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Association between birth outcomes and aflatoxin B_1 biomarker blood levels in pregnant women in Kumasi, Ghana

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Summary

OBJECTIVE To investigate the association between birth outcomes and blood levels of aflatoxin B_1 (AFB1)-lysine adduct in pregnant women in Kumasi, Ghana.

METHOD A cross-sectional study of 785 pregnant women attending antenatal clinic was conducted. Aflatoxin B_1 (AFB_1)-lysine adduct levels were determined by high performance liquid chromatography (HPLC) on blood taken after delivery. The birth outcomes considered were small for gestation age, low birthweight, preterm delivery and stillbirth. Participants were divided into quartiles based on the distribution of aflatoxin B_1-lysine adducts in pg/mg albumin ('low': ≤2.67, 'moderate': >2.67 to ≤4.97, 'high': >4.97 to ≤11.34, 'very high': >11.34). Statistical analysis involved models that included socio-demographic variables and other potential confounders.

RESULTS The average AFB_1-lysine adduct level in maternal serum was 10.9 ± 19.00 pg/mg albumin (range = 0.44–268.73 pg/mg). After adjusting for socio-demographic variables and potential confounding factors, participants in the highest AFB_1-lysine quartile with 'very high' AFB_1-lysine level (>11.34 pg/mg) were more likely to have low birthweight babies (OR, 2.09; 95% CI, 1.19–3.68), and showed a trend of increasing risk for low birthweight (P_{trend} = 0.007) compared to participants in the lowest quartile.

CONCLUSION This study adds to the growing body of evidence that aflatoxins may increase the risk of adverse birth outcomes. The findings have implications for targeted nutritional education of pregnant women in areas with high levels of aflatoxin contamination of foods.

keywords aflatoxins, birth outcomes, pregnancy, Kumasi

Introduction

It is estimated that more than 20 million infants worldwide, representing 15.5% of all births, are born with low birthweight. Among these cases, 96% occur in developing countries (Unicef 2004). A baby's low weight at birth (defined as birthweight < 2500 g) is either the result of preterm birth (before 37 weeks of gestation) or the result of small for gestational age size (sex-specific birthweight at or below the 10th percentile for the weight-for-gestational-age of an international reference population) (Lee *et al.* 2003). Babies who are small for gestational age are also predisposed to adverse birth outcomes including stillbirth (STB), which occurs when a foetus is born dead after 20 weeks of gestation (Fretts 2005). Low birthweight is closely associated with foetal and neonatal mortality and morbidity, inhibited growth and cognitive development and chronic diseases later in life (Unicef 2004). Many factors affect the duration of gestation, foetal growth, birthweight and, thus, the future health of the infant. These factors relate to the foetus itself and the nutrition and health of the mother (Unicef 2004). It is known that maternal nutrition and health have an impact on the welfare of the foetus and is a predictor of the infant's health (Wu *et al.* 2004). Although substances such as alcohol, drugs and infections are known to affect pregnancy and its outcome, the role of most toxins on birth outcomes remains largely unknown (Rasmussen *et al.* 2007; Buhimschi Catalin & Weiner Carl 2009). These environmental toxins may find their way to the foetus

through water or food ingested by the mother. Aflatoxins are one of such toxins that can be ingested in food. Indeed, studies suggest that the biochemical, immunological and metabolic derangement caused by aflatoxins in the foetus could lead to intrauterine growth retardation and low birthweight (Abulu *et al.* 1998; Yousef *et al.* 2002, 2004). The biochemical effects of aflatoxins are characterized by inhibition of protein, enzyme and clotting factor synthesis as well as depression of carbohydrate metabolism, fatty acid and phospholipids synthesis (Bushby & Wogan 1981).

