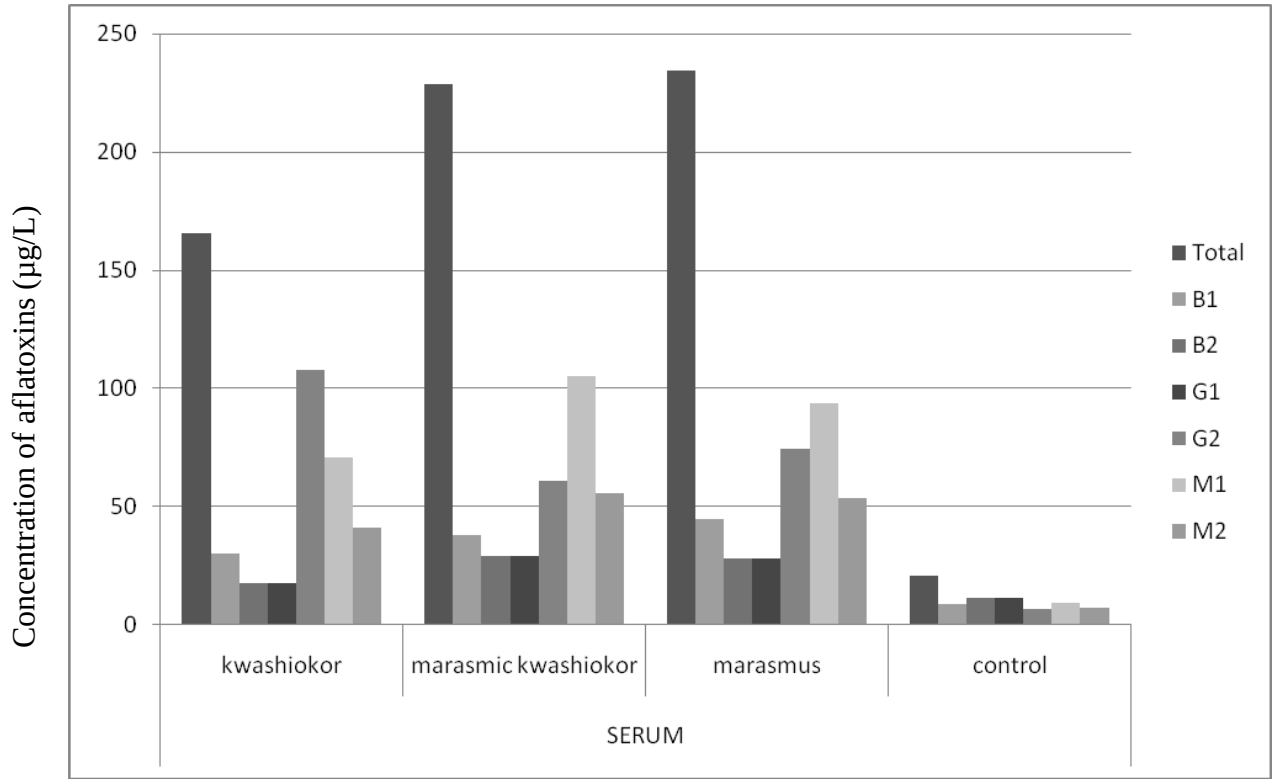

Figure 1: Distribution of median concentrations of various types of aflatoxins in serum (µg/l) of patients and controls

