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Association between birth outcomes and aflatoxin B1-Biomarker levels among pregnant women in Kumasi, Ghana

Faisal Mohammed Barbas Shuaib
University of Alabama at Birmingham

THE ASSOCIATION BETWEEN AFLATOXIN B_1 BIOMARKER LEVELS IN PREGNANT WOMEN AND BIRTH OUTCOMES IN KUMASI, GHANA

By

FAISAL M.B. SHUAIB

PAULINE E. JOLLY, CHAIR

JOHN E. EHIRI

CRAIG WILSON

ELLEN FUNKHOUSER

SHARINA D. PERSON

WILLIAM O. ELLIS

A DISSERTATION

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ABSTRACT

Aflatoxins are fungal metabolites that contaminate staple food crops in many developing countries. Although studies have linked these toxins to adverse health outcomes, few studies have investigated the association between birth outcomes and aflatoxin B_1-lysine adduct levels among pregnant women. The objective of this dissertation was to investigate the association between birth outcomes and aflatoxin B_1-lysine adduct levels among pregnant women in Kumasi, Ghana.

In a cross-sectional study of 755 pregnant women, Aflatoxin B_1-lysine adduct (AF-ALB) levels were determined by High Performance Liquid Chromatography. AF-ALB associations were assessed as both continuous and categorical values. Analysis of variance (ANOVA) was used on log transformed AF-ALB values for bivariate and adjusted analyses. Participants were also divided into quartiles “low”, “moderate”, “high”, and “very high”. Logistic regression was used to examine the association between maternal anemia, birth outcomes and AF-ALB.

From the predictors study, the mean AF-ALB in maternal serum was 10.9 ± 19.00 pg/mg albumin (range=0.44-268.73 pg/mg/ albumin) while the median was 5.0 pg/mg albumin. Participants with weekly income levels above 20 Ghana cedis (GHc) had reduced odds of having high aflatoxin levels (OR, 0.67; 95% CI, 0.50-0.90). Similarly,

participants who were employed (OR, 0.58; 95% CI, 0.40-0.83), had a flush toilet system (OR, 0.56; 95% CI, 0.41-0.79) or had only one child (OR, 0.68; 95% CI, 0.48-0.94) had reduced odds of having high aflatoxin levels.

The second study found that 30.3% of participants were anemic. The odds of being anemic increased 21% (OR, 1.21, p=0.01) with each quartile of AF-ALB reaching an 85% increased odds in the “very high” compared to the “low” category (OR, 1.85; CI, 1.16-2.95). This association was stronger among women with malaria and findings were robust when women with evidence of iron deficiency anemia were excluded.

The study investigating the association between birth outcomes and AF-ALB found that participants in the highest AFB_1-lysine quartile with ‘very high’ AFB1-lysine level (>11.34 pg／mg) were more likely to have low birth weight babies (OR, 2.09; 95% CI, 1.19–3.68), and showed a trend of increasing risk for low birth weight (P_{trend} = 0.007) compared to participants in the lowest quartile.

Key words: Aflatoxins, Pregnancy, birth outcomes, Kumasi, Ghana.

DEDICATION

To *Nira*, my "*rabin-rai"* with all my love

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LITERATURE REVIEW

Introduction

Although it is hypothesized that aflatoxins have adverse effects on birth outcomes,[1] there has been no critical summary of the literature on the subject. Reproductive health addresses the reproductive processes, functions and systems at all stages of life.[2] Central to reproductive health is "the right of access to appropriate health care services that will enable women to go safely through pregnancy and childbirth and provide couples with the best chance of having a *healthy infant*."[2] Globally, one useful indicator of reproductive health is birth weight. It is estimated that more than 20 million infants worldwide, representing 15.5 per cent of all births, are born with low birth weight with 95.6 per cent occurring in developing countries.[3] A baby's low weight at birth (defined as birth weight less than 2500g) is either the result of preterm birth (before 37 weeks of gestation) or due to restricted fetal (intrauterine) growth. Low birth weight is closely associated with fetal and neonatal morbidity and mortality, inhibited growth, poor cognitive development, and chronic diseases later in life.[3]

More than 5 billion people, mostly in developing countries, are at risk of chronic exposure to aflatoxins from contaminated food crops.[4] Aflatoxins are a family of toxic metabolites which are produced by the fungi *Aspergillus parasiticus* and *Aspergillus flavus*.[5] These fungi are ubiquitous in hot and humid environments where mean temperatures are about 27 degrees centigrade, and relative humidity ranges between 80% and 90%.[6] In these settings, Aflatoxins are naturally occurring contaminants of staple

foods such as cereals, groundnuts and other oil seeds. Aflatoxins are classified based on immune-florescent properties. Accordingly, they are classified as blue (B) or Green (G). Of the main aflatoxins B_1 B_2 G_1 and G_2, aflatoxin B_1 is the most potent and carcinogenic.[7] Aflatoxin M_1 is a metabolite of aflatoxin B_1 that can be found in breast milk and urine. Aflatoxins can be measured by a host of methods: Thin Layer Chromatography (TLC) was one of the earliest methods used to detect aflatoxins. Recently, more sensitive and less cumbersome techniques have evolved: the commonest are, High Performance Liquid Chromatography (HPLC), Enzyme Linked Immuno-absorbent Assay (ELISA), and the use of Immuno-affinity columns (IAC).[8-10] Aflatoxin contamination is influenced by high humidity, high temperatures, insect and rodent activity and inadequate drying of crops. This contamination can occur in any stage of food production, from pre-harvest to storage.[8]

In most developing countries, women are involved in subsistence agriculture. They are actively involved in the full range of farming practices from planting, weeding, pest control, to harvesting and storage. They invariably also end up cooking the meals. Consequently, they are primarily exposed to the health hazards of aflatoxins as they may ingest these toxins in high quantities with food in its raw state or during food preparation. Studies have shown that a widely eaten staple food in West African countries such as Ghana; "Kenkey" typically contains large amounts of aflatoxin producing *Aspergillus* even after fermentation.[11] Pica, (an eating disorder among pregnant women which involves the ingestion of non-nutritive substances such as raw maize, soil, gum, ash and other substances that may be contaminated with *Aspergillus* moulds) is another source of increased aflatoxin consumption.[12, 13]

While a host of studies on aflatoxins have associated them with hepatic cell carcinoma, malnutrition, impaired growth[14] and immune suppression,[15] 25 studies have been conducted to show their relationship with reproductive health outcomes.

Cheap and environmentally sustainable methods that can be applied pre or post-harvest to reduce the contamination of aflatoxins are available. These methods include proper irrigation, choice of genetically resistant crop strains and biopesticide management which involves using a non-aflatoxigenic strain of *Aspergillus* that competitively excludes toxic strains.[16, 17] Other methods include sorting and disposal of visibly moldy or damaged seeds, reducing the bioavailability of aflatoxins using clay such as novaSil[18, 19] and chemo-protection with Oltipraz, chlorophylin or vegetables such as broccoli.[20]

Almost a decade after the MDG (Millennium Development Goals) declaration, there has largely been no change in maternal mortality rate and child mortality rates barely decreased by 27%.[21] The continued disparity of health outcomes between nations despite huge investments calls for research on how exposures to toxins like aflatoxins, with their well known health effects, may be contributing to poor health and hampering the attainment of these noble goals.

This review represents an attempt to critically assess and summarize the literature on reproductive health effects of exposure to aflatoxins. It is hoped that the resulting information may be valuable in planning mitigation strategies as part of an overall strategy to promote maternal and child health in resource-poor tropical and sub-tropical countries where aflatoxin contaminated foods abound.

Methods

We searched online databases, scanned reference lists and hand-searched journals for potentially eligible studies. Specifically, we searched PubMed, OVID, MEDLINE, LILACS, EMBASE, Combined Health Information Database (CHID), National Research Register, PsychINFO, ERIC, Science and Social Science Citation Index, Dissertation Abstracts, Online Computer Library Centre (OCLC) and other bibliographic databases. The search covered articles published in English before May 2009. We restricted our retained studies to those with outcomes relating to reproductive health outcomes as previously defined and aflatoxins in all its naturally existing forms. Among the terms and concepts searched are: aflatoxins and reproduction, aflatoxins and birth, aflatoxins and health, aflatoxins and low birth weight, aflatoxins and newborns, aflatoxins and pregnancy, aflatoxins and preterm babies, aflatoxins and stillbirths, aflatoxins and small for gestational age, aflatoxins and infertility, aflatoxins and reproductive organs. To identify studies published in the "gray" literature, we systematically reviewed the bibliographies of all relevant publications, searched the System for Information on Gray Literature in Europe database (SIGLE), the Grey Literature Database of the New York Academy of Medicine, and Grey Literature Network Service (GreyNet) which covers information produced on all levels of government, academia, business and industry in electronic and print formats not controlled by commercial publishing. We also explored online resources (Google and Google Scholar) extensively.

The authors screened titles and abstracts to assess their eligibility for inclusion in the review. Hard copies of studies that were potentially relevant were retrieved for further assessment. The search yielded 121 potential studies. Of these, 25 studies involving 4,942

participants qualified for inclusion and were retained for the review. Most studies evaluated were cross-sectional in nature and of varying quality. Some studies did not document how adjustment for known confounders was conducted while others did not provide effect estimates to quantify relationships between outcomes and independent variables.

Results

Overall, after excluding duplicate studies, we retained 25 studies. In Table 1, we summarize the findings of studies on the effect of aflatoxins on fertility and birth outcomes. In Table 2, we summarize findings relating to the contamination of body fluids by aflatoxins. The tolerable limit of aflatoxin M_1 in breast milk accepted by the European Union and the USA is 25ng/L while the limit accepted by Australia and Switzerland is 10ng/L.[22]

Aflatoxins and fertility

One study examined the possible effect of aflatoxins on fertility. The relationship between aflatoxin levels in serum of infertile men was compared to controls.[23] The investigators found that semen from 40% of infertile men had aflatoxins compared to 8% of semen from fertile men. The concentrations of aflatoxins detected in the semen were consistently higher among infertile compared to the fertile men. Fifty percent of the infertile men with high aflatoxin semen levels also showed abnormalities (sperm count, morphology and motility) of their spermatozoa on semen analysis. On the contrary, 10-15% of the fertile men showed comparative abnormalities of spermatozoa. In the same study, a parallel experiment was conducted in which adult male rats were given aflatoxin contaminated food. Analysis of their semen showed that rats exposed to dietary aflatoxin

(cases) showed changes in their semen which were significantly different from that of the control group ($P<0.01$). The changes were similar to those observed among human semen containing aflatoxins.

