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ORIGINAL ARTICLE

Aflatoxin-lysine adducts in blood serum of the Malawian rural population and aflatoxin contamination in foods (groundnuts, maize) in the corresponding areas

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Abstract

Aflatoxin-lysine (AFB_1-lys) adduct levels in blood samples collected from 230 individuals living in three districts of Malawi (Kasungu, Mchinji, and Nkhotakota) and aflatoxin B_1 (AFB_1) levels in groundnut and maize samples collected from their respective homesteads were determined using indirect competitive enzyme-linked immunosorbent assay (IC-ELISA) methods. AFB_1-lys adducts were detected in 67% of blood samples, with a mean concentration of 20.5 ± 23.4 pg/mg of albumin. AFB_1 was detected in 91% of groundnut samples and in 70% of maize samples, with mean AFB_1 levels of 52.4 and 16.3 μg/kg, respectively. All participants of this study reported consuming maize on a daily basis and consuming groundnuts regularly (mean consumption frequency per week: 3.2 ± 1.7). According to regression analysis, a frequency of groundnut consumption of more than four times per week, being female, and being a farmer were significant ($p < 0.05$) contributors to elevated AFB_1-lys adduct levels in the blood. This is the first report on AFB_1-lys adducts in blood samples of residents in Malawi. The results reinforce the urgent need for interventions, aiming at a reduction of aflatoxin exposure of the population.

Keywords AFB_1? contamination · AFB_1?-lys adduct · Exposure · Women farmers · Groundnuts · Maize

Introduction

In Malawi, the livelihood of the rural population predominantly depends on agriculture, where maize is a major staple food, with a per capita consumption of 382 g/day (Ecker and Qaim 2011). Groundnuts, produced by local smallholders, also play an integral role in the livelihood of many, through provision of dietary proteins and income (Tsusaka et al. 2016; Monyo et al. 2012). Maize and groundnuts are major ingredients of porridge consumed by children. Groundnuts are also the major ingredient of ready-to-use therapeutic food (RUTF) used to treat undernourished children (Fitzgerald 2015). However, these commodities are unfortunately often contaminated with mycotoxins, especially aflatoxins (AFs) (Monyo et al. 2012; Matumba et al. 2015a; Tsusaka et al. 2017).

AFs are secondary metabolites produced mainly by *Aspergillus flavus*, *Aspergillus paraciticus*, and *Aspergillus nomius*. AFs have immunosuppressive, hepatotoxic, carcinogenic, mutagenic, and teratogenic effects in humans and in animals (Wong and Hsieh 1976; Williams et al. 2004; Oswald et al. 2005; El-Nahla et al. 2013). Chronic exposure to AFs causes cancer, contributes to malnutrition and growth faltering in children, suppresses the immune system, and results in a number of disabilities (Gong et al. 2004; Cotty and Jaime-Gracia 2007; Liu et al. 2012; Magoha et al. 2014;

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Shirima et al. 2015). Further, increasing evidences suggest that AFs increase the rate of progression from HIV infection to AIDS (Jiang et al. 2008; Jolly et al. 2013; Jolly 2014).

There are many analogs of AFs, but aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2) are commonly detected in food, with AFB_1 being the major and most potent compound (IARC 1993). Human exposure to AFB_1 occurs through dietary intake of contaminated food products (Eaton and Groopman 1994) as well as inhalational intake and contact with skin (Degen 2011). Once ingested, AFB_1 is absorbed into the body and oxidized by cytochrome P450 enzymes to yield a variety of metabolites including the highly toxic exo-AFB1 8,9-epoxide and the less or nontoxic aflatoxicol, aflatoxin M_1 (AFM_1), aflatoxin P_1 (AFP_1), and aflatoxin Q_1 (AFQ_1) (Eaton and Gallagher 1994; Wild and Turner 2002; Verma 2004; Do and Choi 2007). The major reactive metabolite, the exo-AFB_1 8,9-epoxide, may bind to double stranded DNA to form the promutagenic AFB_1-N7-guanine adduct which is an unstable adduct that undergoes rapid excretion in urine or may be hydrolyzed by an expoxide hydrolase to AFB1-8,9-dihydrodiol (AFB_1-dhd), which is capable of reacting strongly with proteins such as albumin (Groopman et al. 1993; Wild et al. 1986; Johnson et al. 1996). Both urinary AFB_1-N7-guanine and serum AFB_1-lys levels are correlated with dietary intake of aflatoxin and are successfully used to estimate the exposure to AFB_1 in humans (Ediage et al. 2012; Shirima et al. 2013; Turner et al. 2005; Chen et al. 2001). The serum AFB_1-lys levels are assumed to reflect exposure to aflatoxin over the previous 2–3 months, based on the half-life (IARC 2002).

