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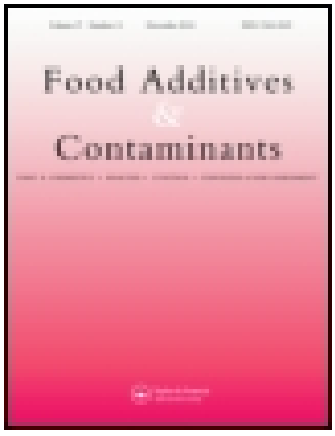

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Longitudinal evaluation of aflatoxin exposure in two cohorts in south-western Uganda

Min-Su Kang[a], Peter Nkurunziza[b], Richard Muwanika[c], Guoqing Qian[a], Lili Tang[a], Xiao Song[a], Kathy Xue[a], Allan Nkwata[a], John Ssempebwa[d], Tom Lutalo[c], Gershim Asiki[b], David Serwadda[cd], Janet Seeley[be], Pontiano Kaleebu[b], Fred Nalugoda[c], Robert Newton[bf], Jonathan H. William[a] & Jia-Sheng Wang[a]

[a] College of Public Health and Peanut CRSP, University of Georgia, Athens, GA, USA

[b] Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) Research Unit on AIDS, Entebbe, Uganda

[c] Rakai Health Sciences Program, Entebbe, Uganda

[d] School of Public Health, Makerere University, Kampala, Uganda

[e] London School of Hygiene and Tropical Medicine, London, UK

[f] University of York, York, UK

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[a]*College of Public Health and Peanut CRSP, University of Georgia, Athens, GA, USA;* [b]*Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) Research Unit on AIDS, Entebbe, Uganda;* [c]*Rakai Health Sciences Program, Entebbe, Uganda;* [d]*School of Public Health, Makerere University, Kampala, Uganda;* [e]*London School of Hygiene and Tropical Medicine, London, UK;* [f]*University of York, York, UK*

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Aflatoxins (AF) are a group of mycotoxins. AF exposure causes acute and chronic adverse health effects such as aflatoxicosis and hepatocellular carcinoma in human populations, especially in the developing world. In this study, AF exposure was evaluated using archived serum samples from human immunodeficiency virus (HIV)-seronegative participants from two cohort studies in south-western Uganda. AFB_1–lysine (AFB-Lys) adduct levels were determined via HPLC fluorescence in a total of 713 serum samples from the General Population Cohort (GPC), covering eight time periods between 1989 and 2010. Overall, 90% (642/713) of the samples were positive for AFB-Lys and the median level was 1.58 pg mg^{-1} albumin (range = 0.40–168 pg mg^{-1} albumin). AFB-Lys adduct levels were also measured in a total of 374 serum samples from the Rakai Community Cohort Study (RCCS), across four time periods between 1999 and 2003. The averaged detection rate was 92.5% (346/374) and the median level was 1.18 pg mg^{-1} albumin (range = 0.40–122.5 pg mg^{-1} albumin). In the GPC study there were no statistically significant differences between demographic parameters, such as age, sex and level of education, and levels of serum AFB-Lys adduct. In the RCCS study, longitudinal analysis using generalised estimating equations revealed significant differences between the adduct levels and residential areas (p = 0.05) and occupations (p = 0.02). This study indicates that AF exposure in people in two populations in south-western Uganda is persistent and has not significantly changed over time. Data from one study, but not the other, indicated that agriculture workers and rural area residents had more AF exposure than those non-agricultural workers and non-rural area residents. These results suggest the need for further study of AF-induced human adverse health effects, especially the predominant diseases in the region.