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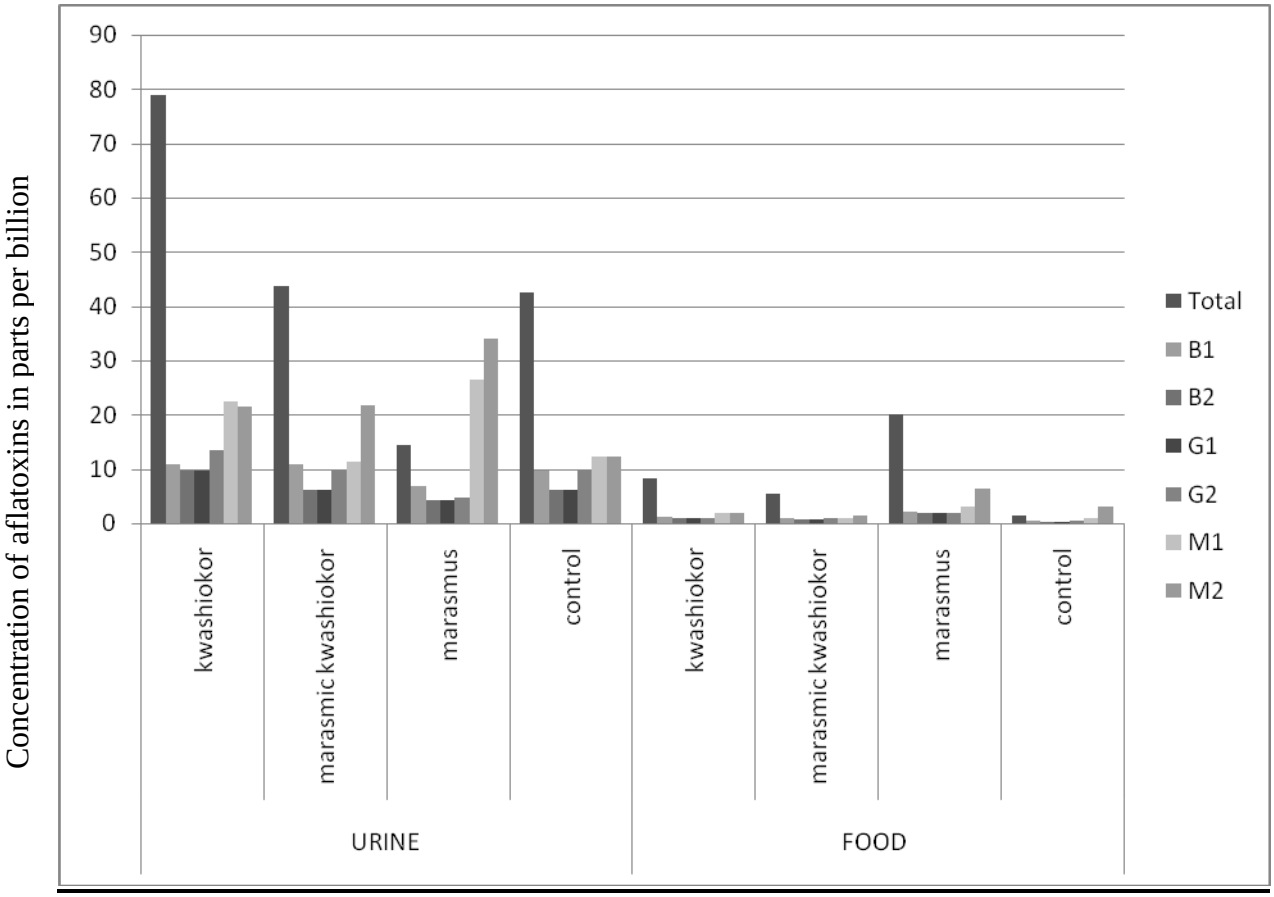

Figure 2: Distribution of median concentration of various types of aflatoxins in urine (µg/l) and food (µg/kg) of patients and controls