In Malawi, however, despite the plenty of information available on crop AFB_1 contamination levels as exemplified by Monyo et al. (2012), no study has been conducted to date to determine the exposure in human to explore its linkage with food consumption. In this regard, the current study was undertaken to fill that knowledge gap to determine the AFB_1-lys in human blood of the individuals living in major groundnut-producing areas of Malawi, namely, Kasungu, Mchinji, and Nkhotakota districts. The study further endeavored to elucidate the relationship between maize and groundnut consumption patterns, AFB_1 contamination in these food commodities, and the presence of AFB_1-lys adduct in human blood samples.

Materials and methods

Study participants and blood sample collection

The study was undertaken in the year 2012 in three major groundnut-producing districts of Malawi (Fig. 1). Purposive sampling was used to select these districts. The criteria were (i) volume of groundnut production and (ii) AFB_1 contamination in groundnuts and maize being mapped earlier (Monyo et al. 2012; Matumba et al. 2015a). The study with human volunteers was approved by National Health Sciences Research Committee (NHSRC) of Malawi. Two hundred and thirty blood samples were collected from volunteers who visited the health centers in Kasungu (106), Mchinji (59), and Nkhotakota (65) districts during May to July 2012 (Harvesting period), and who had asserted to have produced and consumed maize and groundnuts. The age of participants ranged from 14 to 60 years. All objectives of the study were clearly explained to the participants by medical professionals, and consent was obtained from each volunteer before collecting 10 ml of blood. In cases of minors, written consent was obtained from parents and guardians. Demographic and food consumption frequency data for maize and groundnuts were asked from volunteers and were documented. However, the health status of the participants was not recorded as it was beyond the scope of the current study. Immediately after collecting the blood samples, serum was separated from blood by centrifuging at 5000*g* for 10 min (Sabbioni et al. 1990). The centrifuge tubes were then kept in liquid nitrogen and transported to the Kamuzu Central Hospital (KCH). These samples were properly labeled and stored at − 80 °C until further processing. These serum samples were then transported to the International Crops Research Institute for the Semi-arid Tropics (ICRISAT) and analyzed with indirect competitive enzyme-linked immunosorbent assay (IC-ELISA).

Synthesis of the coating conjugate and AFB_1-lys standard for IC-ELISA

AFB_1-ovalbumin (AFB_1-ova) coating conjugate was prepared in two steps, where in the first step, AFB_1-8,9 epoxide was produced by mixing 775 μg of AFB_1 in 250 μl of dichloromethane with 0.1 mol/l phosphate buffer washed 5.68 mg of m-chloroperbenzoic acid (MCPBA) in 250 μl of dichloromethane. The reaction was allowed to proceed for 1 h at 4 °C. Then, 5 mg of ovalbumin, dissolved with 250 μl of 0.1 mol/l phosphate buffer, was added to the AFB_1-8,9 epoxide reaction mixture and the reaction was allowed to proceed for 2 h at 4 °C. The protein concentration of the AFB_1-ovalbumin was determined spectrophotometrically (Beckman DU-50) at 280 nm. The polyclonal antiserum against AFB_1-lys adduct was used as described by Anitha et al. (2011).

Similarly, AFB_1-lys was prepared in two steps. First, 0.6 mg of MCPBA was dissolved in 250 μl of dichloromethane and then it was washed three times with 500 μl of 0.1 mol/l phosphate buffer. In another tube, 100 μg of AFB_1 was dissolved in 250 μl of dichloromethane and added to the MCPBA solution, to generate the AFB_1-8,9 epoxide. The reaction was allowed to proceed for 1 h at 4 °C under gentle mixing. Second, 1 mg of *N*-α-acetyl lysine, dissolved in 250 μl of 0.1 mol/l phosphate buffer,