Keywords: aflatoxins; aflatoxin B_1–lysine adduct; human exposure; Uganda; cohort studies

Introduction

Aflatoxins (AF), mainly produced by *Aspergillus flavus* and *A. parasiticus*, represent a group of naturally occurring fungal metabolites (mycotoxins) that have long been recognised as hazardous contaminants of food, especially in peanuts, corn/maize and dried cassava (Busby & Wogan 1984; Wang et al. 1998; Williams et al. 2004). Aflatoxin B_1 (AFB_1) is a potent hepatotoxic and genotoxic agent (Essigmann et al. 1977; Wang & Groopman 1999), and has been evaluated as a Group I human carcinogen (sufficient evidence of carcinogenicity to humans) by the IARC, WHO (IARC 1993, 2002). Exposure to high levels of AFB_1 via the diet causes acute hepatotoxicity (aflatoxicosis) and death in humans, as demonstrated by recent outbreaks in Kenya, which were responsible for the deaths of more than 150 people (Azziz-Baumgartner et al. 2005; Lewis et al. 2005). Chronic exposure to low levels of AFB_1 is a risk factor in the aetiology of human hepatocellular carcinoma (HCC) in several regions of Africa and Southeast Asia, particularly in conjunction with hepatitis B virus infection (IARC 1993, 2002; Wild & Hall 2000; Wogan et al. 2012). Importantly, AFB_1 has also been shown to be an anti-nutritional agent that reduces concentrations of vitamins and proteins in animals and humans (Gong et al. 2002; Williams et al. 2004; Wild 2007; Tang et al. 2009). Further, it is a potent immunotoxic agent in animals and also changes T-cell phenotypes in humans, which may aggravate the burden of infectious diseases in the developing world (Williams et al. 2004; Jiang et al. 2005; Wild 2007).

AF contamination is an important problem in relation to food quality/food safety in Africa as a result of poor production and storage practices for major staples of the African diet (Hell et al. 2000; Williams et al. 2004, 2010; Lewis et al. 2005). Studies in Uganda indicate that AF exposure is broadly similar to that reported from other African countries (Kaaya & Warren 2005). The environmental conditions in parts of Uganda are ideal for *Aspergillus* growth during crop cultivation and storage in the subsequent post-harvest period due to improper drying

*Corresponding author. Email: jswang@uga.edu

and inadequate storage conditions. AF contamination has been reported in maize, cassava, groundnuts and locally manufactured baby foods including baby soya and rice porridge (Williams et al. 2004, 2010; Kaaya & Eboku 2010; Kitya et al. 2010).

Human exposure to AF and its linkage to adverse health effects in various human populations have been well studied in some West African countries (Williams et al. 2004, 2010), as well as in Kenya (Azziz-Baumgartner et al. 2005; Lewis et al. 2005; Yard et al. 2013), a neighbouring country to Uganda. However, no human exposure studies have ever been assessed in Uganda with the exception of an earlier pilot investigation in small numbers of children and a recent pilot study (Wild et al. 1990; Asiki et al. 2014), although a link between AF and hepatoma was suggested decades ago in Uganda (Alpert et al. 1968, 1971; Serck-Hanssen 1970). In order to explore further the relationships between AF exposure, archived serum samples previously collected from two existing cohort studies in the central and south-west regions of Uganda were analysed for AFB_1–lysine adduct (AFB-Lys) levels to evaluate AF exposure in these human populations.

Materials and methods

Study populations and study samples

The geographic locations of both cohort study sites are indicated on the map of Uganda (Figure 1). The General Population Cohort (GPC) was established by the UK Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) in 1989, and sampling was initially conducted in 15 rural villages (expanded to 25 villages in 2000) in a sub-county of Masaka district located in south western Uganda (Asiki et al. 2013). This is an open-cohort study, with new births, deaths, inward and outward migration reported at each round of follow-up. The total population covered is about 22 000 people, and there is no age limit. Every year since its inception, data were collected on annual household censuses of the resident population covering age, sex, education and relationship to household head. A medical sero-survey was conducted in participants aged 13 years and above (although some rounds also include all children), including collection of blood specimens for human immunodeficiency virus (HIV) testing and a brief behavioural questionnaire.

Figure 1. Uganda and the geographic distribution of GPC and RCCS study sites.