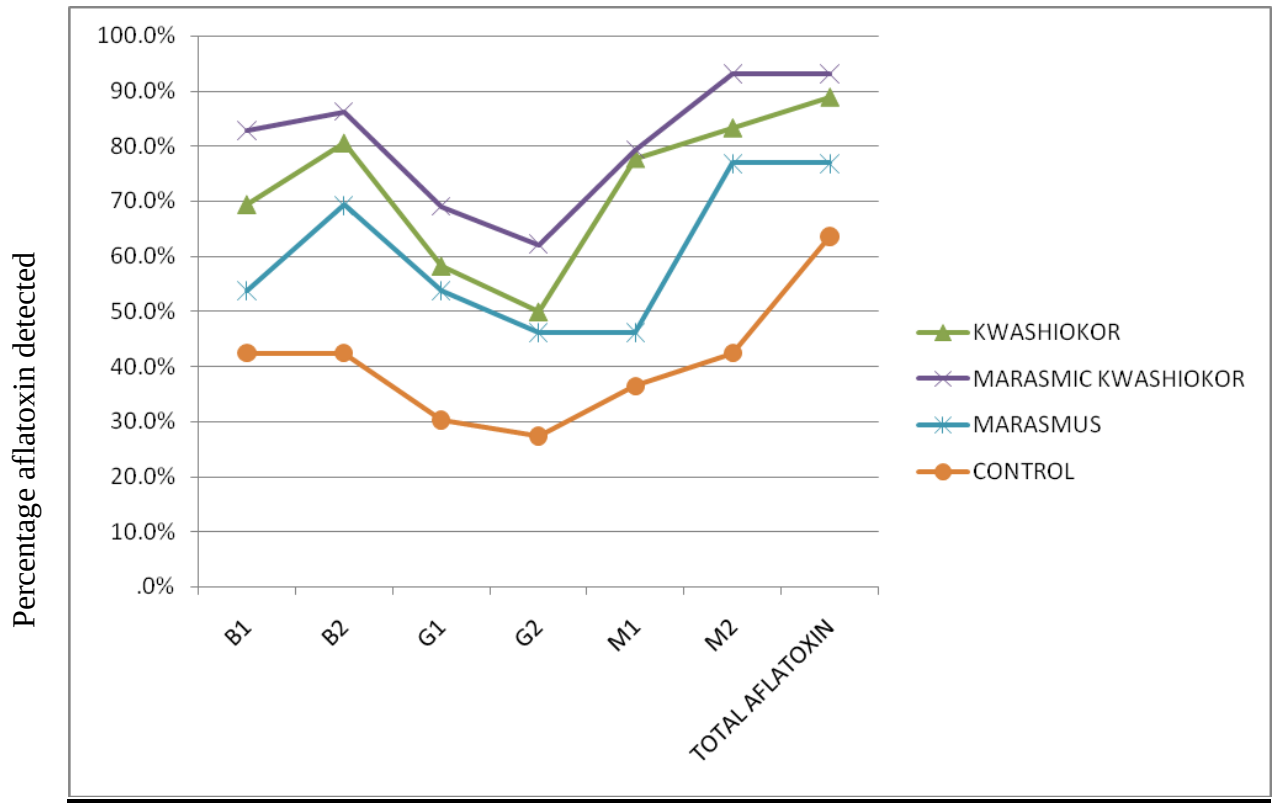

Figure 3: Frequency of detection of various types of aflatoxins in serum of patients and controls

Aflatoxins detection in agro-ecological zones

To determine the possible regional differences in aflatoxin detection rates, the rates of detection of total aflatoxins in sera were compared in 31 healthy controls from 2 agro-ecological regions (Guinea savannah (n=18) and Sudan savannah (n=13). Rain forest was excluded in view of very small sample size of controls (n=2).

The detection rates were higher in the Guinea Savannah (72.2%) than in the Sudan Savannah (53.8%). However, the differences were not statistically significant ($p>0.05$, Chi square).

Aflatoxins in serum

The rate of detection of total aflatoxin was highest in marasmic kwashiorkor and kwashiorkor patients, lower in children with marasmus and lowest in controls (table 2, Figure 1). These differences were statistically significant ($p=0.013$, $X^2=10.85$). The rates of detection of B1 aflatoxin followed a similar trend viz. kwashiorkor (69.4%), marasmic kwashiorkor (82.4%), marasmus (53.8%) and controls (42.4%), $p=0.007$, $X^2= 11.96$. Of all types of aflatoxins detected in serum, M2 had the highest rates of detection in all patient groups and controls (figure 3).

The median concentrations of total aflatoxins (table 2) were significantly higher in each patient group when compared to controls: kwashiorkor vs. control p<0.0001, marasmic kwashiorkor vs. control p<0.0001, marasmus vs. control, p=0.031 (Mann Whitney test). However, there was no statistical difference in median total aflatoxin concentrations between patients (P>0.05 all groups). Similarly, the median concentrations of aflatoxin B1 were significantly higher in each patient group when compared to controls (P<0.05, all groups Mann Whitney) but no statistically different between patients (p>0.05, all groups).