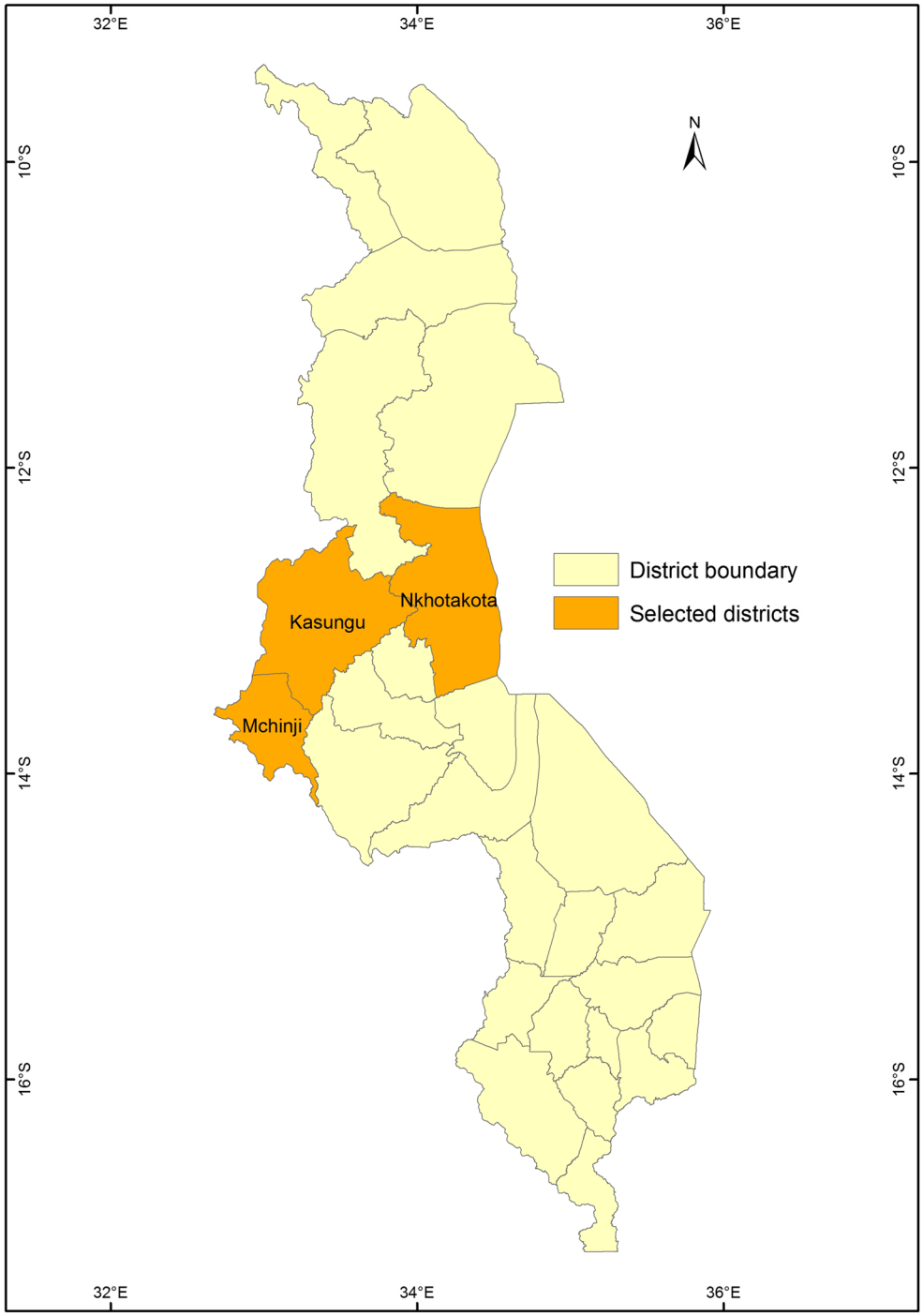

Fig. 1 Map of Malawi showing districts included in this study

was added to the AFB_1-8,9 epoxide reaction mixture and further mixed for 2 h at 4 °C. The concentration of AFB_1-lys formed was measured spectrophotometrically (Beckman DU-50) by scanning at 200–500 nm using methanol as blank. For ELISA standards, the concentration of AFB_1-lys was determined using the extinction coefficient (at pH 7.4 at 346 nm $e = 15{,}821$) of Sabbioni (1990).

Serum sample preparation

The AFB_1-lys adduct was extracted by Pronase (Roche) digestion of 2 mg of albumin that was obtained from 500 μl of the serum sample. The undigested proteins were removed by using Sep-Pak cartridges (WAT051910, Waters Ltd., UK) (Wild et al. 1990), according to manufacturer's instruction, and eluted the AFB1-lys, and the eluent was evaporated in a