Aflatoxins and birth outcomes

All studies were cross-sectional in design. We found twelve (12) studies [1, 22-32] that dealt with the effects of aflatoxin contamination of body fluids on birth outcomes. While seven studies [1, 24, 26, 27, 29, 31, 32] reported on relationship between aflatoxins and birth weights, others reported a mixture of other findings such as birth height,[22] gestational age,[30] and jaundice,[24, 25, 28, 31] Among these, two studies examined the relationship between aflatoxins in maternal blood and cord blood.[26, 31] While one study found no relationship,[26] the other study[31] was not clear about the outcome of this assessment. It is noteworthy that various studies used different body fluids (such as umbilical cord, maternal serum, and breast milk) to measure aflatoxin metabolites such as aflatoxin B_1, M_1 and their association with birth outcomes. Consequently, the comparability of these outcomes must be interpreted with caution.

There was no consensus on findings regarding the relationship between aflatoxins and birth weight. While four studies[1, 24, 29, 31] reported a negative correlation between birth weight and aflatoxin levels (with P values ranging from <0.001 to <0.05), two studies found this relationship only when the sex was female ($p<0.5$).[26, 32] One study conducted in Ibadan Nigeria did not find any correlation between the presence of aflatoxins and birth weight.[27] Similarly, De Vries et al., did not find any correlation between aflatoxins in maternal blood and cord blood.[26] Two studies reported the occurrence of stillbirths among mothers who had significantly high levels of maternal serum aflatoxins[33] or both

maternal and neonatal serum aflatoxin.[26] One study by Sedeghi et al., in Iran found an association between aflatoxin M_1 concentration in breast milk and height of the infant at birth ($P<0.01$).[22] Yousef et al.,[30] did not find any significant correlation between aflatoxin M_1 and gestational age, postnatal age, gender or clinical condition.

Four studies [24, 25, 28, 31] reported findings relating aflatoxins and jaundice among newborns. Only one found that the serum levels of aflatoxin in the infant is a risk factor for neonatal jaundice, (OR, 2.68; CI, 1.18-6.10).[28] Of the two studies that did not find any statistically significant correlation between aflatoxins and jaundice, one used serum from the neonate[25] while the other used cord blood.[31] The fourth study reported that aflatoxins were associated with jaundice in low birth weight babies but did not state whether any association exists between aflatoxins and jaundice among babies of normal weight.[24] It is noteworthy that the aflatoxin levels in body fluids vary by season as was demonstrated by three studies that noted that the frequency of detection of aflatoxins was higher during the wet than the dry season.[24, 26, 33]

Aflatoxins and contamination of body fluids

Body fluids which were found to be contaminated by aflatoxins include maternal breast milk, cord blood, and maternal blood.

Eleven (11) studies demonstrated the presence of aflatoxins in breast milk.[32, 34-43] These were all cross-sectional studies. Fresh breast milk was obtained from puerperal women for the studies except in one study, where the breast milk was obtained from a milk bank in Sao Paulo, Brazil.[37] Three cross-sectional studies investigated aflatoxins in maternal blood, and cord blood. [33, 44, 45] Of these three, one also examined aflatoxin contamination of breast milk.[33] Methods for detection of aflatoxins varied between studies. Six studies

used High Performance Liquid Chromatography (HPLC) while others used Enzyme Linked Immuno Absorbent assay (ELISA). These have different detection limits but the results from both methods are generally comparable.[46] On the whole, there were significant differences in contamination of breast milk between studies that were conducted in developing countries and those conducted in developed countries. While breast milk samples from three studies conducted in developed countries had contamination rates ranging from zero percent in France to five percent in Italy (mean concentration 55.35 ng/L), 34%-99.5% of those from developing countries were contaminated with aflatoxins.[33, 40] The mean concentrations of aflatoxins found in these samples ranged from 130ng/l-8218ng/L in Accra, Ghana, to 2 pg/ml-3ng/mL in Abu Dhabi, UAE. In many cases, several types of aflatoxins and other mycotoxins were found to contaminate milk. The predominant mycotoxins detected were aflatoxin M_1, M_2, B_1 and ochratoxin A. Two studies investigated the presence of aflatoxins in maternal blood. One study conducted in Songkhla, Thailand,[45] found aflatoxins in two of 35 (6%) maternal sera (mean concentration of 0.62nmol/mL), while the other in Jos, Nigeria, found aflatoxins in 21% of 77 maternal samples (range 33-10,390 ng/L).[33] Cord blood examined in the aforementioned Thai study contained between 0.074-13.6 nmol/mL of aflatoxins with a mean of 3.1nmol/L. In two other studies, the presence of aflatoxins in cord blood was demonstrated: Hsieh and Hsieh[44] working in an area of high liver cancer risk (Taipei Chang Gung, Taiwan) found that 57% of 120 placenta samples contained AFB-DNA adducts in the range of 0.6-6.3 μmol/mol DNA. In the same study, 8.9% of 56 cord blood samples contained AFB_1-DNA adducts (range 1.4-2.7 μmol/mol DNA). In the second study, Lamplugh et al.[33] who studied samples from Ghana and Nigeria found that

34% (63 out of 188) of the Ghanaian cord blood specimens contained aflatoxins, while 12% (9 out of 78) of the Nigerian cord blood samples contained aflatoxins.

Discussion

This systematic review of the reproductive health effects of aflatoxins indicates that a significant proportion of people living in low income countries are exposed to environmental and food- borne toxins which may compromise reproductive health. While the study on aflatoxin levels among infertile men is interesting, details were not provided of how the selection of cases and controls were made to avoid bias. Nevertheless, animal studies suggest that aflatoxins are spermatotoxic[47-49] therefore, it is reasonable to theorize on the possible link between sperm cell dysgenesis and aflatoxins in humans. Numerous mechanisms for these effects have been postulated. The toxic effects of aflatoxins on the liver may inhibit enzyme synthesis, fatty acid metabolism and production of sex hormones precursor molecules[23] It has also been suggested that aflatoxins cause a direct lysis of membrane of sperm cells, which results in the loss of lysozyme, an enzyme which facilitates the penetration of the ova by spermatozoa.[23, 50] There is further evidence that suggests that aflatoxins may cause DNA damage and mutations.[51, 52] The pathway for this toxicity is thought to involve epoxide intermediates which bind DNA and RNA. The resulting metabolites interfere with DNA-dependent RNA polymerase, thereby inhibiting RNA and protein synthesis.[53] This could lead to interference with spermatogenesis, maturation of spermatozoa and consequently result in abnormal sperm cells. Further research in this area is necessary to assess these relationships.

We found only five studies that have been conducted on the relationship between birth weight and aflatoxins and all of these were of cross-sectional design with limitations with respect to causality. Though four of the five investigators found a correlation or association between aflatoxins and low birth weight, the results must be interpreted with caution in view of the obvious paucity of studies and issues with the quality of the studies. It is noteworthy that some studies did not adjust for other possible causes of low birth weight such as malaria, and other infectious diseases. At best, findings from these studies ought to lay the groundwork for further studies with more rigorous study designs. By the same token, the inconclusive evidence on the association between aflatoxins and neonatal jaundice would benefit from research which employs more rigorous study designs. However, these studies should also be reviewed in the light of a growing body of evidence that indicate that conditions in-utero set the stage for how the offspring develop throughout their lifetime.[54] Furthermore, only two studies have commented on the possible link between birth outcomes such as stillbirths and aflatoxins. These two studies had too few events and no statistical analysis was conducted to draw any conclusions on the association between aflatoxins and stillbirths.[26, 33] One study found no relationship with gestational and post natal age, but the equivocal pattern of outcomes seen in other studies suggest that further work needs to be done to clarify these relationships.

Table 1 indicates that apart from the highly industrialized countries that have taken steps to curb the contamination of food-stuff by aflatoxins, these mycotoxins are widely consumed in developing countries in amounts that exceed the maximum allowable limits (10ng/ml-25ng/ml) by several factors. The discovery of aflatoxins in maternal blood and cord blood further attests to the ubiquitous nature of these toxins. Selected studies

indicate a pathway that may be characterized by ingestion of food contaminated by aflatoxins, absorption into the systemic circulation via the digestive system and sequestration in the mammary glands and placenta, so that these aflatoxins become evident in breast milk and cord blood respectively.[39, 40] The disparity in the concentrations of aflatoxins between maternal serum and cord blood has been shown by various authors. Denning et al. showed higher levels of aflatoxins in cord blood compared to maternal sera, which indicates not only the transfer of the toxins but also their concentration by the feto-placental unit.[45] This may be the pathway for deleterious health effects such as low birth weight and stillbirth, which has been reported by other investigators.

While the importance of breast milk for the nutrition, and indeed survival, of the infant in developing countries cannot be overemphasized, its potential for negative health outcomes is indicated by these studies. In recent times, UNICEF and other international organizations have been in the fore-front of campaigns to promote exclusive breast feeding. In rural settings, where infant formula are not available, breast feeding for not only 6 months but almost 24 months is the norm.[55] Given the well known effects of aflatoxins in causing immune suppression, chronic liver disease and malnutrition,[4] it is perhaps not surprising that children in these areas are often caught up in the vicious web of illness, poor education and poverty. The reproductive effects that result from growth retardation are well documented.[56]

The finding that aflatoxin levels are higher during the wet season is not surprising since this is when crops that have been stored for long periods under hot and humid conditions (with increased potential for contamination by the aflatoxin producing fungi) are eaten.

This is the pattern that has been documented in other studies conducted in West Africa.[57] However, it may also indicate that freshly harvested crops are contaminated very rapidly. The seasonality (i.e. wet vs. dry season) in the contamination rate of food stuff presents a window of opportunity for policy makers and program managers, to plan interventions that will decrease exposure to these toxins during periods when communities are most at risk. Such interventions may benefit the most vulnerable population of women and children and thus contribute towards achieving millennium development goals 4 and 5 which are targeted at reducing maternal and child morbidity and mortality.

The studies reviewed were limited by the fact that they were cross-sectional in design. These have their obvious drawback of being unable to link causality to observed associations. Most of the studies were conducted in developing countries where the equipment and personnel to conduct experiments requiring highly skilled expertise may be in short supply. As afore-mentioned, there were also variations in measurement methods of the aflatoxins. Thus, it is difficult to comment on the accuracy of results obtained from some of the measurements on aflatoxins. Although some studies adjusted for social class, a well known confounder of birth weight, it is possible that residual confounding due to measurement error persisted.