At least 4.5 billion people, mostly in resource-poor countries, are at risk of chronic exposure to aflatoxins from contaminated food crops (Williams *et al.* 2004). Aflatoxins are a family of toxic metabolites, which are derived from the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi are ubiquitous in hot (25–35 °C) and humid (≥77%) conditions that provide conducive environments for fungal proliferation. These environments typify crop storage conditions prevalent in tropical settings such as the Kumasi region of Ghana (Ghana Homepage 2008). Studies from the 10 regions in Ghana have shown that up to 37% of stored crops such as groundnuts, maize and oil seeds, which form a major part of the diet, may be contaminated with the aflatoxin-producing *Aspergillus* fungi and aflatoxin levels in quantities far exceeding the USDAs regulatory limit of 20 ppb (Awuah & Kpodo 1996; ISID 2000).

The carcinogenic, immunosuppressive and growth-retarding effect of long-term ingestion of aflatoxins in diet have been documented (Fung & Clark 2004). However, few studies have been conducted to investigate the association of birth outcomes with aflatoxins but they have not been conclusive on specific outcomes. For instance, of seven studies that reported on the relationship between birthweight and aflatoxin levels, four indicated significant negative correlations with P values ranging from $P < 0.001$ to $P < 0.05$ (Abulu *et al.* 1998; Yousef *et al.* 2002, 2004; Turner *et al.* 2007), two studies indicated this negative correlation only among female live births (De Vries *et al.* 1989; Jonsyn *et al.* 1995), while one study found no association between birthweight and aflatoxins in serum (Maxwell *et al.* 1994). Furthermore, one study found a significant association ($P < 0.01$) between height at birth and aflatoxins (Sedeghi *et al.* 2009).

To date, only two reports have speculated on a possible association between STBs and aflatoxin: two STB cases were reported where high levels of aflatoxins were found in both maternal peripheral blood and cord blood (De Vries *et al.* 1989). Similarly, one STB was reported by Lamplugh *et al.* (1988) in their study, which was based on aflatoxin in maternal blood. Only Yousef *et al.* (2003), working in the UAE, reported the absence of a significant correlation between aflatoxin M_1, and gestational age.

Other investigators have evaluated the association between clinical conditions such as neonatal jaundice, and aflatoxins in neonatal serum. While two studies conducted in UAE (Yousef *et al.* 2004) and Zaria, Nigeria (Ahmed *et al.* 1995), found no correlation, one study conducted in Ibadan, Nigeria (Sodeinde *et al.* 1995) found serum aflatoxin to be a risk factor for neonatal jaundice (OR, 2.68; 95% CI, 1.18–6.10). Poignantly, the aflatoxin measurements involved different aflatoxin metabolites and various body fluids such as maternal serum, umbilical cord blood and breast milk. These make the results from these studies difficult to compare. Most studies did not adequately account for the effect of other plausible causes of birth outcomes such as malaria, anaemia and intestinal worm infections, which have been associated with birth outcomes (Egwunyenga *et al.* 2001; Guyatt & Snow 2004).

Although the above studies have advanced the literature on the relationship between birth outcomes and aflatoxins, much remains to be known on the contribution of aflatoxins to the burden of low birthweight, preterm delivery, small for gestational age and STB. Indeed, to our knowledge, no study has investigated the association between STBs and aflatoxins. If developing countries are to attain the target of the Millennium Development Goal (MDG) number four of reducing childhood mortality, more directed research is needed to unravel unknown or suspected causes of low birthweight in resource-poor and sub-tropical countries.

The objective of this study was to examine the association between aflatoxin B_1-lysine adduct levels in blood of pregnant women and specific birth outcomes such as low birthweight, preterm, small for gestational age and STB deliveries. We hypothesized that higher blood levels of aflatoxin B_1 will be associated with these adverse birth outcomes.

Methods

Study setting

The study was conducted in Kumasi the capital city of the Ashanti region of Ghana, West Africa, which has a population of approximately 1.2 million (CIA 2005). Its climate is tropical with two rainy seasons occurring from April to June and from September to October (Ghana Districts 2006).

Study design and participants

As described in an earlier study (Yatich *et al.* 2009), this was a cross-sectional study of women presenting for delivery at two hospitals in Kumasi: Komfo Anokye Teaching Hospital

(KATH) and Manhyia Polyclinic, during November and December 2006. All women who had a singleton, uncomplicated pregnancy were invited to participate. Women were identified from admission records. Women who had multiple or complicated pregnancy were excluded from the study. Written informed consent was obtained from participants. Participation in the study was voluntary and no incentives were provided. A total of 785 women were eligible for the study, and all consented. The Institutional Review Board of the University of Alabama at Birmingham and the Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, reviewed and approved the study protocol.