The Rakai Community Cohort Study (RCCS), initiated in 1987 in Rakai District, south-western Uganda, is a collaborative study conducted by the Ministry of Health through the UVRI, Makerere University and Johns Hopkins University. The current cohort, which consists of some of the adult residents in 50 villages, was established in 1994–95 (Wawer et al. 1991; Sewankambo et al. 1994; Gray et al. 1998). All participants are followed annually in their homes, at which time they provide personal information (demographics, education, current and past sexual behaviours, and sexually transmitted infection history) through surveys, and blood samples were collected for detection of HIV, sexually transmitted diseases (STDs) and other infections. This is an open cohort of adults aged 15–49 years, which enrols new immigrants and age-eligible residents at each survey visit annually. The numbers of participants under surveillance are maintained between the ranges of 12 000–16 000 annually. The main objectives of these two cohort studies are similar: to study the dynamics of HIV infection; to identify major risk factors; and to measure the impacts of HIV infection on mortality and fertility. However, there are two important differences between the GPC and the RCCS. Firstly, the GPC is concentrated in one sub-county of Masaka District, working in contiguous villages, while the RCCS study communities were selected to represent different parts of the district, so are scattered across the area. Secondly, the GPC includes all age groups, from infants to the very old, and the RCCS focuses on collecting data in age groups between 15 and 49 years.

Archived serum samples were randomly selected from GPC samples collected in 1989, 1992, 1995, 1998, 2001, 2004, 2007 and 2010, and from the RCCS samples collected in 2000, 2001, 2002 and 2003, respectively. A total of 713 and 374 archived sample were thawed and aliquots were separated, stored on dry ice, and shipped via air for the analysis of AFB-Lys adducts at the College of Public Health, University of Georgia (UGA), Athens, Georgia, USA, with the import permit issued by the US Centers for Disease Control and Prevention (CDC). All samples selected for this analysis were HIV negative.

Materials for laboratory analysis

AFB1 (> 98% purity), albumin determination reagent, bromocreosol purple and normal human serum were purchased from Sigma Aldrich Chemical Co. (St Louis, MO, USA). Protein assay dye reagent concentrate and protein standards were purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA). Pronase (25 kU, Nuclease-free) was purchased from Calbiochem (La Jolla, CA, USA). Mixed-mode SPE cartridges were purchased from Waters Corp. (Milford, MA, USA). Authentic AFB-Lys was synthesised as previously described (Sabbioni et al. 1987). The authentic AFB-Lys standard was purified and characterised by UV absorption and mass spectrometry (Sabbioni et al. 1987; Wang, Abubaker, et al. 2001). All other chemicals and solvents used were of the highest grade commercially available.

Measurement of serum AFB-Lys adducts

Serum samples were coded separately and analysed with a newly developed HPLC-fluorescence method (Qian et al. 2013). Briefly, thawed human serum samples were heated to 56°C for 30 min to inactivate infectious microbial agents. Total protein and albumin concentrations were determined using a modification of a previously described method (Wang et al. 1996). A portion of each serum sample (150 µl) was digested by pronase (pronase:total protein, 1:4, w/w) at 37°C for 3 h to release the AFB-Lys adduct. The serum sample digests were subjected to SPE clean-up using Waters MAX SPE cartridges, which had been conditioned with methanol and equilibrated with water prior to loading with the AFB-Lys containing digests. The loaded cartridges were sequentially washed by water, 70% methanol and 1% ammonium hydroxide in methanol at a flow rate of 1 ml min^{-1}. Purified AFB-Lys was eluted with 2% formic acid in methanol. The eluent was vacuum dried with a Labconco Centrivap concentrator (Kansas City, MO, USA) and reconstituted for HPLC-fluorescence detection. The analysis of AFB-Lys adduct was conducted in an Agilent 1200 HPLC-fluorescence system (Santa Clara, CA, USA). The mobile phases consist of buffer A (20 mM $NH_4H_2PO_4$, pH 7.2) and buffer B (100% methanol). The Zorbax Eclipse XDB-C18 reverse phase column (5 µm, 4.6 × 250 mm) equipped with a guard column was used. Column temperature was maintained at 25°C during analysis, and a volume of 100 µl was injected into the HPLC. The flow rate was kept at 1 ml min^{-1}. A gradient was generated to separate the AFB-Lys adduct within 25 min of injection. The AFB-Lys adduct was detected by fluorescence at maximum excitation and emission wavelengths of 405 and 470 nm, respectively. Calibration curves of authentic standard were generated weekly, and the standard AFB-Lys was eluted at approximately 13.1 min. Quality assurance and quality control procedures were conducted during analyses, which include the analysis of one authentic standard and a quality control sample daily in the same sequence. The LOD was 0.4 pg mg^{-1} albumin. The average recovery rate with various spiked concentrations of AFB-Lys adduct standard was 90%. The serum AFB-Lys concentration was normalised to albumin content for statistical analysis and report. The technicians who perform the analyses had no information about sample sources, which guaranteed confidentiality.