In sum, we found that few studies have been conducted to investigate the relationship between reproductive health and aflatoxins. The available studies have largely focused on birth outcomes such as low birth weight and contamination of breast milk by aflatoxins. Even so, the lack of rigorous study designs limits the drawing of conclusions about causality. Our findings show a higher rate of contamination of breast milk in developing countries by aflatoxins, at levels beyond the acceptable limits. Although the reviewed

studies were unable to draw definitive conclusions about the effects of aflatoxins on reproductive health, the high contamination rate of breast milk by aflatoxins and the known adverse effects of aflatoxins on other organ systems require stakeholders in affected countries, to take urgent steps to reduce exposure of vulnerable populations to these toxins.

Table 1: Summary of findings on aflatoxins, infertility and birth outcomes

Study ID	Objective	Design	Population	Outcomes	Results	Remarks
Ibeh et al. 1994 Benin city, Nigeria[22]	To discover the relationship between aflatoxin levels in serum of infertile men compared to controls.	Cross sectional	50 infertile men and 50 normal individuals from the same community on the same staple diet.	Mean aflatoxin concentration of semen.	40% of semen from infertile men had aflatoxins. 50% of spermatozoa were abnormal. Eight percent of semen from fertile individuals had aflatoxins. 10%-15% were abnormal in the fertile men. 1.660±0.04 micrograms/mL (infertile men) and 1.041±0.01 micrograms/mL (fertile men)	-
Turner et al. 2007 West Kiang region, Gambia[1]	To investigate the effect of in-utero aflatoxin exposure on birth weight and infant growth.	Cross-sectional	138 singleton infants	Aflatoxin in maternal blood, cord blood, infant blood, birth weight and height gain in the first year of life	Aflatoxin-albumin in maternal blood predicts birth weight and height gain in the first year of life. P=0.012 for birth weight and P=0.044 for height gain	Maternal AF-alb level was significantly higher in blood samples collected in December–March, than in April–July or in August–November (P<0.001)
Maxwell et al. 1994 Ibadan, Nigeria[26]	To determine the extent of fetal exposure to aflatoxins and naphtols and influence on birth weight	Cross-sectional	625 babies	Aflatoxins in serum	14.6% of serum samples were contaminated with aflatoxins. No correlation between the presence of either compound and birth weight.	
Yousef et al. 2002 United Arab Emirates[28]	To determine whether fetuses had been significantly exposed to aflatoxins	Cross-sectional	201 women	Umbilical cord blood levels of aflatoxins.	Aflatoxins were detected in 54.7% of samples. Negative correlation between birth weight and levels of aflatoxins (r=-0.63).	P<001
Yousef et al. 2004 United Arab Emirates[30]	To assess whether aflatoxin M_1 concentrations in newborn infants correlated with those of their mothers and to determine whether the presence of aflatoxin M_1 in	Cross-sectional	250 samples taken from women admitted to labor wards.	M_1 in maternal and umbilical cord blood	There was a strong correlation between aflatoxin levels and birth weight (r=-0.565), p<0.001) but there was no association between aflatoxin M_1 concentration in maternal or cord blood and rates of jaundice or infection.	

	cord blood was associated with an increase in morbidity in the newborn					
Yousef et al. 2003 United Arab Emirates[29]	To determine whether breast-milk of mothers from UAE contained aflatoxins and if there was any correlation with gestational age.	Cross-sectional	140 lactating mothers	Aflatoxin M_1 in breast milk	No significant correlation between aflatoxin M_1 and gestational age, postnatal age, gender, and clinical condition.	
Sedeghi et al., 2009 Tehran, Iran[21]	Exposure of infants to aflatoxin M_1 and of lactating mothers to aflatoxin B_1, using AFM_1 in breast milk as a biomarker for exposure to AFB_1	Cross-sectional	Breast milk sample from 160 women	AFM_1 concentration in milk	AFM_1 detection in 157 samples (98.1%) average concentration=8.2±5.1ng/kg. Range 0.3-26.7ng/kg.	Significant association between AFM_1 concentration and height at birth ($P<0.01$). Direction of association unclear
Abulu et al. 1998 Edo, Nigeria[23]	To investigate the presence of aflatoxins in cord blood.	Cross-sectional	164 neonates	Aflatoxin in cord blood and neonatal jaundice.	Neonates with jaundice have a high mean concentration of aflatoxin B_1. There was significant reduction in birth weight ($P<0.05$) of jaundiced neonates with aflatoxin.	Rate of detection was higher in wet (81.8%) than dry season (50.0%)

Ahmed et al. 1995 Zaria, Nigeria[24]	To determine the relationship between perinatal aflatoxin exposure and neonatal jaundice.	Prospective study	77 neonates	Aflatoxins in cord and peripheral blood and neonatal jaundice	Aflatoxins in cord blood of 37.8% of jaundiced neonates and in 22.5% of controls. Mean cord aflatoxins concentration was highest in jaundiced neonates with septicemia but difference not stat. significant. No statistically sig. difference between aflatoxin in peripheral blood of jaundiced and non-jaundiced babies. No correlation between severity of hyperbilirubinemia and serum aflatoxin levels	
De Vries et al. 1989 Embu district, Kenya[25]	To determine foetal and neonatal exposure to aflatoxins	Cross-sectional	125 primigravidae	Aflatoxins in maternal and cord blood	53% of maternal blood contained aflatoxins. 37% of 101 cord blood contained aflatoxins. There was no relationship between aflatoxins in maternal and cord blood. The mean birth weights of females born to aflatoxin positive mothers were significantly lower than those of aflatoxin free mothers.	Two stillbirths were recorded in cases with aflatoxins in both maternal and cord blood. The frequency of detection was significantly higher in maternal and cord blood during the 'wet' rainy season than 'dry' months.
Sodeinde et al. 1995 Ibadan, Nigeria[27]	To investigate the prevalence of naphthols and aflatoxins in the sera of babies with neonatal jaundice and their mothers in order to determine whether they contribute to the occurrence of unexplained neonatal	Cross-sectional	327 jaundiced neonates and 80 of their mothers, and 60 non-jaundiced controls and seven of their mothers	Aflatoxins in blood.	Aflatoxins were detected in 27.4% of jaundiced neonates, 17% of their mothers, 16.6% of controls and 14.4% of control mothers. serum aflatoxin is a risk factor for neonatal jaundice, OR=2.68 (CI: 1.18-6.10),	

	jaundice in Ibadan.					
Jonsyn et al. 1995 Southern province, Sierra Leone.[31]	To examine breast milk for mycotoxin content.	Cross-sectional	113 mothers in two under five clinics.	Aflatoxins, ochratoxins A and other mycotoxins in breast milk.	Eighty-eight percent contained various aflatoxins and 35% contained ochratoxin A; 15% had a single mycotoxin; 32% had two mycotoxins and 40% had three or more.	Girl infants exposed to OTA and aflatoxins have lower birth weights (P<0.05)

Table 2: Summary of findings on contamination of breast milk and body fluids by aflatoxins

Study ID	Objective	Design	Population	Outcomes	Results	Remarks
Turconi et al. 2004 Lombardy, Italy [40]	To assess the presence of aflatoxins, ochratoxin A, lead and cadmium in human milk.	Cross sectional	231 puerperal women.	Aflatoxin and ochratoxin A levels in breast milk	Aflatoxin B_1 (11.4ng/l) and aflatoxin M_1 (194ng/l) were found in one sample, while ochratoxin A (6.01± 8.31ng/l) was detected in 198 (85.7%) samples.	Study shows mycotoxins are present in maternal milk
Denning et al. 1990 Songkhla, Thailand[44]	To determine aflatoxin levels in human cord sera at birth and maternal serum immediately after birth.	Cross-sectional	35 mothers and their babies	Aflatoxin (AFB_1, AFG_1 and AFQ_1) levels in human cord sera at birth and maternal serum immediately after birth.	17 out of 35 cord sera (48%) contained aflatoxin, mean 3.1nmol/ml (range 0.064-13.6nmol/ml). By comparison, only two (6%) of maternal sera contained aflatoxin (mean 0.62 nmol/ml)	Results demonstrate transplacental transfer and concentration of aflatoxin by the feto-placental unit.
Polychronaki et al. 2006 Qalyubiyah, Egypt[38]	To assess the level and frequency of breast milk aflatoxin M_1 as a biomarker of maternal exposure to aflatoxins.	Cross-sectional	388 lactating women	AFM_1 levels in breast milk	36% of the 388 mothers tested positive for AFM_1 (median 13.5pg/ml)	AF contamination of breast milk is frequent, albeit at moderate levels.
Galvano et al. 2008 various sites, Italy[35]	To determine contamination of human milk by aflatoxins and ochratoxin A.	Cross-sectional	82 samples of human mature milk form various Italian hospitals.	Aflatoxin M_1 and ochratoxin A in breast milk.	AFM_1 detected in 4 (5%) of milk samples (mean level: 55.35 ng/L). OTA was detected in 61 (74%) of milk samples (mean level: 30.43 ng/L). OTA levels significantly higher in people who consume lots of bread, bakery products and cured pork meat.	Findings support possibility of dietary recommendations to women during pregnancy, aimed at reducing the OTA in milk.
Hsieh and Hsieh 1993 Taipei Chang Gung, Taiwan[43]	To investígate metabolism, bioactivation and transplancental transfer of procarcinogens through human placental and cord blood.	Cross-sectional	120 placentas and 56 cord bloods from term, uncomplicated pregnancies.	Alflatoxins in placenta and cord blood.	Of the 120 samples of placentas, 57% contained AFB_1-DNA adducts of range 0.6-6.3 µmol/mol DNA. Of the 56 samples of cord bloods, 5 (8.9%) contained AFB_1-DNA adducts range 1.4-2.7	Results indicate a significant number of individuals in an area of high liver cancer risk have been exposed to AFB_1, through the transplacental unit.