vacuum evaporator and the pellets were reconstituted in 500 μl of PBS. This digested and purified amino acid product that contained the AFB_1-lys was subjected to IC-ELISA for AFB_1-lys. The wells of microtiter plate (F96 MaxiSorp, Thermo Fisher Scientific, Denmark) were coated each with 150 μl of an AFB_1-ovalbumin solution (10 mg/ml, in 0.2 mol/l carbonate buffer, pH 9.6) and incubated overnight at 4 °C. Free protein binding sites were blocked by incubation with 0.2% bovine serum albumin (BSA) dissolved in phosphate buffer saline containing Tween-20 (PBST). In each subsequent step, the plates were incubated at 37 °C for 1 h, and then the wells were washed with PBST three times. Serial dilutions of AFB_1-lys standard solution (100 μl per well, 2000 to 7.8 pg/ml) or serum sample extract (diluted 1:5 in PBST) were added in duplicate wells against standard. Then, 50 μl per well of AFB_1-lys antiserum (1: 40,000, diluted in PBST) was added per well. Then, the plate was incubated and washed again. Then, alkaline phosphatase-conjugated (AR-ALP) anti-rabbit IgG antibodies (Sigma-Aldrich, St. Louis, USA) (diluted 1:2000 in PBST) were added (150 μl per well). The plate was washed again, and 1 mg/ml of enzyme substrate (para-nitro phenyl phosphate, pNPP, Sigma-Aldrich, St. Louis, USA) dissolved in 10% diethanolamine buffer (pH 9.8) was added (150 μl per well) and incubated the plate at 37 °C for 1–2 h. The colorimetric reaction was measured in an ELISA plate reader (Multiscan reader, Thermoscientific, China) fitted with a 405-nm filter. The detection limit of this methodology is 2.5 pg/mg of albumin (Anitha et al. 2011). The AFB_1-lys concentration was calculated using the formula $(AXDXE)/G$ pg/mg of albumin, where A is the concentration of AFB_1-lys (pg/ml), D the dilution factor (ml), E the extraction solvent volume (ml), and G the sample weight (mg). The 10-point calibration was used from 2000 to 7.8 pg/ml. The calibration curve was considered valid when $R^2 \geq 0.99$.

Quality control was monitored through artificial spiking, wherein 10, 50, 100, 500, 1000, and 2000 pg of AFB_1-lys was added to healthy human serum albumin (Sigma) and digested with pronase and processed in a similar way that we process the samples. The recovery of AFB_1-lys has been presented in Table 1. The unspiked control human serum albumin (Sigma) was processed similar to other samples by digesting it with pronase, purified, and then used in ELISA as known negative control. The reproducibility of the method for both intra-day and inter-day is presented in Table 2. The method was validated by comparing six selected positive samples ranging between 10 and 75 pg/mg of albumin using Shimadzu Liquid Chromatography LC-10AT VP coupled to a Shimadzu RF-10 AXL florescence detector. The calibration standards were prepared by fortifying digested black human serum albumin (Sigma) to the concentration of 5, 25, 50, 75, and 100 pg of AFB_1-lys/mg of albumin. The chromatographic separation was achieved using a 25 cm × 4.6 mm reversed-phase C18 HPLC column (Waters Ltd., Watford, UK) protected by a C18 guard cartridge (4 × 3 mm internal diameter, Agilent Technologies). The solvent flow rate was 0.8 ml/min with 40–100% (*v*/*v*) methanol in 0.02 M sodium phosphate buffer (pH 7.2) generated over 18 min linear gradient. The column oven temperature was maintained at 40 °C and the injection volume was 20 μl for both standards and samples. Fluorescence excitation and emission wavelength were set at 360 and 440 nm, respectively.

Groundnut and maize sample collection

Shelled groundnut and maize samples were collected from all the participating households. The samples were collected only as kernels/grains and not in any other form such as powder or paste. The representative sample was obtained by mixing 10 samples collected from multiple parts of the bag, each weighing about 10 g, collected from different parts of each bag to constitute 100 g of sample. Likewise, from every five bags, 100 g of sample was collected and pooled to make 500 g and was used to assay for AFB_1 contamination.

Determination of AFB_1 in groundnuts and in maize by IC-ELISA

The procedure for detection of AFB_1 in crop samples is different from that for detection of AFB_1-lys albumin in human samples, though the underlying indirect competitive ELISA test principle is the same. The AFB_1 assay was carried out using an IC-ELISA procedure which had been successfully validated in inter-comparison trials (Reddy et al. 2001; Waliyar et al. 2009). Briefly, two replicates of milled samples of about 20 g each were mixed with 100 ml of 70% methanol in water (*v*/*v*), augmented with 0.5% potassium chloride (KCl), and blended further. The mixture was transferred to a 250-ml conical flask, shaken at 250 g for 30 min (Gallenkamp Orbital Shaker, CAT # SCM 300 0101, England), and filtered through a Whatman No. 41 filter paper (GE Healthcare, UK). The 100 μl of filtrate was assayed for AFB_1 using IC-ELISA with a 96-well ELISA plate (F96 Maxisorp, Thermo Fisher Scientific, Denmark). The wells of the microtiter plate were coated each with 150 μl of AFB_1-BSA (Sigma) conjugate solution (10 mg/ml, in 0.2 mol/l carbonate buffer, pH 9.6) and incubated overnight at 4 °C. Free protein binding sites were blocked by incubation with 0.2% BSA dissolved in PBST. In each subsequent step, the plates were incubated at 37 °C for 1 h, and then the wells were washed with PBST three times. Serial dilution of AFB_1 standard solution (100 μl per well, 25 to 0.65 ng/ml) or extracted crop samples (1:5 in PBST) were added in duplicate wells against the standard. Then, 50 μl per well of AFB_1-BSA antiserum (1: 60,000 in PBST) was added per well and incubated. The antiserum produced at ICRISAT by Reddy et al. (2001) was used in this