Statistical analysis

An Excel database was established to include the AFB-Lys adduct level and other demographic information. Data analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC, USA). Levels of AFB-Lys adducts in the groups with different demographic parameters such as age, gender, area, occupation and education were compared. Since participants in both cohorts provided serum samples multiple times, longitudinal analysis for repeated measure was used. Generalised estimating equations were used to handle the inconsistent participation of some participants and non-normal distribution of the AFB-Lys adduct in serum. We separately analysed the datasets from the GPC and RCCS due to the discrepancy of baseline demographic factors in both cohorts, which was tested by χ^2- and *t*-tests. The monotonic trend of AFB-Lys across rounds (time) in participants was estimated by a Wilcoxon-type test for trend and generalised estimating equations (Cuzick 1985). Temporal variation of AFB-Lys was analysed using a Kruskal–Wallis test. Box plot analysis was done through SigmaPlot 10.0 (San Jose, CA, USA). A *p*-value of less than 0.05 was considered to be statistically significant.

Ethics

Ethical approval for this study was granted by the UVRI Science Ethics Committee and the Uganda National Council of Science and Technology. The laboratory study protocol of serum sample analysis for the AFB-Lys adduct was approved by the Institutional Review Board for Human Subject Protection at the University of Georgia.

Results

Demographic information for study participants at both cohort sites is described in Table 1. As shown, there were significant differences between the two cohort study sites on basic demographic parameters in terms of age, sex, area of residency, occupation and education level. Thus, the statistical analysis was done separately.

Table 1. Demographic information for participants in two study sites.

	GPC site	RCCS site	*p*-value
Sex			
Male	183	151	0.0034
Female	174	223	
Age (mean ± SD)	44.3 ± 18.6	30.2 ± 9.76	< 0.001
Area of residency			
Rural	357	314	< 0.0001
Semi-urban	–	60	
Occupation			
Agriculture	323	233	< 0.0001
Others	34	141	
Level of education			
Never	54	23	< 0.0001
Primary	261	227	
Secondary and above	42	124	
HIV status			
Negative	357	374	
Positive	–	–	

Note: *p*-values were obtained by a χ^2 test (sex, area, occupation, education) and a *t*-test (age).

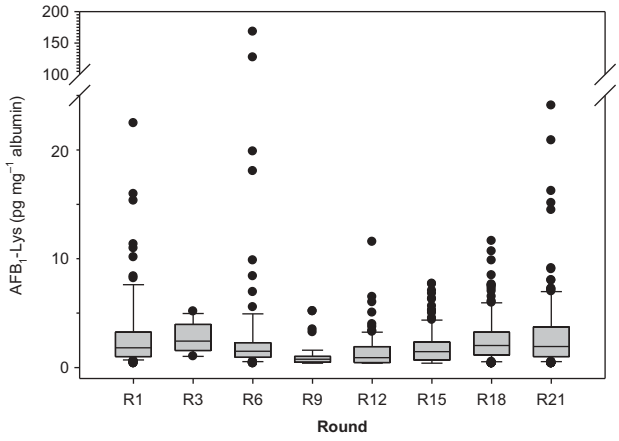

Figure 2. Temporal variations of AFB-Lys adduct level in GPC study participants. The box plots show the distribution of serum AFB-Lys adducts levels in each round. Box values ranged from the 25th to the 75th percentile of total samples, the line within it indicating the median value. Bars on both sides of a box represent values ranging from the 5th to 25th percentile and from the 75th to the 95th percentile, respectively.