					µmol/mol DNA.	
Lamplugh et al. 1988 Accra, Ghana and Jos, Nigeria.[32]	To confirm the presence of aflatoxins in human breast milk and if they cross the human placental membrane.	Cross-sectional	*In Ghana*: 264 breast milk and 188 cord blood samples. *In Nigeria*: venous blood from 77 pregnant women and cord blood samples from their infants after delivery.	Aflatoxins in breast milk, cord blood and venous blood samples.	In Ghana: 90 (34%) of the 264 milk samples contained AF. Aflatoxins were detected in 63 (34%) of the cord blood specimens. In Nigeria: Blood samples showed AF in 16 (21%) of 77 maternal samples and 9 (12%) of 78 cord blood samples. AF were found in maternal and cord blood in 7 instances.	Frequency of detection of AF was more in the wet season than the dry season. The mean concentration was also higher during the wet "rainy" season. One stillbirth was recorded in the study (maternal blood contained aflatoxin B_1 553ng/l)
Coulter et al. 1984 Sudan[33]	To determine the occurrence of aflatoxins in breast milk, maternal serum and the blood and urine of their infants	Cross-sectional	Breast milk from 99 Sudanese mothers. 80 children	Aflatoxin in breast milk, blood and urine.	Aflatoxin M_1 and/or M_2 were detected in 37 of the milk samples. M_1 occurred in 13 of the milk samples (mean 19.0 pg/ml), while M_2 was detected in 11 of the milk samples (mean 12.2pg/ml). Aflatoxin was detected in the blood of three children while only urine of two children contained aflatoxin.	There appears to be a linear relationship between M_1 and M_2 where both were excreted. No correlation with the presence of aflatoxin in mothers' blood or the infant's blood and urine.
Saad and Moss 1995 Abu Dhabi, UAE[39]	To determine the occurence of AFM_1 in donated breast milk	Cross-sectional	445 breast milk donations from women attending 2 hospitals.	Aflatoxins in milk	99.5% of the samples contained aflatoxins at concentrations ranging between 2 pg ml-1 to 3 ng/ ml	
El-nezami et al., 1995 Victoria, Australia and Thailand[34]	To examine the exposure of infants to aflatoxin M_1 (AFM_1) and the lactating mothers to aflatoxin B_1 (AFB_1), using AFM_1 in breast milk as a biomarker for exposure to AFB_1	Cross-sectional	73 women from Victoria, Australia and 11 women from Thailand	Aflatoxins in milk	AFM_1 was detected in 11 samples from Victoria and five samples from Thailand at median concentrations of 0.071 ng/ml (range 0.028 to 1.031 ng/ml) and 0.664ng/ml (range 0.039 to 1.736 ng/ml), respectively.	AFM_1 in Thai milk samples significantly higher than in milk samples from Victoria.

Navas et al., 2005 Sao Paulo, Brazil[36]	To determine aflatoxin M_1 and ochratoxin A in milk from the Human Milk Bank of the Southern Regional Hospital, São Paulo, Brazil	Cross-sectional	Total of 50 samples analysed.	Aflatoxins and Ochratoxin A in stored human milk.	Only one was contaminated with AFM1, at 0.024 ng/ml, and two with OTA, at 0.011 and 0.024 ng/ ml.	Although the incidence observed was low, it is recommended that the study be extended to other milk banks of the city of São Paulo
Wild et al., 1987 Zimbabwe and France[41]	Detection of AF in human breast milk	Cross-sectional	54 samples from Zimbabwe rural women, and 42 women from France	Aflatoxins in breast milk using ELISA	6 breast milk samples from rural villages in Zimbabwe were found to be positive (11%) with levels up to 50 pg AF per ml. No positive samples were detected out of 42 milk samples obtained from women in France.	-
Zarba et al. 1992 Gambia, West Africa.[42]	To explore the relationships between dietary intake of aflatoxins and a number of aflatoxin biomarkers including aflatoxin metabolite excretion into breast milk.	Cross-sectional	5 breast milk samples	Aflatoxin M_1 in breast milk by a preparative monoclonal antibody immunoaffinity column /HPLC method	3 out of the 5 breast milk samples contained aflatoxins M_1	The proportion of aflatoxin in the diet excreted as AFM_1 in milk ranged from 0.09% to 0.43%.
Nyathi et al., 1989 Zimbabwe[37]	Human exposure to aflatoxins in Zimbabwe	Cross-sectional	54 breast milk samples	Aflatoxins in breastmilk and urine	AFM_1 detected in 11% of samples	-

Keys: *OTA =Ochratoxin A, AF=Aflatoxin*

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RESEARCH QUESTIONS/STUDY AIMS

The overall objective of the study is to determine if there is an association between birth outcomes and aflatoxin B_1-lysine adduct levels among pregnant women. If aflatoxin birth outcomes are associated with biomarker B_1-lysine adduct levels, then it would be apparent at some levels of aflatoxin in the blood. However, there is a dearth of evidence linking these toxins to reproductive health or other health outcomes. Besides, very few studies have been done on special populations such as pregnant women to describe prevalence and levels of serum aflatoxins in blood and health related outcomes. While the carcinogenicity of aflatoxins may represent one end of the spectrum of health outcomes, more directed research is required to evaluate their more subtle, yet probably significant impact on the prevalence of anemia in pregnancy and birth outcomes.

The specific aims and hypotheses are as follows:

1. To determine the aflatoxin B_1-lysine adduct levels in blood of pregnant women and assess its relationship with socio-demographic factors in Kumasi.

Based on studies conducted in non-pregnant population, we hypothesize that aflatoxin levels will vary by socio-demographic factors (age, marital status, educational level, income bracket and ethnicity) in pregnant women in Kumasi. (Jolly, Jiang et al. 2006)

2. To determine the association between aflatoxin B_1-lysine adduct levels in blood and anemia in pregnant women.

We hypothesize that higher aflatoxin blood levels in pregnant women will be associated with anemia as observed experimentally in other primates. (Verma 2004)

3. To establish the association between aflatoxin B_1 biomarker blood levels in pregnant women and birth outcomes such as low birth weight (less than 2500g), preterm (born before 37 completed weeks of pregnancy) and small for gestational age deliveries (sex specific birth weight at or below the 10th percentile for the weight-for-gestational-age of an international reference population).

From available literature, we hypothesize that higher blood levels of aflatoxin B_1 will be associated with increased Low birth weight deliveries and possibly other adverse birth outcomes. (Yousef, Osman et al. 2002)

MATERIALS AND METHODS

The study was conducted in the Kumasi region of Ghana, West Africa, which has a population of approximately 1.2 million.24 Kumasi is the second largest city in Ghana. Its climate is hot and humid with two rainy seasons occurring from April to June and from September to October.25 The climate and poor pre- and post- harvest handling of crops contributes to fungal growth and aflatoxin contamination of staple crops.26, 27

This was a cross-sectional study of women presenting for delivery at two hospitals in Kumasi, the Komfo Anokye Teaching Hospital (KATH) and the Manhyia Polyclinic, between November and December 2006. As described in an earlier report,13 all women who had a singleton, uncomplicated pregnancy were identified from admission records and invited to participate. Women who had multiple or complicated pregnancy, who were positive for syphilis, who had hemoglobinopathy, or infections known to affect maternal health or birth outcome were excluded from the study. Informed consent was obtained from participants. Participation in the study was voluntary and no incentives were provided. All 785 women who were eligible for the study participated; adequate blood and stool samples for laboratory tests could only be obtained for 755. 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 prior to its implementation.

After informed consent was obtained, a questionnaire was administered by a trained interviewer. The questionnaire elicited information on demographic characteristics (age, education, annual income, employment status, residence, number of

children, and type of toilet facilities), obstetric history for current and previous pregnancies (stillbirth, ectopic pregnancy, preterm delivery, and low birth weight), 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).28 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, hematinics, antihelminthic medication, illnesses, and treatment during pregnancy. A single blood sample was collected in EDTA by venepuncture for determination of complete blood count (CBC) and differentials, red blood cell indices, hemoglobin, malaria antigen, folate, and AF-ALB levels. Stool samples were obtained for determination of intestinal helminths.

Laboratory procedures and definition of variables

Determination of aflatoxin B1- lysine adducts. Serum AFB1-lysine adduct, the major form of AFB1-albumin adducts and reflecting AF exposure in the previous 2–3 months, was measured by a modified HPLC-fluorescence method.29 In brief, 150 µl 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 injected to HPLC.

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 X 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
AFB1-lysine adducts was monitored at wavelengths of 405 nm (excitation) and 470 nm (emission). The peak of authentic AFB1-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 AFB1-lysine adducts concentration was adjusted by serum albumin level.

Malaria, intestinal worm infection and folate deficiency have been associated with anemia, thus these conditions were also evaluated. Determination of malaria antigen in plasma was done using the Malaria Antigen CELISA assay with a sensitivity and specificity of 98% and 96% respectively.30 Malaria status was described as the presence or absence of malaria antigens in peripheral blood at delivery.

Intestinal worm infection connotes the presence of helminths (Hookworm, Ascaris lumbricoides, Strongyloides stercoralis, and Trichuris trichura) eggs or larvae (S. stercoralis) in stool samples obtained from the participants. These intestinal helminths are known to cause anemia. Determination of hookworm, Ascaris lumbricoides and Trichuris trichura ova in stool samples was done using the Kato-Katz thick smear technique.31 Samples for detection of Strongyloides stercoralis were processed using the Baermann method.32 Hemoglobin level was measured in an automatic cell counter (Sysmex M-2000; Digitana AG, Hamburg, Germany). Anemia was defined as hemoglobin level is < 11 g/dl.4 Serum folate concentrations were measured by radioimmunoassay Quantaphase

II, Bio-Rad, Hercules, California, USA). The reference interval for folate in women is 3.9–18.1 ng/ml.33 Folate deficiency was based on serum levels of less than 3.9ng/ml. Since we were interested in examining hemolytic anemia, possibly due to aflatoxins, we attempted to separate participants with IDA from those with hemolytic anemia using measures of

hemoglobin in red blood cells. In the absence of plasma iron measurements, these measures were used as surrogates of IDA. These tests were conducted by the KATH hospital laboratory. They included the following: MCV (mean corpuscular volume) with reference range of 80-96 fL, MCHC (mean corpuscular hemoglobin concentration) with reference range 32-36 g/dl, and MCH (mean corpuscular hemoglobin) with reference range of 27-33pg.34 Based on laboratory results, participants were classified as having IDA if they had any one of the MCV, MCH, or MCHC indices below the reference range. In hemolytic anemia and anemia of other origins, MCV, MCH and MCHC indices may either be increased or normal.

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.

Aim 1: Socio-demographic determinants of aflatoxin B_1-lysine adduct levels among pregnant women in Kumasi, Ghana.

Missing values were excluded from the analysis, thus only 755 of the 785 women were used for the final analysis. AF-ALB assocations were assessed as both continuous and categorical values. Analysis of variance (ANOVA) was used on log transformed AF-ALB values for bivariate and adjusted analyses to assess differences in mean ln AF-ALB values across socio-demographic characteristics. The upper quartile of AF-ALB (>11.34pg/mg) was categorized as "high." Chi-square tests were used for bivariate analysis, then multiple logistic regression was used to identify and characterize independent associations of socio-demographic characteristics with high levels of AF-ALB. Categorization of socio-demographic variables for full models, ANOVA and ligistic, were informed by bivariate analyses. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the logistic regression models. Comparison of findings from analyses based on continuous and the categorical (high) AF-ALB measures were compared to evaluate the robustness of the findings.