Aflatoxins in urine

All types of aflatoxin were detected in urine samples of patients and controls at varying concentrations (figure 2). Although controls had highest detection of aflatoxins (90.9%), followed by kwashiorkor (84.6%), marasmus (81.8%) and marasmic kwashiorkor (60%) in descending order, the observed differences in rates of detection in urine were not significant (p>0.05).

The median concentration of total aflatoxins in urine was highest in kwashiorkor patients, followed by marasmic kwashiorkor, controls and marasmus in descending order (table 2). Kwashiorkor patients had a significantly higher concentration of urinary aflatoxin when compared to marasmus patients (p=0.011, Mann Whitney). However, the differences in the median total aflatoxin concentrations between kwashiorkor, marasmic kwashiorkor and controls were not statistically significant (P>0.05, all groups Mann Whitney test).

Ratio of serum urinary aflatoxin concentration

To determine possible differences in excretion of aflatoxins in urine, the ratio of simultaneous samples of serum and urinary aflatoxin concentrations were determined in 24 patients (kwashiorkor n=6, marasmic kwashiorkor n=10, marasmus n=6) and controls (n=6). Controls had very low serum/urinary aflatoxin ratio compared to patients with PEM. The median ratio of total serum /urine aflatoxin concentration for kwashiorkor, marasmic kwashiorkor, marasmus and controls were 1.2, 5.2, 1.5 and 0.73, respectively (p>0.05, Kruskal Wallis test). These findings may suggest that aflatoxins excretion in urine is more efficient in controls than in patients with PEM.

Aflatoxins in food on plate

At least one type of aflatoxin was detected in the foods of all study subjects at varying concentrations with total aflatoxins concentrations ranging from 0.4ug/kg to 20.3ug/kg (figure 2). One case of marasmus had total aflatoxin above 20ug/kg (the recommended limit for food- [2], while others were below this value. Although sample size was small for objective statistical analysis, controls seem to have the lowest concentrations of aflatoxins in food followed by marasmic kwashiorkor, kwashiorkor and marasmus in ascending order. Of all types of aflatoxins detected in food, B1 and G2 were detected in all food samples analysed but M1 and M2 had the highest median concentrations in food samples analysed, possibly due to high rates of consumption of ‘fura’, - cow milk derived local delicacy.

DISCUSSION

This is the first study from Nigeria highlighting the presence of aflatoxins in body fluids and diet of Nigerian children with PEM as well as in normal healthy children. The high prevalence of aflatoxins detected in sera of healthy children from the evaluated agro-ecological regions probably reflects high rates of ingestion of aflatoxins in diet of Nigerian children. This assertion is supported by detection of aflatoxin at varying concentrations in their diet. However, aflatoxins have also been detected in sera of healthy adult Nigerians, indicating an age independent exposure to aflatoxins in Nigerians [16, 18].

The level of aflatoxin contamination of food crops in Nigeria have been shown to be related to differences in humidity of the various agro-ecological with the humid south having higher contamination than the drier north [19]. In this study, it is plausible that higher humidity in the Guinea savannah relative to the Sudan Savannah may be responsible for higher rates of detection of aflatoxins in Guinea savannah than in the Sudan savannah.

The study data indicate that aflatoxins were more frequently detected in sera of patients with marasmic kwashiorkor and kwashiorkor as compared to other PEM group and controls. These findings are in agreement with studies from Sudan, Kenya and Egypt where significantly higher concentrations of aflatoxins were detected in sera of children with kwashiorkor as compared to other nutritional groups and controls [6-9]. However, these previous studies detected higher concentrations of aflatoxins in sera of children with kwashiorkor as compared to other PEM groups and controls. In contrast, this study detected no significant differences in the serum concentrations of aflatoxins between patients with the various types of PEM. Rather, for each type of PEM, serum aflatoxin concentrations were significantly higher than those of controls ($p<0.05$). Consequently, elevated aflatoxin levels, and by extension the suggested pathogenic role of aflatoxin in malnutrition, are associated with all types of PEM and not necessarily restricted to kwashiorkor. The findings of this study are similar to those of Tchana *et al*. [20] who found no significant difference in the frequency and concentrations of aflatoxins detected in Cameroon children with kwashiorkor and marasmic kwashiorkor patients, although the children with PEM had significantly lower aflatoxins compared to healthy children.