AFB-Lys adduct levels in a total of 713 archived GPC serum samples were determined at eight separate time points between 1989 and 2010. Longitudinal temporal variations of AFB-Lys adduct levels in these serum samples are shown in Table 2 and Figure 2. The averaged detection rate was 90% (642/713) and the median level was 1.58 pg mg^{-1} albumin (range = 0.40–168 pg mg^{-1} albumin) over all analysed cohort samples. There was statistically significant variation across the various rounds ($p < 0.0001$), showing the great increase of AFB-Lys adduct for rounds 9–21, representing 1998–2010, However, there was no monotonic increase in detection rates and the median level of serum AFB-Lys adduct among eight rounds ($P_{trend} = 0.073$), Further longitudinal analysis using generalised estimating equations for the AFB-Lys adduct did not show significant differences between demographic parameters, such as age, gender, residence, occupation and level of education (Table 4).

AFB-Lys adduct levels in a total of 374 archived Rakai RCCS serum samples were determined according to four consecutive rounds of sample collections, representing 2000–03. Longitudinal temporal variations of AFB-Lys adduct levels in these serum samples are shown in Table 3 and Figure 3. The averaged detection rate was 92.5% (346/374) and the median level was 1.18 pg mg^{-1} albumin (range = 0.40–122.5 pg mg^{-1} albumin) over all analysed cohort samples. There was a significant variation across four round ($p = 0.0006$), showing increasing AFB-Lys adducts in 2001–03, but there was no statistical significance in the increasing trend of the AF exposure in RCCS (Figure 3, $P_{trend} = 0.061$). Although there were no significant differences between demographic parameters such as age, gender and level of education with sorted AFB-Lys adduct levels (Table 4), analysis using generalised estimating equations revealed significant differences between levels of AFB-Lys adducts and

Table 2. AFB-Lys adduct levels (pg mg^{-1} albumin) in the GPC study participants over time.

	Round (year)								
	R1 (1989)	R3 (1992)	R6 (1995)	R9 (1998)	R12 (2001)	R15 (2004)	R18 (2007)	R21 (2010)	Total
Number	87	11	86	49	80	128	141	131	713
Mean	3.09	2.71	5.62	1.06	1.51	1.89	3.00	3.12	2.85
SD	3.70	1.43	22.46	1.05	1.70	1.62	4.07	3.69	8.37
Median	1.82	2.44	1.50	0.77	0.92	1.46	2.03	1.93	1.58
Minimum	0.40	1.02	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Maximum	22.5	5.16	168	5.18	11.6	7.7	32.3	24.08	168.0
Prevalence of detectable AFB-Lys (%)	95.4 (83/87)	100 (11/11)	95.3 (82/86)	83.7 (41/49)	81.3 (65/80)	85.2 (109/128)	91.5 (129/141)	93.9 (123/131)	90.0 (642/713)

Table 3. AFB-Lys adduct levels (pg mg^{-1} albumin) in RCCS study participants over time.

	Round (year)				
	R6 (2000)	R7 (2001)	R8 (2002)	R9 (2003)	Total
Number	84	146	128	14	374
Mean	5.71	2.35	2.95	6.55	3.46
SD	11.8	4.22	11.2	8.82	9.25
Median	1.86	1.11	1.12	2.31	1.18
Minimum	0.40	0.40	0.40	0.40	0.4
Maximum	94.8	26.0	122.5	30.9	122.5
Prevalence of detectable AFB-Lys (%)	95.2 (80/84)	94.5 (138/146)	89.8 (115/128)	78.6 (11/14)	92.5 (346/374)