Aim 2: Association between aflatoxin B_1-lysine adduct levels in blood and anemia in pregnant women in Kumasi, Ghana

Analysis was restricted to the 755 women from whom adequate samples were obtained. Multiple logistic regression analysis was used to investigate the association between anemia (based on hemoglobin levels), the dependent variable, and AF-ALB levels, the independent variable of primary interest. Participants were divided into quartiles based on the distribution of AF-ALB in blood ("low": ≤ 2.67pg/mg, "moderate": > 2.67 to ≤ 4.97pg/mg, "high": > 4.97 to ≤ 11.34pg/mg, and "very high" : > 11.34pg/mg). Variables

that were statistically significant at $P < 0.05$ on bivariate analysis and those known to be associated with anemia based on extant literature were incorporated into models using the backward step-wise technique.35 We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for each variable entered into the model.

Strata specific analyses were conducted to assess differences in the association between anemia and AF-ALB levels according to presence of malaria parasitemia, intestinal helminth infections

and folate deficiency. In addition, in order to eliminate the effect of IDA on the relationship between anemia and AF-ALB, all analyses were repeated excluding the participants with laboratory test results suggestive of IDA. A diagram showing the statistical analysis plan is presented in figure 2.

Aim 3: Birth outcomes and birth outcomes and aflatoxin B_1-lysine adduct levels

Participants were divided into quartiles based on the distribution of aflatoxin B1-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, 2001 #407} We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for each variable entered into the model.

SOCIO-DEMOGRAPHIC DETERMINANTS OF AFLATOXIN B_1-LYSINE ADDUCT LEVELS AMONG PREGNANT WOMEN IN KUMASI, GHANA.

FAISAL M.B SHUAIB, JOHN E. EHIRI, ELLEN FUNKHOUSER, CRAIG WILSON, SHARINA D. PERSON, WILLIAM O. ELLIS

PAULINE E. JOLLY

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Abstract

Background: Aflatoxins are fungal metabolites that contaminate staple food crops in many developing countries. Although studies have linked these toxins to adverse birth outcomes and poor infant development, no study has investigated the socio-demographic and economic determinants of aflatoxin levels among pregnant women living in sub-Saharan Africa. The objective of this study was to investigate the socio-demographic determinants of aflatoxin B_1- lysine levels among pregnant women in Kumasi, Ghana

Methods: A cross-sectional study was conducted among 785 pregnant women. Aflatoxin B_1 lysine adduct levels (AF-ALB) were determined by High Performance Liquid Chromatography. Participants were divided into quartiles of serum AF-ALB in pg/mg albumin. Logistic regression was used to assess the relationship between aflatoxin levels and socio-demographic variables.

Results: AF-ALB levels ranged from 0.44 pg/mg to 268.73 pg/mg albumin with a median level of 5.0 pg/mg. Bivariate analyses indicates that mean ln AF-ALB as well as the percent of women having high AF-ALB levels (>=11.34pg/mg; upper quartile) were inversely associated with indices of higher SES, namely, higher education and income, being employed and having a flush toilet. They were also inversely associated with having only one child.

Findings present in bivariate analyses remained in multivariable models using ANOVA to assess the differences in mean ln AF-ALB levels and using multiple logistic regression to assess associations with high AF-ALB levels. In the latter, higher income, being employed, having one child (verses no children) and having a flush toilet (verses no toilet

facilities) were each independently associated with a 30-40% reduced odds of high AF-ALB levels.

Conclusion: The results of this study add to the growing body of evidence that aflatoxins exposure is increased by low resource availability in the household. Additional research is needed to investigate how socio-demographic and economic factors interact to influence aflatoxin ingestion by individuals in regions with high aflatoxin crop contamination. This knowledge can be used to formulate and implement policies that will reduce exposure of women and their unborn children to these toxins.

Key words: Aflatoxins; Socio-demographic; economic; pregnancy; Kumasi; Ghana

Introduction

Fungal contamination of food is a worldwide phenomenon. Globally, at least 4.5 billion people are thought to be chronically exposed to aflatoxins, a by-product of fungal contamination mainly *Aspergillus flavus* and *Aspergillus parasiticus* of certain foods.[4] In humans, aflatoxins have been implicated in the pathogenesis of conditions such as primary liver cell carcinoma, immunosuppresion, malnutrition, infertility and growth retardation.[53]

Aflatoxin contamination of crops (especially cereals) is ubiquitous in hot and humid environments with temperatures averaging 30 degrees Celsius and humidity exceeding 77%, conditions which favor fungal growth. Along with humidity and temperature, other factors such as inadequate drying of the crops, insect and rodent activities also promote fungal contamination of food stuff. 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 aflatoxins in quantities that far exceed the United States Department of Agriculture's (USDA) regulatory limit of 20 ppb.[58]

While chronic, sub-clinical exposure to aflatoxins is more common, acute exposure to aflatoxin can result in aflatoxicosis with case fatality rates (CFR) of 25% or more.[59] Acute aflatoxin outbreaks are a recurring public health problem in developing countries. In 2004, the most notable outbreak of aflatoxicosis occurred in Kenya leading to 125 deaths among 317 cases (CFR=39%).[60] In sub-Saharan Africa where the health infrastructure is weak and the sick have limited access to healthcare, the reported morbidity and mortality from aflatoxicosis may represent the tip of the iceberg.[61] While contamination of grains and cereals by aflatoxins is known to occur all over the world,

studies have shown that ingestion of aflatoxin contaminated foods occurs mostly in developing countries.

Studies conducted among pregnant women from developing countries indicate very high levels of aflatoxin in maternal serum, neonatal umbilical cord blood, and in breast milk.[22, 24, 26-28, 30, 31, 33] Lamplugh and others conducted a study in Accra, Ghana and Jos, Nigeria to determine the presence of aflatoxins in human breast milk and to ascertain if they cross the human placental membrane. In Ghana, they found aflatoxins in 34% (n=90) of the 264 milk samples (AFM_2 concentration=16ng/l-2075ng/l) and 34% (n=63) of the umbilical cord blood specimens (AFB_1 concentration=185ng/l-43,822ng/l). In the Nigerian study, aflatoxins were detected in 21% (n=16) of 77 maternal samples (AFB_1 concentration=553ng/l-10,390ng/l).[33] These finding along with others conducted in the United Arab Emirates provide evidence that newborns are exposed to unacceptable levels of aflatoxins before birth and in breast milk.[29-31, 40] Furthermore, Denning and colleagues detected high levels of aflatoxins in human cord sera at birth and maternal serum immediately after birth in Songkhla, Thailand. Seventeen of 35 cord sera (48%) contained aflatoxin (mean 3.1 nmol/ml) and 6% percent of the maternal sera contained aflatoxin (mean 0.62 nmol/ml). The latter result demonstrates the possibility of trans-placental transfer of aflatoxins and its accumulation in the fetus.[45] These levels of aflatoxins detected in some umbilical cord blood samples immediately after birth are among the highest

levels ever recorded in human tissue and fluids.[62] On the other hand, only few studies have reported more than trace amounts of aflatoxins in the breast milk of women from developed countries such as Italy and France. In the former study, only one sample out of

231 puerperal women was contaminated with 11.4ng/l of aflatoxin B_1 and 194ng/l of aflatoxin M_1[41]). A study conducted by Wild and colleagues in France did not report any aflatoxin contamination of 42 samples of breast milk.[42] Clearly, limitation in environmental conditions that lead to aflatoxin proliferation, education, and enforcement of strict restrictions on the allowable limits for aflatoxins in food (25ng/kg for infant milk)[63] may be partly responsible for these observed disparities in aflatoxin contamination of breast milk in developed and less developed countries.

No studies have been conducted to assess the role of socio-demographic variables in aflatoxin exposure among pregnant women in sub-Saharan Africa, where aflatoxin contamination of food is common. A study conducted to investigate pertinent socio-demographic factors that determine aflatoxin contamination of body fluids involved samples obtained from the general population and did not involve pregnant women.[64] The study found that the participants' ethnicity, village of residence and the number of individuals in the household were significant predictors of high AF-ALB levels. Studies of this nature are important given that the diet may change during pregnancy and some of the non-nutritive substances ingested by these pregnant women may be contaminated with *Aspergillus* moulds.[12, 13]

The objective of this study is to determine the aflatoxin B_1-lysine adduct (AF-ALB) levels in blood of pregnant women in Kumasi, Ghana and assess the relationship with socio-demographic factors. We hypothesized that majority of pregnant women will have AF-ALB in blood and that AF-ALB levels will vary by socio-demographic factors such as age, marital status, educational level, and employment status.

Materials and methods

Study setting

The study was conducted in the Kumasi the capital city of the Ashanti region of Ghana in West Africa, which has a population of approximately 1.2 million.[65] Kumasi is the second largest city in Ghana with an area of about 254 square kilometers. It lies about 270Km North of Accra, the capital city of Ghana. Its climate is tropical with two rainy seasons occurring from April to June and from September to October.[66]

Study design and participants

As described in an earlier study,[67] this was a cross-sectional study of women presenting for delivery at two hospitals in Kumasi, the Komfo Anokye Teaching Hospital (KATH) and the Manhyia Polyclinic, during November and December 2006. The women were identified from admission records and all women who had a singleton, uncomplicated pregnancy were invited to participate. 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, all consented; blood and stool samples adequate for analysis were obtained from 755. 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.

Study instruments and data collection

After informed consent was obtained, a questionnaire was administered by a trained interviewer. The questionnaire elicited information on demographics (age, education, socio-economic status, residence, and type of toilet facilities), obstetric history for current

and previous pregnancies, 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).[68] Obstetric information was obtained from the women's antenatal care (ANC) charts and is published elsewhere.[69] A single blood sample was collected in EDTA by venepuncture for determination of aflatoxin levels.

Laboratory procedures

Determination of aflatoxin B_1 lysine adducts. Serum AFB_1-lysine adduct, the major form of AFB_1-albumin adducts which reflects aflatoxin exposure in the previous 2–3 months, was measured by a modified HPLC-fluorescence method.[70] In brief, 150 µl 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 injected to HPLC.

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 X 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 adduct's concentration was adjusted by serum albumin level.