Table 1 Recovery of AFB_1-lys from a spiked human serum albumin sample by IC-ELISA (two replicates)

Sample number	AFB_1-lys spike in human serum albumin (pg/mg)	Mean recovery (pg/mg) (Std. Dev.)	Mean percentage recovery (%)
1	10	10.5 (0.7)	105.0
2	50	53.0 (1.4)	106.0
3	100	94.0 (4.2)	94.5
4	500	460.0 (9.8)	92.0
5	1000	916.0 (7.0)	91.6
6	2000	1790.0 (14.1)	89.5
Mean percentage recovery of AFB_1-lysine from 6 samples (Std. Dev.)			96.3 ± 7.2

current study. Then, alkaline phosphatase-conjugated (AR-ALP) anti-rabbit IgG antibodies (Sigma-Aldrich, St. Louis, USA) were added (1:4000 in PBST, 150 μl per well). The plate was washed after incubation and 1 mg/ml of enzyme substrate (para-nitro phenyl phosphate, pNPP Sigma-Aldrich, St. Louis, USA) dissolved in 10% diethanolamine buffer (pH 9.8) was added (150 μl/well) and incubated in the plate at 37 °C for 1–2 h. The colorimetric reaction was measured in an ELISA plate reader (Multiscan reader, Thermo Fisher Scientific, China) using a 405-nm filter. The method has a detection limit of 1 μg/kg and on-going control of the method was monitored using naturally contaminated corn reference materials (4.2 and 23.0 μg/kg of AFB_1, product no. TR-A 100, batch number A-C-268 and A-C 271; R-Biopharm AG, Darmstadt, Germany). Further confirmation of the presence of AFB_1 in selected samples was performed by thin-layer chromatography (TLC) using silica gel-coated 20 × 20 cm glass plates (Fluka Analytical, Sigma-Aldrich, St. Louis, USA), which was developed in a mixture of chloroform and acetone (93:7, *v*/*v*) under vapor saturated conditions. AFB_1 was detected directly under long-wave ultraviolet (UV) light by their fluorescence (Park et al. 1994; Abass et al. 2004).

Statistical analysis

The dataset included continuous and binary variables representing AFB_1 contamination in crops, AFB_1-lys adduct level in the blood, awareness of AFB_1, gender, age, body weight of the participant, frequency of groundnut and maize consumption, and primary occupation, among others. STATA version 14 and SAS version 9.2 were used to process and analyze the data. Descriptive statistics were used to outline the status of AFB_1 contamination in crops and humans, while multivariate analysis was employed to determine the effects of various factors on AFB_1-lys adduct levels in blood samples, in particular, frequency of groundnut consumption and AFB_1 contamination in crop produce. Frequency of maize consumption was not included because all the participants consumed maize 7 days a week, and therefore there was no variation among the participants. The dependent variable in the multivariate analysis, i.e., AFB_1-lys adduct level, has multiple zero values since some participants have no exposure, meaning that the variable is left-censored; in which cases, the estimated coefficients have a downward bias and the error terms are not normally distributed, unless appropriate models are adopted. To counteract this problem, the tobit model was adopted, which is a type of a sample selection model that fits our case of left-censoring occurring at the value of zero (McDonald and Moffitt 1980; Ranjan and Phanindra 2014). Further, heteroscedasticity robust standard errors were modeled to minimize potential biases in statistical significance when

Table 2 Intra-day and inter-day reproducibility of IC-ELISA results for AFB_1-lys

Sample number	ELISA plate 1 AFB_1-lys (pg/mg)	ELISA plate 2 AFB_1-lys (pg/mg)	Mean (pg/mg) (Std. Dev.)	Coefficient of variation (%)
Intra-day reproducibility				
1	23.0	22.8	22.9 (0.1)	0.6
2	40.6	40.7	40.7 (0.1)	0.2
3	33.8	32.6	33.2 (0.8)	2.5
4	60.5	60.1	60.3 (0.3)	0.5
5	12.4	11.5	12.0 (0.6)	5.3
Inter-day reproducibility	Day 1	Day 2		
1	23.0	24.9	24.0 (1.3)	5.6
2	40.6	39.8	40.2 (0.6)	1.4
3	33.8	33.0	33.4 (0.6)	1.6
4	60.5	57.4	59.0 (2.1)	3.7
5	12.4	14.2	13.3 (1.3)	9.6