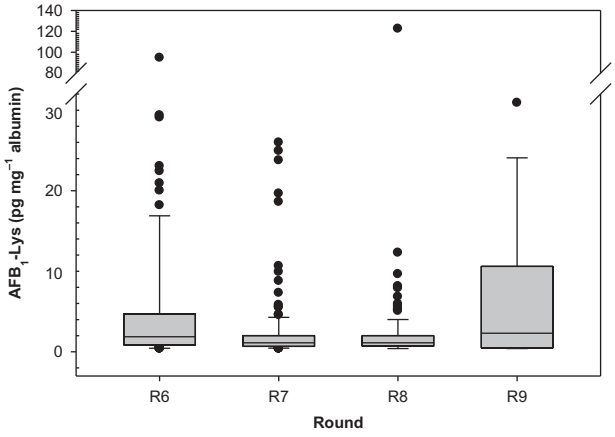

Figure 3. Temporal variations of AFB-Lys adduct levels in RCCS study participants. Box plots show the distribution of serum AFB-Lys adducts levels in each round. Box values ranged from the 25th to the 75th percentile of the total samples, the line within it indicating the median value. Bars on both sides of a box represent values ranging from the 5th to the 25th percentile and from the 75th to the 95th percentile, respectively.

residential areas ($p = 0.045$) and occupations ($p = 0.016$) in this cohort samples, as demonstrated in Table 4.

Discussion

This study showed that AF exposure is almost ubiquitous in study participants from both cohorts, as evidenced by the detection rate above 90% and the constant detection of AFB-Lys adduct in these cohort serum samples throughout different times. These results also suggested the universal contamination of AF in the food supply in south-western regions of Uganda. AF contamination in food has been a long-term problem in Uganda (Alpert et al. 1971; Kaaya & Warren 2005; Kitya et al. 2010).

While contamination by the AF-producing *Aspergillus* fungi may be universal within a given geographical area, the levels or final concentration of AF in the grain product can vary from < 1 µg kg^{-1} (1 ppb) to > 12 000 µg kg^{-1} (12 ppm) (Gan et al. 1988). For this reason, the measurement of human consumption of AF by sampling foodstuffs is imprecise. Further, obvious contamination of a commodity with the fungi does not necessarily demonstrate the presence of AF, and the appearance of a sound, uninfected sample of commodity does not preclude the existence of significant quantities of AFs (IARC 2002; Kaaya & Kyamuhangire 2006). Therefore, accurate assessment of human AF exposure using the biomarker approach has been highly recommended in the past 20 years. AFB-Lys adduct in serum albumin has been proven to be the most reliable exposure biomarker (Sabbioni et al. 1987; Qian et al. 2010, 2013). As a result of its longer *in vivo* half-life as compared with other urinary AFB_1 metabolites, such as AFM_1 and AFB-N^7-guanine, the AFB-Lys adduct can reflect integrated exposures over 2–3 months. The long-term stability (up to 20 years) in frozen serum or plasma made this adduct a first choice of biomarkers to study human AF exposure and its link to diseases (Wang et al. 1996; Wang, Abubaker, et al. 2001; Scholl & Groopman 2008). Highly significant associations between AFB-Lys adduct level and dietary AFB_1 exposure have been found in human populations from several regions of the world. In addition, AFB-Lys adduct has been used as a biological response indicator of acute and chronic human diseases, such as aflatoxicosis in Africa, risks of HCC in Taiwan, China and Africa, and infectious disease-linked immune suppression (Wang et al. 1996, 1998; Wang, Huang, et al. 2001; Turner et al. 2003; Azziz-Baumgartner et al. 2005; Lewis et al. 2005). Moreover, the AFB-Lys adduct has been used as the primary biomarker to assess the efficacy of several human chemoprevention and intervention trials (Tang et al. 2008; Wild & Hall 2000; Wang, Abubaker, et al. 2001; Wang et al. 2008). Therefore, application of the AFB-Lys adduct is a significant tool as an optimal biomarker for studying global public health concern on AF-associated human adverse health effects and evaluating efficacies of various intervention or prevention strategies, especially in the developing world.

The present data are consistent with the previous reported cross-sectional pilot study in GPC samples collected in 2011 (Asiki et al. 2014). The geometric means of

Table 4. AFB-Lys adduct levels in all participants according to selected demographic characteristics.