Statistical analysis

Missing values were excluded from the analysis, thus only 755 of the 785 women were used for the final analysis. AF-ALB assocations were assessed as both continuous and categorical values. Analysis of variance (ANOVA) was used on log transformed AF-ALB values for bivariate and adjusted analyses to assess differences in mean ln AF-ALB values across socio-demographic characteristics. The upper quartile of AF-ALB (>11.34pg/mg) was categorized as "high." Chi-square tests were used for bivariate analysis, then multiple logistic regression was used to identify and characterize independent associations of socio-demographic characteristics with high levels of AF-ALB. Categorization of socio-demographic variables for full models, ANOVA and ligistic, were informed by bivariate analyses. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the logistic regression models. Comparison of findings from analyses based on continuous and the categorical (high) AF-ALB measures were compared to evaluate the robustness of the findings. Data analysis was performed using SAS software version 9.2 (SAS Institute, Cary, NC).[71]

Results

AF-ALB levels ranged from 0.44 pg/mg to 268.73 pg/mg albumin with a median level of 5.0 pg/mg. Figure 1 shows the distribution of the aflatoxin levels among the 755 participants. The average age of the respondents was 27± 6.3 years (range: 14-48 years). Nearly half had a junior high school education; only 16 (2.1%) had a higher level of education. Most participants were employed and most were married. About a third were nulliparous and a third had some type of flush toilet (Table 1).

Bivariate analyses indicates that mean ln AF-ALB as well as the percent of women having high AF-ALB levels (>=11.34pg/mg; upper quartile) were inversely associated with indices of higher SES, namely, higher education and income, being employed and having a flush toilet. They were also inversely associated with having only one child (Table 1).

Findings present in bivariate analyses remained in multivariable models using ANOVA to assess the differences in mean ln AF-ALB levels (Table 2) and using multiple logistic regression to assess associations with high AF-ALB levels (Table 3). In the latter, higher income, being employed, having one child (verses no children) and having a flush toilet (verses no toilet facilities) were each independently associated with a 30-40% reduced odds of high AF-ALB levels.

Discussion

We investigated the socio-demographic factors that determined AF-ALB levels among pregnant women in Kumasi, Ghana. Our findings suggest that the key determinant may be related to the economic situation of the participant's household.

The average age of respondents (27± 6.3 years) is quite similar to the mean age (28.9 ± 5.8 years) of pregnant women who were part of a study conducted in the Korle Bu Teaching Hospital in Accra, Ghana.[72] This adds to the external validity of the study given that samples from both studies provided similar results with respect to the ages of women who were found to be attending antenatal clinic or who had just delivered an infant. Most participants (47%) reported having junior high school education. This proportion is quite close to that reported as the official female literacy rate of 49.8% for Ghana.[73]

In light of the fact that Ghana is a developing country with 28% living below the poverty line (living on less than 1 US dollar per day as at 2007) [73] it is interesting that majority of the participants (65%) reported earning 20 GHc or more (1.4 GHc is equivalent to 1 US dollars) per week. This indicates that more than a substantial proportion of the participants earn at least 14 to 25 US dollars per week. Except for those participants from large number households, this earning should place them above the poverty line. Considering all participants had AF-ALB detected in their blood, it implies that the aflatoxin contamination of body fluids is a challenge faced not only by the very poor. Additionally, about 30% of respondents reported not being employed; the National unemployment rate in Ghana is 11%. In West Africa, more women than men are likely to be unemployed; nevertheless, it is surprising that the unemployment rate among these women is as high as 30%. A lower unemployment rate was expected. It is possible that this sample is made up of women who were largely housewives or women who stopped working during their pregnancy. On the other hand, it is also possible that many women who are involved in small scale commercial activities to augment the earnings of their households may not have considered this an "occupation" to report.

The total fertility rate (defined as the number of children born per woman) in Ghana is 3.68. It is therefore not surprising that participants (mean age of 27 years) who are having their second child are in the majority (42.1%) and may be nearing the peak of their reproductive years.

The mean AF-ALB of 10.9 pg/mg albumin is lower than the values obtained from a study of males and non-pregnant women in the Ejura-Sekyeredumase district of the Ashanti region, Ghana.[64] The likely reason for this disparity is that a larger proportion of

participants in our study were Akans (69%) who eat more yam tubers along with other food crops unlike participants in the Ejura-Sekyeredumase study who were largely non-Akan ethnic groups (74%) who mainly eat maize, peanuts and other cereals. The mean AF-ALB level in this study is also lower than that reported in a study from Benin, West Africa[74] and those reported by Wang et al. from areas with high liver-cancer risk in China.[75] It is however higher than levels reported by in a study among Gambians.[76]

The results of multivariate analysis indicate that those participants who have higher incomes are less likely to have high levels of serum AF-ALB levels. This finding corroborates that of Jolly et al.[64] who found that the number of individuals in the household (a surrogate for available financial resources per head) was significantly associated with aflatoxin B_1 -lysine adducts in Ejura Sekyedumase district of Ghana. The latter study was however done among both males and non-pregnant females. It is possible that participants who have higher incomes may be better able to afford food stuffs other than maize and maize flour "Kenkey" which is a cheap staple food eaten by most residents and frequently contaminated with aflatoxins.[11] Perhaps food-stuff that is obviously contaminated by fungi is cheaper than those that are not. In which case, individuals who have more financial resources are able to afford higher quality foods and thereby reduce their exposure to the toxins. It may be reasonable to conjecture that the protective effect of being employed compared to being unemployed may be determined by identical factors as the income levels. Furthermore, correlation analysis of education with income (not shown) revealed strong correlation between both variables ($P<0.0001$). Consequently, we may deduce that individuals with higher income and education have access to relevant information and may be aware of the general health risk of eating

moldy food stuff, though they may not necessary be aware of the relationship between this and specific disease conditions caused by the metabolic by-products of fungi. As a result, they avoid exposure to obviously contaminated grains and nuts. Women who reported being employed had reduced odds of having high levels of aflatoxins compared to the unemployed. While this may again be linked to their different economic circumstances, it is also possible that some individuals who list themselves as unemployed include farmers who may be more exposed to aflatoxin-producing fungi because they are restricted to eating the crops they grow irrespective of their wholesomeness. It is noteworthy that our results demonstrate that having one child was comparatively protective against having higher aflatoxin levels. It is possible that couples who have one child may be more cautious about consuming and sharing contaminated food with their young offspring. Nevertheless, this finding is contrary to what Sedeghi and colleagues found in Tehran, Iran where there was no association between aflatoxin B_1 in breast milk and the parity of participants.[22] On the other hand, a study (in Qalyubiyah, Egypt) which also used breast milk as the source of aflatoxin B_1 found that detection of aflatoxin in breast milk was more likely if the participant had more than one child.[39] Perhaps the different levels of aflatoxins in breast milk or serum have an influence on the relationship with socio-demographic variables. The vastly different socio-demographic context of the studies may also be a factor responsible for these different observations.

The finding that participants who have a flush type toilet system were less likely to have higher aflatoxins compared to those without, seems to be related to the socioeconomic status of the participants. Since the type of toilet system utilized by participants was used

as an indicator of socioeconomic status, it is expected that those with better financial means are able to afford the more expensive flush type toilet system in their homes. Houses that do not have this facility may be cheaper and more affordable to the poor. Poor individuals may also not be prudent about safeguarding their health, and may not take steps to avoid fungal growth on their food-stuff or refrain from eating obviously contaminated food. Further research could investigate how socio-demographic and economic determinants interact and how this knowledge can be used to reduce exposure to aflatoxins.

Our study has some potential limitations. Firstly, it did not report the income level of spouses. This may be a confounder of the socio-economic status of the participants. Secondly, by measuring the AF-ALB levels at only one point in time, we do not have a complete picture of what the concentrations may be at other times of the year when a mixture of fresh and stored crops may be eaten. Eating freshly harvested crops invariably reduces exposure to higher aflatoxin levels. However, since the AF-ALB levels measured reflect the blood levels of the toxin over a period of at least 2-3 months, it still gives us an idea of the exposure rates of these participants to aflatoxins. Our relatively large sample size was an advantage of the study and enhanced the probability of detecting otherwise small associations. The fact that our sample was drawn from a population of women with varied socio-demographic and economic circumstances, promotes the genaralizability of our results to the general population of women in Kumasi city, Ghana.

In conclusion, our study adds to the body of evidence linking increased aflatoxin exposure to poor resource availability in the household. It uniquely advances the field in terms of documenting the prevalence of high aflatoxin levels among pregnant women in

Ghana and the possible role of household resource availability on these levels. The very high AF-ALB levels buttress the fact that efforts to reduce exposure of women and unborn children to these toxins cannot be overemphasized.

Table 1. Demographic characteristics of 755 pregnant Ghanaian women and corespondng mean log aflatoxin B_1-lysine adduct levels (AF-ALB) and percent in highest quartile of AF-ALB.

Variable	All		Mean log aflatoxin level	ln highest AF-ALB quartile‡	
	N	Col %		N	Row %
Age category					
<20	103	13.6	1.84	29	28.2
20-24	189	25.0	1.69	48	25.4
25-29	218	28.9	1.60	49	22.5
≥30	245	32.5	1.72	63	25.7
			p=0.37		*p=0.96*
Formal education					
None	170	22.7	1.90	58	34.1
Primary or middle school	212	28.3	1.65	47	22.2
Junior high school	352	46.9	1.65	63	17.9
≥Senior high school	16	2.1	1.13	21	18.3
			p=0.02		*p=0.12*
Weekly income (GHc)†					
<10	205	27.4	1.98	68	33.2
10-19.9	55	7.4	1.83	18	32.7
20.–35.4	287	38.4	1.59	60	20.9
≥ 35.5	201	26.9	1.54	42	20.9
			p<0.001		***p=0.07***
Marital status					
Single	159	21.2	1.79	47	29.6
Living in union	142	18.9	1.78	42	29.6
Married	449	59.9	1.65	100	22.3
			p=0.29		*p=0.25*
Employment					
Unemployed	225	30.0	1.69	56	24.9
Employed	526	70.0	1.71	133	25.3
			p= 0.85		*p=0.94*
No. of children					
0	268	36.9	1.80	81	30.2
1	306	42.1	1.57	60	19.6
2 or more	153	21.1	1.81	42	27.5
			p= 0.02		***p= 0.02***
Toilet facilities					
None	278	37.0	1.89	84	30.2
Simple Pit latrine	120	15.9	1.82	27	22.5
Ventilated Pit latrine	116	15.5	1.78	30	25.9
Pour flush toilet	37	4.9	1.39	6	16.2
Flush toilet	200	26.6	1.37	42	21.0
			p < 0.001		***p = 0.003***

†= GHc= Ghana cedi; 1.4 Ghana cedi is equivalent to 1 US dollar
‡ (> =11.34pg/mg)

Table 2. Bivariate and adjusted mean log aflatoxin B_1-lysine adduct levels (AF-ALB) by demographic characteristics of 755 pregnant women in Kumasi, Ghana.