Study instruments and data collection

After informed consent was obtained, a questionnaire was administered by a trained interviewer. The questionnaire included information on demographic characteristics (age, education, socio-economic status, residence and type of toilet facilities), obstetric history for current and previous pregnancies [STB, ectopic pregnancy, preterm delivery and Low birthweight (LBW)], illnesses and treatments during the current pregnancy. Items of the study instrument were derived from a model questionnaire recommended for use by Roll Back Malaria Monitoring and Evaluation Reference group (malaria indicator survey, women's questionnaire) (Roll Back Malaria and World Health Organization, and United Nations Children's Fund 2004). Obstetric information was obtained from the women's antenatal care (ANC) charts. ANC charts provided information on gestational age at first ANC visit, number of antenatal care visits, gestational age as assessed by palpation or ultrasound at first ANC visit, tetanus immunization, malarial prophylaxis, antihelminthic medication, illnesses and treatment during pregnancy. A single blood sample was collected in EDTA by venepuncture for determination of malarial antigen, haemoglobin, Folate and aflatoxin levels. Stool samples were obtained for determination of intestinal helminths.

Laboratory procedures

Determination of AFB1-lysine adduct

Serum aflatoxin B1-lysine adduct, reflecting aflatoxin exposure in the previous 2-3 months, was measured by a modified High Performance Liquid Chromatography (HPLC)-fluorescence method (Qian et al. 2009). Briefly, 150μl serum samples were digested by Pronase and loaded onto an Oasis Max cartridge from Waters Co. (Milford, MA, USA). The cartridge was sequentially washed, and eluted with 2% formic acid in methanol. The eluents were evaporated to dryness and reconstituted with 150 μl 10% methanol before HPLC analysis. HPLC analysis was carried out on an 1100 liquid chromatography system (Agilent Technologies Wilmington, DE, USA). Chromatographic separation was performed on an Agilent C18 column (5 μm particle size, 250 × 4.6 mm). The mobile phase consisted of 20 mM ammonium phosphate monobasic (pH 7.2) and methanol in a linear gradient profile. The concentration of AFB_1-lysine adducts was monitored at wavelengths of 405 nm (excitation) and 470 nm (emission). The peak of authentic AFB_1-lysine adduct standard or samples was co-eluted with the retention time around 12.7 min. The detection limit of this method is 0.5 pg/ml. The results of AFB_1-lysine adducts concentration was adjusted by serum albumin level.

Given that anaemia, malaria and intestinal helminth infections have been associated with birth outcomes, the following laboratory procedures were conducted to evaluate these conditions and results were entered fit into models as confounding variables: (i) determination of malarial antigen in plasma using the Malaria Antigen Celisa assay (Cellabs 2005), (ii) determination of hookworms, *Ascaris lumbricoides* and *Trichuris trichura* using the Kato-Katz thick smear technique (WHO 1991) and detection of *Strongyloides stercoralis* using the Baermann method (Garcia 2001), and (iii) measurement of haemoglobin level in an automatic cell counter (Sysmex M-2000; Digitana AG, Hamburg, Germany). The cut-off level for the third trimester was set at 11 g/dl.

Definition of variables

Preterm delivery (PTD) refers to births which occurred before 37 completed weeks of gestation. LBW was defined as birthweight <2500 g. Small for gestational age (SGA) delivery was defined as sex-specific birthweight at or below the 10th percentile for the weight-for-gestational-age of an international reference population. A stillborn delivery was defined as a baby born dead after 20 weeks of gestation (Fretts 2005).

Malaria status was described as the presence or absence of maternal peripheral blood malaria antigens at delivery. Worm (intestinal) infection connotes the presence of helminth eggs or larvae in stool samples obtained from the participants. Haemoglobin level was based on three categories: Participants were classed as severely anaemic at haemoglobin levels 8 g/dl and as moderately anaemic at levels between 8 and 11 g/dl. Participants with haemoglobin of >11 g/dl were considered to be without anaemia.