	Number	Detection rate (%)	Geometric mean	Median (95 % CI)	25th percentile	75th percentile	90th percentile	*p*-value[a]
GPCsite								
Sex								
Male	183	87.4	1.63	1.56 (0.4–7.25)	0.81	3.09	7.25	0.367
Female	174	90.8	1.75	1.84 (0.40–8.20)	0.93	2.79	4.54	
Residential area								
Rural	357	89.1	1.69	1.71 (0.4–7.63)	0.87	3.07	5.5	–
Occupation								
Agriculture	323	88.9	1.63	1.66 (0.4–7.16)	0.86	2.85	5.26	0.464
Others	34	91.2	2.19	2.41 (0.4–11.33)	1.06	4.54	7.7	
Education								
None	54	90.7	1.9	1.97 (0.4–11.33)	0.95	3.52	7.47	0.386
Primary	261	88.5	1.66	1.62 (0.4–7.02)	0.87	2.87	5.26	
Secondary or higher	42	90.5	1.69	1.78 (0.4–6.05)	0.79	2.99	5.16	
Age (years)								
< 20	24	79.2	1.2	1.05 (0.4–4.1)	0.66	2.34	3.96	0.348
20–39	56	92.9	1.96	1.75 (0.4–15.34)	1.03	3.13	6.79	
40–59	194	89.7	1.68	1.7 (0.4–7.47)	0.89	2.71	5.89	
> 60	83	88.0	1.64	1.8 (0.4–7)	0.69	3.39	5.29	
RCCS site								
Sex								
Male	151	92.1	1.49	1.11 (0.4–14.2)	0.74	2.48	6.32	0.975
Female	223	92.8	1.53	1.25 (0.4–13)	0.68	2.63	7.39	
Residential area								
Rural	314	93.3	1.58	1.25 (0.4–18.2)	0.72	2.63	6.84	0.045
Semi-urban	60	88.3	1.21	0.95 (0.4–8.34)	0.67	1.87	4.85	
Occupation								
Agriculture	233	91.4	1.59	1.19 (0.4–20.92)	0.68	2.66	9.93	0.016
Others	141	94.3	1.39	1.17 (0.4–6.32)	0.75	2.43	5.08	
Education								
None	23	91.3	2.06	1.05 (0.4–5.93)	0.71	1.82	3.14	0.493
Primary	227	93.4	1.46	1.3 (0.4–15.6)	0.73	2.85	8.14	
Secondary or higher	124	91.9	1.52	1.07 (0.4–8.14)	0.69	2.26	5.13	
Age (years)								
< 20	42	90.5	1.33	0.99 (0.4–5.83)	0.58	1.98	4.57	0.404
20–39	257	91.4	1.59	1.30 (0.4–15.59)	0.72	2.95	7.39	
40–59	75	97.3	1.37	1.04 (0.44–20.01)	0.74	2.01	5.39	

Note: [a]Longitudinal analysis using generalised estimating equations.

AFB-Lys in our GPC study was 1.62 pg mg^{-1} albumin and 1.66 pg mg^{-1} albumin in RCCS, which is different from the geometric mean of 11.5 pg mg^{-1} albumin in that report (Asiki et al. 2014). The difference possibly originated from different analytical methods for AFB-Lys measurement: the HPLC-fluorescence detection was used in our study, and the ELISA method was used in that study. Another explanation would be the difference of dietary pattern changes, study designs and source of samples. In contrast to the pilot study using cross-sectional design for samples only collected in 2011, which may reflect recent dietary pattern changes in GPC study participants, our longitudinal study design reflects temporal pattern of the AFB-Lys adduct levels from 1989 to 2010. Additionally, our study used archived serum samples in both cohorts. Even though the AFB-Lys adduct has been shown to have a long-term stability over 20 years under proper storage conditions (Wang et al. 1996; Wang, Abubaker, et al. 2001; Scholl & Groopman 2008), the repeated thaw and freeze cycle of these samples may degrade the adduct level in the original samples. Nevertheless, by using longitudinal study design and a more accurate detection method for AFB-Lys measurement, our study shows the constant AF exposure over 21 years and provides better estimates of AF exposure in central and south-western Ugandan populations.