the variables took values in any certain range (Hayes and Cai 2007; Imbens and Kolesár 2016). The interaction term between frequency of groundnut intake and AFB_1 contamination in groundnut samples was included in regression because the influence of contamination in groundnuts may increase with the frequency of groundnut consumption. Lastly, since age and body weight variables had numbers of missing observations, two specifications were estimated, including and excluding these variables. In other words, the inclusive model is able to control for these variables at the cost of degree of freedom, in addition to potentially suffering attrition bias, while the exclusive model enjoys a higher degree of freedom at the cost of control over these two variables. The two models are juxtaposed in Table 5 for comparison. In the result section, the exclusive model is referred to as specification 1, while the inclusive one is referred to as specification 2. In either case, since linear models are fitted, the estimated coefficients represent marginal effects within the non-zero range of the dependent variable. That is, the coefficient β on an explanatory variable x being statistically significant means that a positive change in x by one unit results in a change in y (dependent variable) by units of β on average, while the effects of all other included variables are controlled for. For instance, if the explanatory variable is age, a unit change means a change by 1 year. If the explanatory variable is a gender dummy variable, which takes the value of one for female and zero for male, then a unit change means a change in gender from male to female.

Results

The coefficient of variation for intra-day testing of AFB_1-ly was 1.8% and that for inter-day testing of AFB_1-lys was 4.4% (Table 2). This shows the high reproducibility of the ELISA testing, and ELISA results were comparable to HPLC (AFB_1-lys $_{ELISA} = 1.0026$, AFB_1-lys $_{HPLC} = -1.2451$, $R^2 = 0.96$).

The demographic and groundnut consumption data concerning the study participants and AFB_1-lys-positive status are described in Table 3. AFB_1-lys was detected in 67% of the human blood samples. There was no significant variation in mean AFB_1-lys levels among study districts (ranging from 16 to 24.4 pg/mg of albumin) and age groups (ranging from 14.3 to 21.4 pg/mg of albumin). Although participants under the age of 20 years seemed to have lower levels of AFB_1-lys, the difference across different age groups was actually statistically insignificant ($p = 0.414$). In contrast, women had significantly ($p = 0.055$) higher AFB_1-lys levels than men. About 96% of the participants' primary occupation was farming, of whom 67% were growing both groundnuts and maize. Individuals whose primary occupation was farming had higher mean AFB_1-lys level 22.8 ± 22.0 ($p = 0.032$) than those whose primary occupation was an off-farm job. A significant difference in mean AFB_1-lys levels was observed between participants who consumed groundnuts frequently (≥ 4 times a week) and those who consumed less frequently (≤ 3 times a week). It is worth noting that the frequency of groundnut consumption varied from one to seven times a week, while all (100%) the participants consumed maize on a daily basis in the form of thick porridge called *nsima*.

Data on AFB_1 contamination in maize and groundnut samples that were collected from the homesteads of the subjects are summarized in Table 4. Most (91%) of the groundnut samples and 70% of the maize samples tested positive for AFB_1, while 44% of the groundnut samples and 32% of the maize samples contained more than 20 μg/kg of AFB_1. District-wise, AFB_1 levels higher than 20 μg/kg were found in 38, 64, and 44% of the fresh groundnut samples collected from the homesteads in Nkhotakota, Mchinji, and Kasungu, respectively. Similarly, AFB_1 levels higher than 20 μg/kg were found in 49, 12, and 22% of the fresh maize samples collected from the homesteads in Nkhotakota, Mchinji, and Kasungu, respectively. The mean AFB_1 contamination level in groundnut samples for the three districts was 52.4 μg/kg, which was about three times higher than that for maize (16.3 μg/kg). Among the three districts, samples collected from Mchinji appeared to have the highest mean level of AFB_1 contamination in groundnuts, though the difference was statistically insignificant.

Table 5 shows the result of the tobit model estimation. The two specifications, i.e., with and without age and body weight variables, exhibited generally consistent results. In both specifications, one third of the observations were left-censored at zero. Age and body weight being insignificant in specification 2 suggest that omission of these variables in specification 1 did not cause a considerable estimation bias. Hence, specification 1 may be more reliable in light of its larger sample size. Gender, frequency of groundnut intake, and primary occupation had statistically significant coefficients. Being a woman resulted in raising the AFB_1-lys level by 10.1 pg/mg on average, holding other variables constant. The more often groundnuts were consumed, the higher the exposure level was. An increase in weekly frequency by once led to an increase in the AFB_1-lys adduct level in the blood by 4.3 pg/mg. Farming as a primary occupation resulted in higher exposure levels by 12.6 pg/mg compared to those whose primary occupation was not farming. Interestingly, AFB_1 levels in crops did not exhibit significant effects on the biomarker level. Given the significant effect of the frequency of groundnut intake, it might be the case that the sampling of crop produce was not representative of the true contamination.