Statistical analysis

Data analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC, USA). Missing values were excluded from the analysis, thus only 755 of the 785 individuals were used for the final analysis. Participants were divided into quartiles based on the distribution of aflatoxin B_1-lysine adducts in pg/mg ('low': ≤2.67 pg/mg, 'moderate': >2.67 to ≤4.97 pg/mg, 'high': >4.97 to ≤ 11.34 pg/mg, 'very high': >11.34 pg/mg). Spearman rank correlations were estimated to ascertain associations of potential confounding variables with aflatoxin levels. Multiple logistic regression analysis was used to investigate the association between birth outcomes and aflatoxin levels. Variables that were statistically significant at $P < 0.05$ on bivariate analysis and those known to be associated with birth outcomes based on extant literature were incorporated into models using the backward stepwise technique (Hosmer & Lemeshow 2001). We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for each variable entered into the model.

Results

The average aflatoxin B_1-lysine adduct level in maternal serum was 10.9 pg/mg albumin (range = 0.44–268.73 pg/mg). It was found that 103 (13.6%) infants were small for gestational age, 153 (20.3%) were of low birthweight, 144 (19.1%) were born preterm and 33 (4.4%) were stillborn.

The average age of those interviewed was 26.8 ± 6.3 years (SD) [range: 14–48 years]. Most participants had attended either junior high school (34%) or primary school (28%). Compared to participants who had higher levels of education, senior high school or beyond (15%), those with no formal education (23%) and junior high school education (34%) had higher levels of aflatoxins. The income bracket represented by GHc 20-GHc 35.4 (1.4 GHc = 1 US Dollar) had the most participants with 38.4%. Higher proportions of participants in the lowest income levels ($P = 0.07$) and in the nulliparous group ($P = 0.03$) had the highest aflatoxin levels (quartile 4) (Table 1).

There were 17% of participants positive for malaria parasite antigen while only 26% of them had any type of worm infection. Of the 30% of participants who were anaemic, 26% were moderately anaemic while 4% were severely anaemic. Other demographic characteristics of the study population are presented in Table 1. Bivariate analysis showed an increased odd of delivering a baby who is SGA, low birthweight, preterm or stillborn with aflatoxin levels in the highest quartile (>11.34 pg/mg albumin; Table 2). However, this greater likelihood was statistically significant only with respect to having a low-birthweight baby (OR, 2.00; 95% CI, 1.22–3.28). Multivariable analysis showed that, while there was greater likelihood for all outcomes when aflatoxin levels were in the highest quartile, this association was significant only with low birthweight as the outcome measure (OR, 2.09; 95% CI, 1.19–3.68). Furthermore, when compared to subjects in the lowest quartile, there was a trend of increasing risk for low birthweight ($P_{trend} = 0.007$).

Discussion

We investigated the possible association between birth outcomes and serum levels of aflatoxin B_1 lysine adduct among pregnant women at delivery. Our findings suggest an association between 'very high' levels of aflatoxin exposure and low birthweight.

The average age of participants (27 ± 6.3 years) indicates that the women were in the prime of their reproductive years and is similar to findings by Duda *et al.* (2007) in Accra, Ghana. They found that the average age of first delivery for women in their study was 22 years. Most of our participants (79%) were either having their first or second child.

Pregnant women with aflatoxin levels in the highest quartile were twice as likely to have low birthweight infants (OR, 2.09; 95% CI, 1.19–3.68) as women in the lowest quartile; there was a trend of increasing risk for low birthweight with increasing aflatoxin levels ($P_{trend} = 0.007$). This association remained after adjusting for known confounders, i.e. malaria parasitemia, anaemia and worm infections. This result supports the hypothesis that exposure to aflatoxins may play a role in the incidence of low birthweight. This result is similar to those obtained by Abulu *et al.* (1998) in Edo, Nigeria and Yousef *et al.* (2002, 2004) in the UAE. While our results also corroborate the findings by two other studies conducted in Kenya (De Vries *et al.* 1989) and Sierra Leone (Jonsyn *et al.* 1995), they differ from the latter two because these studies found an association between low birth and aflatoxins only among female births while our study did not find any difference based on gender. This study failed to find an association between STBs and aflatoxins, although serum of mothers who delivered STBs had aflatoxin B_1-lysine adduct levels ranging from 0.68 to 229.49 pg/mg albumin (mean 24.37 pg/mg albumin). It is possible that the small number of events (33 STBs) made it difficult to detect any associations. It is also possible that much higher doses and longer duration of exposure to aflatoxins at critical periods during foetal life are required to produce STBs.