Other investigators have also suggested a role of aflatoxins in malnutrition not related to kwashiorkor. In one study from Gambia, aflatoxin exposure in utero was associated with subsequent growth retardation in infants [11]. Similarly, chronic aflatoxin exposure among children in Togo and Benin was causally implicated in growth stunting and underweight as strong dose– response relationships were observed between exposure and the extent of stunting and being underweight [10].

Some mechanisms may underlie, to a large extent, the elevated serum aflatoxin levels found in studied patients with PEM as compared to controls. First, it may be attributable to higher dietary exposures to aflatoxins in patients since relatively lower

concentrations of aflatoxins were detected in the diet of controls as compared to the PEM patients. However, the study only measured aflatoxins in a single meal of a small number of patients. A temporal analysis of aflatoxin levels in food samples of larger number of children before and after development of PEM may be more instructive.

Second, the metabolism and excretion of aflatoxins may be slow or impaired in patients with PEM either due to liver disease [5] or suboptimal renal function [21]. In support, the ratio of serum and urinary aflatoxin concentrations was lowest in controls than patients, indicating that healthy children are more likely to excrete aflatoxins in their urine when compared with children with PEM.

Third, by directly causing various micro and macronutrient deficiencies as well as protein malabsorption and liver damage [3], aflatoxins may act in synergy with other aetiological factors to promote development of PEM. In a study by Kocabas *et al*. [22], mice fed low protein diet supplemented with aflatoxin B1 were more likely to develop decreases in total protein and albumin and as well as more remarkable histological changes in their liver than those fed a low protein diet. However, the authors could not prove a direct causal role of aflatoxin in kwashiorkor as liver changes typical of kwashiorkor were associated with a low protein diet with or without aflatoxin exposure.

Clearly, large prospective studies are desirable to establish if aflatoxins are causally related to all forms of PEM in Nigerian children. Furthermore, since study findings reflected only acute exposure to aflatoxins over last 24-72 hours, it will be useful for future studies to explore the associations between chronic aflatoxin exposure and PEM by measurement of aflatoxins-albumin adducts, which is a reflection of chronic exposure over last 2-3 months.

In conclusion, aflatoxins are prevalent in the body fluids of both healthy children and in children with PEM in Nigeria. However, they are detected more frequently and at higher concentrations in children with the types of PEM groups compared to healthy children without PEM, possibly due to decreased excretion or increased exposure. Future prospective studies are desirable to confirm if aflatoxins may contribute to the pathogenesis of all types of PEM and not necessarily kwashiorkor alone.

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Table 1: Distribution of study subjects according to agro-ecological regions and types of samples

Agro-ecological regions surveyed	Type of sample n%			Samples survey
	Serum (n=111)	Urine (n=55)	Food on plate (n=17)	
Sudan Savannah (Malumfashi, Funtua-)	47(42.3%)	14(25.5%)	7(41.2%)	68
Guinea Savannah (Zaria, Jos)	50(45.1%)	30(54.5%)	10(58.8%)	90
Rain forest (Owerri, Afikpo)	14(12.6%)	11(20%)	-	25

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Table 2: Distribution of aflatoxin detection rates and concentrations of total aflatoxin in various samples of patients and healthy controls

	Serum aflatoxin in ug/L (n=111)			Urine aflatoxin in ug/L (n=55)			Food on plate aflatoxin in ug/kg(n=17)		
Subject category	screened/ detected	% detection	Median (range)	screened/ detected	% detection	Median (range)	screened/ detected	% detection	Median (range)
Kwashiorkor	32/36	88.9%	165.6	11/13	84.6%	79	6/6	100%	8.3
			5.3-7646			15-361			0.4-16.7
Marasmic	27/29	93.1%	228.4	12/20	60%	43.8	4/4	100%	5.4
kwashiorkor			12.6-8045			0.3-35.3			0.4-10.4
Marasmus	10/13	76.9%	234.3	9/11	81.8%	14.4	3/3	100%	20.1
			5.4-1246.7			6.6-210			2.3-20.3
Controls	21/33	63.6%	20.7	10/11	90.9%	42.6	4/4	100%	1.3
			7-161.6			1.2-140			0.75-8.7

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