Discussion

The AFB_1-lys levels found in our study are comparable to existing studies in other parts of Sub-Saharan Africa. The

Table 3 AFB_1-lys adduct levels by gender, age, district, and consumption patterns in Malawi

Variable/category	No. of samples tested for AFB_1-lys	Proportion of positive AFB_1-lys (%)	Mean AFB_1-lys (pg/mg) ± Std. Dev.[1]	*p* value[2]
Gender				0.055
Female	114	73	23.5 ± 23.8	
Male	116	62	17.6 ± 13.4	
Age (years)				0.414
< 20	22	50	14.3 ± 22.5	
20–65	161	71	21.4 ± 23.7	
> 65	47	30	20.3 ± 22.5	
Primary occupation				0.032
Farming	155	82	22.8 ± 22.0	
Not farming	75	42	15.6 ± 23.7	
Frequency of groundnut consumption				0.021
≤ 3 times a week	135	52	17.5 ± 21.1	
≥ 4 times a week	95	77	24.7 ± 25.7	
District				0.135
Nkhotakota	65	58	24.4 ± 30.0	
Mchinji	59	71	16.0 ± 17.6	
Kasungu	106	71	20.6 ± 21.2	
Total	230	67	20.5 ± 23.4	

[1] The limit of detection is 2.5 pg/mg of albumin

[2] The *p* values are with respect to the two-sample *t* test for gender, primary occupation, and frequency of groundnut consumption, and the one-way ANOVA test for district and age groups

mean AFB_1-lys level found in the current study (20.4 pg/mg of albumin) was higher than what was reported for the counterpart in the neighboring country of Tanzania (4.7 pg/mg of albumin) (Shirima et al. 2015). However, a higher mean AFB_1-lys (32.8 pg/mg of albumin) level was reported in children from Togo and Benin (Gong et al. 2003), and a wide range of AFB_1-lys (31.4 to 119.3 pg/mg of albumin) was reported in children from Benin (Gong et al. 2004).

AFB_1-lys levels reported are an indication of risk of AFB_1 exposure through contaminated food. In fact, our result shows that the more often groundnuts were consumed, the higher the AFB_1-lys level was in the human blood sample. It is important to note that although the mean AFB_1 level in groundnuts was found to be three times higher compared to maize, it is likely that maize contributed a greater quantity of AFB_1 to humans in total than groundnuts did, given the higher per capita consumption of maize (382 g/day) compared to groundnuts (less than 100 g/day).

The significantly higher AFB_1-lys level detected in women compared to men contradicts the previous studies from Taiwan (Sun et al. 2002) and the UK (Turner et al. 1998). This contrast could be attributed to the cultural settings of our study area where women are predominantly involved in groundnut post-harvest handling such as harvesting, drying,

Table 4 AFB_1 contamination levels in groundnut and maize samples

Commodity	District	Number	*n* of AFB_1-positive samples (%)	*n* of samples with AFB_1 > 20 μg/kg (%)	Range of AFB_1 (μg/kg)[1]	Mean AFB_1 (μg/kg) (Std. Dev.)
Groundnut	Nkhotakota	65	62 (95)	25 (38)	0–868	54.2 (121.5)
	Mchinji	59	57 (97)	38 (64)	0–568	67.2 (104.8)
	Kasungu	106	90 (86)	38 (36)	0–584	43.0 (89.1)
	Total	230	209 (91)	101 (44)	0–868	52.4 (103.2)
Maize	Nkhotakota	65	58 (89)	31 (49)	0–90	22.7 (23.4)
	Mchinji	59	39 (66)	16 (12)	0–87	13.9 (21.4)
	Kasungu	106	64 (60)	26 (22)	0–91	13.8 (24.3)
	Total	230	161 (70)	73 (32)	0–91	16.3 (23.6)

[1] The limit of detection for groundnuts and maize is 1 μg/kg

Table 5 Estimates of tobit regression of AFB_1-lys adduct levels in the blood

Explanatory variable	Coefficients	
	Specification 1 (without age and body weight)	Specification 2 (with age and body weight)
Age		0.002 (0.982)
Gender dummy[1] (1 if female)	10.157** (0.018)	9.028 (0.142)
Frequency of groundnut intake	4.293*** (0.003)	15.656*** (0.000)
AFB_1 in groundnut produce	− 0.043 (0.443)	− 0.086 (0.384)
(Frequency of groundnut intake) × (AFB_1 in groundnut produce)	0.001 (0.962)	− 0.0495 (0.144)
AFB_1 in maize produce	− 0.044 (0.630)	0.030 (0.802)
Dummy for aflatoxin awareness	− 4.300 (− 0.372)	− 6.300 (0.355)
Body weight		0.235 (0.446)
Occupation dummy (1 if farming)[2]	12.588** (0.009)	20.904*** (0.001)
Intercept	− 10.683 (0.132)	− 44.672*** (0.001)
Number of observations	229	117
Number of observations left-censored at zero	75	39
Likelihood ratio test	χ^2 (6) = 30.75 $p = 0.000$	χ^2 (9) = 44.71 $p = 0.000$