Our findings should be interpreted with caution in view of the fact that it was a cross-sectional study, which limits

Table 1 Demographic characteristics of 755 Ghanaian women by aflatoxin B_1-lysine adduct level

	All		Quartile# 1 (≤2.67 pg/mg albumin)		Quartile 2 (>2.67 to ≤4.97 pg/mg albumin)		Quartile 3 (≥4.97 to ≤11.34 pg/mg albumin)		Quartile 4 (>11.34 pg/mg albumin)		
Variables	No.	%	No.	%	No.	%	No.	%	No.	%	*P*-value
Age years											
<20	103	13.6	25	13.3	23	12.2	26	13.8	29	15.3	0.96
20–24	189	25.0	47	25.0	49	25.9	45	23.8	48	25.4	
25–29	218	28.9	61	32.5	53	28.0	55	29.1	49	25.9	
≥30	245	32.5	55	29.3	64	33.9	63	33.3	63	33.3	
Formal education											
None	170	22.7	42	22.7	39	20.6	31	16.6	58	30.7	0.12
Primary or middle school	212	28.3	52	28.1	58	30.7	55	29.4	47	24.9	
Junior high school	253	33.7	63	34.1	60	31.8	67	35.8	63	33.3	
≥Senior high school	115	15.3	28	15.1	32	16.9	34	18.2	21	11.1	
Weekly income (GHc)†											
<10	205	27.4	43	23.5	44	23.3	50	26.6	68	36.2	0.07
10–19.9	55	7.4	14	7.7	13	6.9	10	18.2	18	9.6	
20–35.4	287	38.4	72	39.3	82	43.4	73	38.2	60	31.9	
≥35.5	201	26.9	54	29.5	50	26.5	55	27.4	42	22.3	
Marital status											
Single	159	21.2	43	23.4	33	17.5	36	19.2	47	24.9	0.25
Living in union	142	18.9	31	16.9	38	20.1	31	16.5	42	22.2	
Married	449	59.9	110	59.8	118	62.4	121	64.4	100	52.9	
Employment											
Unemployed	225	30.0	58	31.4	54	28.6	57	30.3	56	29.6	0.96
Self-employed	478	63.4	118	63.8	123	65.1	118	62.8	119	62.9	
Employed	48	6.4	9	4.9	12	6.4	13	6.9	14	7.4	
No. of Children											
0	268	36.7	63	34.8	63	35.2	61	32.8	81	44.0	0.03*
1	306	41.9	91	50.3	73	40.8	82	44.1	60	32.6	
2	124	17.0	24	13.3	33	18.4	35	18.8	32	17.4	
3	32	4.4	3	1.7	10	5.6	8	4.3	11	5.9	
Ethnic group											
Akan	518	68.6	131	69.7	132	69.8	126	66.7	129	68.3	0.90
Others	237	31.4	57	30.3	57	30.2	63	33.3	60	31.8	

Note: # = Alflatoxin B_1-lysine adduct levels categorized into quartiles
* = Statistically significant. Mean age ± SD = 27 ± 6.3;
† = 1.4 Ghana cedi (GHc) is equivalent to 1 US Dollar.