Variable	Bivariate mean log aflatoxin level	Adjusted $^{\beta}$ mean log aflatoxin level
Age category		
<20	1.84	1.79
20-24	1.69	1.68
25-29	1.60	1.72
≥30	1.72	1.83
	p=0.37	*p=0.31*
Formal education		
None	1.90	1.85
Primary or middle school	1.65	1.66
Junior high school	1.65	1.77
≥Senior high school	1.13	1.44
	p=0.02	***p=0.01***
Weekly income (GHc)†		
<20	1.95	1.95
≥ 20	1.57	1.42
	p<0.001	***p<0.001***
Marital status		
Single	1.79	1.75
Living in union	1.78	1.81
Married	1.65	1.76
	p=0.29	*p=0.32*
Employment		
Unemployed	1.69	1.96
Employed	1.71	1.54
	p=0.85	***p=0.003***
No. of children		
0	1.80	1.92
1	1.57	1.69
2 or more	1.81	1.90
	p=0.02	***p=0.02***
Toilet facilities		
None	1.89	1.91
Simple pit latrine	1.82	1.84
Ventilated pit latrine	1.78	1.85
Any type flush toilet‡	1.37	1.42
	p<0.001	***p<0.001***

Note:
†= GHc= Ghana cedi; 1.4 Ghana cedi is equivalent to 1 US dollar
‡= Flush or pour type toilet system
β = Adjusted for socio-demographic variables: Weekly income, toilet facilities, employment, and Number of children

Table 3 Associations between aflatoxin B_1-lysine adduct levels and socio-demographic characteristics of 755 pregnant Ghanaian women.

Variables	Crude OR	95% CI	Adjusted OR♣	95% CI
Age in Years				
<20	Ref	Ref	Ref	Ref
20-24	0.88	0.58-1.36	0.94	0.59-1.49
25-29	0.78	0.52-1.19	0.92	0.57-1.47
≥30	0.96	0.63-1.44	1.19	0.71-2.00
Formal education				
None	Ref	Ref	Ref	Ref
Primary or middle school	0.76	0.53-1.09	0.84	0.55-1.24
Junior high school	0.82	0.59-1.14	0.99	0.70-1.41
≥Senior high school	**0.38**	**0.15-0.98**	0.55	0.21-1.43
Weekly income (GHc)†				
< 20	Ref	Ref	Ref	Ref
≥ 20	**0.66**	**0.50-0.87**	**0.67**	**0.50-0.90**
Marital status				
Single	Ref	Ref	Ref	Ref
Living in union	1.07	0.72-1.61	1.11	0.72-1.70
Married	0.88	0.64-1.22	0.96	0.65-1.43
Employment				
Unemployed	Ref	Ref	Ref	Ref
Employed	0.97	0.73-1.28	**0.58**	**0.40-0.83**
Number of Children				
0	Ref	Ref	Ref	Ref
1	**0.68**	**0.51-0.92**	**0.68**	**0.48-0.94**
2 or more	1.08	0.78-1.54	1.03	0.66-1.61
Toilet facilities				
None	Ref	Ref	Ref	Ref
Simple pit latrine	0.77	0.53-1.13	0.77	0.51-1.14
Ventilated improved pit latrine	0.92	0.62-1.36	0.95	0.64-1.42
Any type flush toilet‡	**0.53**	**0.39-0.73**	**0.56**	**0.41-0.79**

Note: Values in bold are statistically significant

†= GHc= Ghana cedi; 1.4 Ghana cedi is equivalent to 1 US dollar

♣ =Full model adjusted for socio-demographic variables: Weekly income, toilet facilities, employment, and Number of children

‡= Flush or pour type toilet system

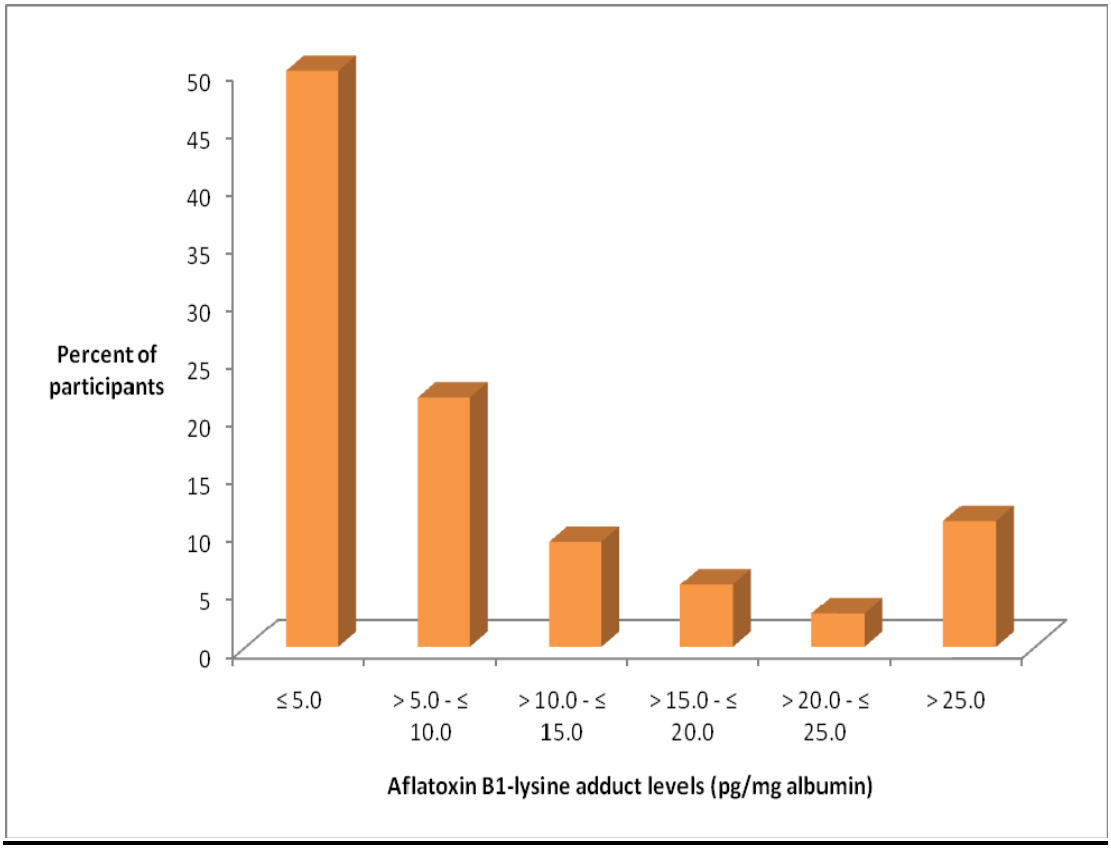
Figure 1 legend.

Aflatoxin B_1- lysine adduct levels in serum of 755 pregnant Ghanaian women.

Mean: 10.9 pg/mg albumin

Median: 5.0 pg/mg albumin

Figure 1


Percent of participants
50
45
40
35
30
25
20
15
10
5
0
≤ 5.0
> 5.0 - ≤ 10.0
> 10.0 - ≤ 15.0
> 15.0 - ≤ 20.0
> 20.0 - ≤ 25.0
> 25.0
Aflatoxin B1-lysine adduct levels (pg/mg albumin)

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ASSOCIATION BETWEEN ANEMIA AND AFLATOXIN B-LYSINE ADDUCT LEVELS AMONG PREGNANT WOMEN IN KUMASI, GHANA.

FAISAL M.B SHUAIB, JOHN E. EHIRI, ELLEN FUNKHOUSER, CRAIG WILSON, SHARINA D. PERSON, WILLIAM O. ELLIS

PAULINE E. JOLLY

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ASSOCIATION BETWEEN ANEMIA AND AFLATOXIN B_1 BIOMARKER LEVELS AMONG PREGNANT WOMEN IN KUMASI, GHANA

Abstract

Background: Aflatoxins are fungal metabolites that contaminate staple food crops in many developing countries.

Method: In a cross-sectional study of 755 pregnant women, Aflatoxin B_1-lysine adduct (AF-ALB) levels were determined by High Performance Liquid Chromatography. Participants were divided into quartiles "low", "moderate", "high", and "very high". Anemia was defined as hemoglobin levels < 11g/dl. Logistic regression was used to examine the association of anemia with AF-ALB.

Results: The mean AF-ALB level was 10.9 pg/mg (range=0.44-268.73 pg/mg); 30.3% of participants were anemic. The odds of being anemic increased 21% (OR, 1.21, p=0.01) with each quartile of AF-ALB reaching an 85% increased odds in the "very high" compared to the "low" category (OR, 1.85; CI, 1.16-2.95). This association was stronger among women with malaria and findings were robust when women with evidence of iron deficiency anemia were excluded.

Conclusion: This study found a strong, consistent association between anemia in pregnancy and aflatoxins.

INTRODUCTION

Figures jointly released by the United Nations Population Fund (UNFPA), World Health Organization (WHO), United Nations Children's Fund (UNICEF) and the World Bank show that a woman dies every minute from pregnancy or childbirth.[77] More than 99% of these deaths are in developing countries, particularly sub-Saharan Africa where there is a 1 in 13 chance of a woman dying during childbirth compared to 1 in 4,100 in industrialized countries.[77] The Millennium Development goal #5 is to reduce the maternal mortality ratio by 75% between 1990 and 2015[78] but there has been less than a 1% decline in global maternal mortality in the last decade. Indeed, the World Bank estimates that only one developing region (Middle East and North Africa) is likely to achieve this target.[78]

Anemia in pregnancy is one of the leading causes of maternal mortality. It is a clinical condition characterized by reduced ability of the red blood cells to carry oxygen to body tissues, usually as result of their decreased hemoglobin content. The WHO defines anemia as a hemoglobin level of less than 11g/dl during the first and third trimester while the cut-off is 10.5g/dl in the second trimester.[13]

Anemia afflicts about two billion people worldwide.[79] An estimated 40% of all maternal deaths at childbirth are linked to anemia. Although anemia is a global health problem, it occurs more frequently among children and pregnant women in developing countries.[80] Only 23% of pregnant women in industrialized countries are anemic compared to 52% of their counterparts in less developed countries. Up to 18% of women in developed

countries and 43% of those in developing countries are already anemic at the time of conception.[81] Anemia in pregnancy is

ranked among the top 4 causes of morbidity and mortality among pregnant women in Africa and is the second leading cause in Asia.[82] About 40% of women attending prenatal clinics in Africa suffer from anemia at different stages of pregnancy.[83] Sixty-two reports compiled by WHO on the proportion of maternal deaths associated with anemia indicate that maternal deaths could directly be linked to anemia in 26% of these reports. Seventy four percent of the reports also show that anemia is a contributory factor in the documented maternal mortality.[84] Furthermore, a review of anemia and pregnancy related mortality by Brabin et al, showed estimates for all-cause anemia attributable mortality (both direct and indirect) of 6.3% for Africa, 7.3% for Asia and 3.0% for Latin America. The case fatality rates, which were based on hospital data, varied from 1% to 50%.[85] While these figures underscore the magnitude of the burden of anemia in pregnancy, they are only the tip of the iceberg in many developing countries where women do not obtain adequate prenatal care. Indeed, the WHO estimates that about 55% of women have antenatal care and 60% of all deliveries in developing countries occur outside of a health facility.[86] Consequently, many women who die during pregnancy or delivery from causes related to anemia are not reported.