Dependent variable: AFB_1-lys level in blood samples (pg/mg of albumin). The tobit model is estimated with the left-censoring occurring at zero. Heteroscedasticity robust standard errors are estimated. The p values are in the parentheses

*Dummy is a binary variable (0 or 1). **$p < 0.05$; ***$p < 0.01$

[1] The indication is put back

[2] It is a binary variable that takes the value of one for those whose primary occupation is farming, and zero otherwise

stripping, and shelling. Speculatively, women being involved in all these processes could consume groundnuts in higher amounts than other women. In fact, in 207 out of the 230 households sampled, it was discovered that women were responsible for producing groundnuts, and they reportedly ate groundnuts during the harvest and shelling points. Moreover, at the household level, the mean frequency of consumption of groundnuts is slightly higher in women (3.5 ± 1.9) compared to men (3.1 ± 1.9). However, eating groundnuts during harvest and shelling points may not be the only route of exposure in women: inhalational intake of AF containing dust may also occur especially for the field labors (Degen 2011). In any case, high AF biomarker levels in the blood of women can have an implication on infant health since one of the AFB_1 metabolites, namely AFM_1 is transferred with breast milk to infants (Leong et al. 2012; Warth et al. 2016). Moreover, children continue to be exposed to high levels of aflatoxin considering that maize and groundnuts are used in weaning and post-weaning foods. In fact, exposure to aflatoxin from these foods is often several times higher than that from milk. Unfortunately, exposure to aflatoxin early in life has been linked to stunted growth in children (Turner 2013). Further interventions are currently in progress to elucidate the link between food AFB_1 contamination, AFB_1-lys in the blood, and the rate of stunting in children which is currently estimated as 37% (NSO 2017) in Malawi. Other available evidences indicate that a primary risk factor for childhood cancer involves trans-placental exposure to either mutagenic or promutagenic agents including AFs (Chawanthayatham et al. 2015).

In general, the AFB_1 levels observed in groundnut samples in all three districts are higher than what was previously reported by Monyo et al. (2012) in Malawi and Seetha et al. (2017) in neighboring Tanzania. Moreover, the observed lack of correlation between AFB_1 levels in food commodities and blood AFB_1-lys levels could have arisen from the fact that maize and groundnut samples were collected only once and yet AFB_1-lys in the blood is a reflection of cumulative exposure to AFB_1 over a period of the past 2–3 months (Chen et al. 2001). Furthermore, as for maize, the present study did not capture information on how the crop was processed prior to milling. Some processing methods such as dehulling may significantly lower AFB_1 levels in maize (Matumba et al. 2015b). Therefore, processing may have lowered AFB_1 levels and thus contributed to the insignificant association between the raw maize samples and AFB_1-lys levels observed in this study.

The higher mean AFB_1-lys adduct level in blood samples found in Nkhotakota corroborates the earlier report which indicates that the lakeshore ecological zone (e.g., Nkhotakota) is riskier than the mid-altitude zone (e.g., Mchinji and Kasungu) (Matumba et al. 2015a) in terms of

AFB_1 contamination in food. Therefore, our result further affirms the need for continuous monitoring of AFB_1 occurrence in food along with development of site (agro-ecology)-specific pre- and post-harvest management strategies in order to reduce dietary risks (Matumba et al. 2015a).

It is worth mentioning that this study has two major limitations. First, the study did not collect samples repeatedly, which could have helped to determine seasonal variation of AFB_1 contamination of food commodities and AFB_1-lys levels. Second, the daily intake of AFB_1 and their correlation with AFB_1-lys were not estimated in this study. That could have contributed to elucidating the unpacked relationship in different ways. Nonetheless, our results are alarming and necessitate appropriate interventions to reduce both the exposure of humans and contamination of crop produce. It is recommended that future studies should focus on establishing the link between the AFB_1-lys level and malnutrition in Malawi with special focus on child stunting.

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Compliance with ethical standards

The study with human volunteers was approved by National Health Sciences Research Committee (NHSRC) of Malawi. All objectives of the study were clearly explained to the participants by medical professionals, and consent was obtained from each volunteer before collecting 10 ml of blood. In cases of minors, written consent was obtained from parents and guardians.

Conflict of interest The authors declare that there is no conflict of interest.

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