Compared with other African countries, the averaged AFB-Lys level found in this study was among those lower levels reported. Yard et al. (2013) suggested that the estimated AFB-Lys adduct values from the ELISA method is typically 4.6 times higher than that from the HPLC-fluorescence measurement. If taking their proposed 4.6 conversion, the AFB-Lys adduct level in our study using GPC study samples is similar to that of Asiki et al. (2014). The averaged adduct level is actually higher than the levels found in children from Kikelelwa, Tanzania (0.78 pg mg^{-1} albumin after conversion by division of 4.6) (Shirima et al. 2013; Yard et al. 2013). However, the adduct level is still lower than averaged levels found from neighbouring countries, such as Kenya and Tanzania, and West African countries, such as Ghana, Gambia, Benin and Togo (Gong et al. 2002, 2012; Ofori-Adjei 2012; Shirima et al. 2013). This may be again due to different diet patterns in study participants or different AF exposure levels, or the degradation of AFB-Lys adducts in our serum samples analysed. Also, direct comparisons between countries may not be valid because of different study designs, various analytical methods used and seasonal variations of sample collections. However, considering the consistency in our data and the data from Asiki et al. (2014), there is no doubt that human populations residing in south-western Uganda have been constantly exposed to AF via their diets.

Exposure to AF has been documented in Uganda since 1967 when AF contamination was found in peanuts sold for human consumption (Lopez & Crawford 1967). Approximately 30% of the sampled human food items (n = 480) were positive for AF contamination and 7.7% (37/480) had concentrations of total AF between 100 and 1,000 ppb (Alpert et al. 1971; Kaaya & Warren 2005). About 60–88% of the corn samples collected from three agro-ecological zones contained detectable levels of AF contamination with AFB_1 as the dominant type found in 86–100% of corn samples cross zones (Kaaya & Kyamuhangire 2006). A more recent study found AF contamination in common processed food samples collected from south-western Uganda, covering ground nuts, cassava, millet, sorghum flour and Eshabwe sauce, which is a traditional food in this region made from milk and cheese (Kitya et al. 2010). The mean of AF levels in these food items (n = 90) was 15.7 ppb (range = 0–55) with 82.2% (74/90) exceeding the regulatory levels of the European Union (4 ppb for processed food).

In this study we also found that the serum samples from participants of rural areas had a significantly higher AFB-Lys adduct levels than samples from semi-urban residents in the RCCS cohort, whereas no such a difference can be examined in GPC samples because all study participants were recruited from rural areas. Food resources of rural residents are usually limited, dependent on local productions and highly reliant on natural environmental conditions (Huang & Bouls 1996; Romanik 2008; Mukwaya et al. 2012). Their food production in rural Uganda is vulnerable to toxigenic *Aspergillus* growth during crop cultivation, and higher AF contamination may occur due to inadequate post-harvest drying and improper storage practices. Thus, rural populations are likely to be exposed to higher levels of AF. On the other hand, residents in semi-urban areas usually have better economic status and have a better chance to access diverse food items such as meat, fish and fruit rather than just cereal or other staples such as bananas (Huang & Bouls 1996; Mukwaya et al. 2012). According to a survey conducted in Malawi, 90% of food consumed by households in urban area is purchased, whereas only 30% of food consumed in rural areas is purchased in markets (Romanik 2008). The diverse food items other than AF-contaminated grains will protect residents in semi-urban areas from high AF exposure. Also, rural populations suffer poverty more frequently than urban populations in Uganda (Mukwaya et al. 2012). The association of AF exposure with poverty has been well proven (Shuaib et al. 2012; Adejumo et al. 2013).

In summary, our study showed that AF exposure has been a constant problem over decades in central and south-western Ugandan. It has been well documented that AF exposure is correlated to an increased risk of HCC, immune modulations and anti-nutrition in human populations (Williams et al. 2004; Wild 2007). How AF exposure impacts human health in these regions needs to be further explored, especially for its linkage to the aggravation of infectious diseases, such as HIV, and the disturbance of growth and development in children.

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No potential conflict of interest was reported by the authors.

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