the ability to draw causal or temporal associations. However, the study does at least provide a framework to theorize on relationships between the variables investigated. Secondly, we used maternal serum collected at delivery to assess aflatoxin exposure levels, while other investigators have used cord blood or even maternal breast milk to assess the exposure of the foetus to the effects of aflatoxins in-utero. Nevertheless, our results are comparable and these findings may be one of the stepping stones towards convening a study on the relationship between birth outcomes and aflatoxins using more rigorous study designs. Further research may consider longitudinal studies that investigate the impact of these aflatoxins on the incidence of miscarriages and possibly congenital birth defects. The biological mechanisms and pathways also need to be elucidated. A further limitation of the study is that by measuring the aflatoxin B_1-lysine adduct levels at only one point in time, it does not reflect the concentration at other times during the 9 months of pregnancy when crops may be eaten fresh. Eating food stuffs when freshly harvested may avoid the growth of moulds. However, because the AFB_1 levels measured reflect the blood levels of the toxin over a period of 2–3 months, it still gives us an idea of the exposure of these participants to aflatoxins. Our relatively large sample size was a strong feature of this study, because it may have increased the ability to detect some associations

Table 2 Odds ratios and confidence intervals for association between birth outcomes and aflatoxin B_1-lysine adduct levels in Kumasi, Ghana

	SGA (*n* = 103)		LBW (*n* = 153)		PTD (*n* = 144)		STB (*n* = 33)	
Variable	Crude OR (95% CI)	Adjusted OR (95% CI)$^{\alpha}$	Crude OR (95% CI)	Adjusted OR (95% CI)$^{\alpha}$	Crude OR (95% CI)	Adjusted OR (95% CI)$^{\alpha}$	Crude OR (95% CI)	Adjusted OR (95% CI)$^{\alpha}$
Aflatoxin levels								
Low	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**
Moderate	0.92 (0.50–1.68)	1.05 (0.55–1.99)	1.07 (0.63–1.82)	1.34 (0.74–2.45)	0.92 (0.53–1.59)	1.07 (0.59–1.93)	0.61 (0.19–1.90)	0.64 (0.20–2.03)
High	0.79 (0.43–1.49)	0.85 (0.44–1.65)	0.99 (0.58–1.70)	1.25 (0.69–2.28)	1.31 (0.78–2.19)	1.51 (0.87–2.62)	0.99 (0.37–2.71)	0.92 (0.34–2.54)
Very high	1.40 (0.79–2.47)	1.23 (0.67–2.27)	**2.00 (1.22–3.28)**	**2.09 (1.19–3.68)**	1.39 (0.84–2.32)	1.30 (0.75–2.27)	1.53 (0.61–3.82)	1.35 (0.52–3.50)

Note:
Numbers in bold are significant.
'Low' aflatoxin B_1-lysine adduct level: ≤2.67 pg/mg albumin;
'Moderate' aflatoxin B_1-lysine adduct level: >2.67 to ≤4.97 pg/mg albumin;
'High' aflatoxin B_1-lysine adduct level: >4.97 to ≤11.34 pg/mg albumin; 'Very high' aflatoxin B_1-lysine adduct level: >11.34 pg/mg albumin
OR, Odds ratio;
CI, Confidence interval;
α, adjusted for baby's gender, maternal income level, No. of children, maternal educational level, anaemia status, malarial status, and worm infection;
LBW, Low birthweight;
PTD, Preterm delivery;
SGA, Small for gestational age;
STB, Stillbirth.

that may have been missed by similar studies which generally had smaller sample sizes (De Vries *et al.* 1989; Jonsyn *et al.* 1995; Yousef *et al.* 2004).

Because our sample participants were recruited from a secondary medical facility and a tertiary (teaching) hospital, which attend to women from different backgrounds and communities, this study can be generalized to the population of Ghanaian women in Kumasi and surrounding areas, and possibly other women in the West African sub-region with similar food and dietary conditions.

In conclusion, the results of this study add to the growing body of evidence that show an association between low birthweight and aflatoxin exposure. Additional research is needed to document the mechanism responsible for this association. While no association was found with small for gestational age, preterm and STB deliveries, our findings have practical policy implications in terms of the need for policy makers in developing countries to put in place well researched and documented methods to reduce the exposure of their populace to aflatoxins. These measures may help in the bid for these nations to achieve MDG goal four of reducing child morbidity and mortality, because low birthweight predisposes these infants to poor growth and development and adverse health outcomes.

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