Although Iron Deficiency Anemia (IDA) is the most common cause of anemia in pregnant women, there are many other causes including nutritional causes such as folate and vitamin B_{12} deficiencies, infective causes such as malaria, and intestinal helminth infections, severe hemorrhage, hemoglobinopathies, chronic diseases, G6PD deficiency, and hemolytic anemia from drugs or toxins.[67, 83, 87] Consequently, any assessment of the

cause of anemia must put these into consideration in order to delineate cause-specific etiology. Besides, programs to prevent maternal anemia are more likely to be effective when they identify and address the multiple causes of anemia through multi-disciplinary and integrated interventions.

Contamination of food-stuff and animal feeds by aflatoxin producing molds is a recurrent public health problem. Globally, about 4.5 billion people are at risk of chronic exposure to aflatoxins.[4] Aflatoxins are toxic metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. They are classified based on immune-fluorescent properties; B_1, B_2, G_1 and G_2. Aflatoxin B_1 (AFB_1) is the most potent and carcinogenic of all aflatoxin sub-types.[88, 89] Staple foods such as cereals, groundnuts and other oil seeds are most prone to aflatoxin contamination. [53, 90] Aflatoxin contamination is influenced by high humidity, high temperatures, insect and rodent activity, and inadequate drying of the crops. Contamination occurs frequently in countries of Africa and Asia. This contamination can occur at any stage of food production from pre-harvest to storage.[8] Measurements of aflatoxin levels in food crops have been conducted in some West African countries.[8, 91] One study which collected samples from major processing sites in Accra, Ghana reported aflatoxin levels that ranged from 2 to 662µg/kg.[91] When these aflatoxins are ingested, AFB_1 binds to albumin to form AFB_1 -lysine adducts (AL-ALB), which persist for up to 2-3 months or longer in the blood and can be detected.[64] Aflatoxins have been associated with hemolytic anemia in experimental animals.[92] Studies with experimental animals have shown that aflatoxins cause hemolysis of red blood cells and may interact with RNA and DNA to cause a depression of hemopoiesis in primates.[93] Although this has not been documented to occur in humans, it is expected that

aflatoxins may cause a similar effect in humans. The objective of this study is to examine the association between anemia and

AF-ALB levels in the blood of pregnant women in Kumasi, Ghana. We hypothesized that higher AF-ALB levels in the pregnant women would be associated with anemia as observed experimentally in other primates.

Methods

Study setting

The study was conducted in the Kumasi region of Ghana, West Africa, which has a population of approximately 1.2 million.[65] Kumasi is the second largest city in Ghana. Its climate is hot and humid with two rainy seasons occurring from April to June and from September to October.[66] The climate and poor pre- and post- harvest handling of crops contributes to fungal growth and aflatoxin contamination of staple crops.[58, 94]

Study design and participants

This was a cross-sectional study of women presenting for delivery at two hospitals in Kumasi, the Komfo Anokye Teaching Hospital (KATH) and the Manhyia Polyclinic, between November and December 2006. As described in an earlier report,[67] all women who had a singleton, uncomplicated pregnancy were identified from admission records and invited to participate. Women who had multiple or complicated pregnancy, who were positive for syphilis, who had hemoglobinopathy, or infections known to affect maternal health or birth outcome were excluded from the study. Informed consent was obtained from participants. Participation in the study was voluntary and no incentives were provided. All 785 women who were eligible for the study participated; adequate blood and stool samples for laboratory tests could only be obtained for 755. 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 prior to its implementation.

Study instruments and data collection.

After informed consent was obtained, a questionnaire was administered by a trained interviewer. The questionnaire elicited information on demographic characteristics (age, education, annual income, employment status, residence, number of children, and type of toilet facilities), obstetric history for current and previous pregnancies (stillbirth, ectopic pregnancy, preterm delivery, and low birth weight), 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).[68] 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, hematinics, antihelminthic medication, illnesses, and treatment during pregnancy. A single blood sample was collected in EDTA by venepuncture for determination of complete blood count (CBC) and differentials, red blood cell indices, hemoglobin, malaria antigen, folate, and AF-ALB levels. Stool samples were obtained for determination of intestinal helminths.

Laboratory procedures and definition of variables

Determination of aflatoxin B_1- lysine adducts. Serum AFB_1-lysine adduct, the major form of AFB_1-albumin adducts and reflecting AF exposure in the previous 2–3 months, was measured by a modified HPLC-fluorescence method.[70] In brief, 150 μl 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 injected to HPLC.

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 X 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 AFB1-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 AFB1-lysine adducts concentration was adjusted by serum albumin level.

Malaria, intestinal worm infection and folate deficiency have been associated with anemia, thus these conditions were also evaluated. Determination of malaria antigen in plasma was done using the Malaria Antigen CELISA assay with a sensitivity and

specificity of 98% and 96% respectively.[95] Malaria status was described as the presence or absence of malaria antigens in peripheral blood at delivery. Intestinal worm infection connotes the presence of helminths (Hookworm*, Ascaris lumbricoides, Strongyloides stercoralis, and Trichuris trichura*) eggs or larvae (*S. stercoralis*) in stool samples obtained from the participants. These intestinal helminths are known to cause anemia. Determination of hookworm, *Ascaris lumbricoides* and *Trichuris trichura* ova in stool samples was done using the Kato-Katz thick smear technique.[96] Samples for detection of *Strongyloides stercoralis* were processed using the Baermann method.[97] Hemoglobin level was measured in an automatic cell counter (Sysmex M-2000; Digitana AG, Hamburg, Germany). Anemia was defined as hemoglobin level is < 11 g/dl.[79] Serum folate concentrations were measured by radioimmunoassay Quantaphase II, Bio-Rad, Hercules, California, USA). The reference interval for folate in women is 3.9–18.1 ng/ml.[98] Folate deficiency was based on serum levels of less than 3.9ng/ml.

Since we were interested in examining hemolytic anemia, possibly due to aflatoxins, we attempted to separate participants with IDA from those with hemolytic anemia using measures of

hemoglobin in red blood cells. In the absence of plasma iron measurements, these measures were used as surrogates of IDA. These tests were conducted by the KATH hospital laboratory. They included the following: MCV (mean corpuscular volume) with reference range of 80-96 fL, MCHC (mean corpuscular hemoglobin concentration) with reference range 32-36 g/dl, and MCH (mean corpuscular hemoglobin) with reference range of 27-33pg.[99] Based on laboratory results, participants were classified as having IDA if they had any one of the MCV, MCH, or MCHC indices below the reference

range. In hemolytic anemia and anemia of other origins, MCV, MCH and MCHC indices may either be increased or normal.

Statistical analysis

Data analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC). Analysis was restricted to the 755 women from whom adequate samples were obtained. Multiple logistic regression analysis was used to investigate the association between anemia (based on hemoglobin levels), the dependent variable, and AF-ALB levels, the independent variable of primary interest. Participants were divided into quartiles based on the distribution of AF-ALB in blood ("low": ≤ 2.67pg/mg, "moderate": > 2.67 to ≤ 4.97pg/mg, "high": > 4.97 to ≤ 11.34pg/mg, and "very high" : > 11.34pg/mg). Variables that were statistically significant at $P < 0.05$ on bivariate analysis and those known to be associated with anemia based on extant literature were incorporated into models using the backward step-wise technique.[71] We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for each variable entered into the model.

Strata specific analyses were conducted to assess differences in the association between anemia and AF-ALB levels according to presence of malaria parasitemia, intestinal helminth infections

and folate deficiency. In addition, in order to eliminate the effect of IDA on the relationship between anemia and AF-ALB, all analyses were repeated excluding the participants with laboratory test results suggestive of IDA. A diagram showing the statistical analysis plan is presented in figure 1.

Results

The modal age group was the "30 years and older" age category and modal education level was junior high school education. Table 1 displays other socio-demographic characteristics of all the participants, and according to whether or not the woman presented with anemia.

AF-ALB were detected in the blood of all pregnant women in the study. The mean AF-ALB level in maternal serum was 10.9 pg/mg (range = 0.44-268.73 pg/mg). The mean hemoglobin level was 11.7 g/dl ± 1.97 (range: 3.4-17.3 g/dl); 30.3% (n = 229) of participants were anemic based on hemoglobin levels below11g/dl. The higher the AF-ALB level (quartile), the higher the percent of women with anemia (24.7% to 37.4%, trend p = 0.006). As seen in Table 2, both the crude and adjusted associations of AF-ALB with anemia status indicate that a linear trend is present. When aflatoxins were assessed as ordinal variables in the logistic model, the odds of anemia increased 21% (OR, 1.21; 95% CI, 1.04-1.39; p = 0.01) with each quartile of AL-ALB.

The mean serum folate concentration was 7.36 ± 4.69 ng/ml. About 30% of participants had blood folate level below the reference range of 3.9-18.1 ng/ml; however, almost all participants (~97%) were on daily hematinics containing iron (30mg) and folate (800mcg). Because no measurements of iron ferritin or other storage forms of iron were available for this study. However, measures of hemoglobin in RBC described above were used as surrogates to identify

study participants who may have IDA. A total of 175 (23.2%) participants were categorized as having laboratory evidence of IDA. A dose-response relationship between quartile of AF-ALB and increased odds of anemia remains after exclusion of these 175 participants (Table 2). Malaria antigen test revealed that 17% of participants were positive for malaria although these women had no symptoms of malaria infection. About 23% of participants were infected with at least one of the helminth parasites known to contribute to anemia. This included 7.9% who had hookworm, 12.3% who had *Ascaris lumbricoides*, 5.8% who had *Trichiuris trichura* and 3.7% who had *Strongyloides stercoralis* infection.

Table 3 presents stratification of all 580 women without laboratory diagnosis of IDA, by presence or absence of malaria parasitemia, intestinal helmith infection and serum folate status. A much stronger association of very high levels of AF-ALB with anemia is indicated among women with malaria; however, these strata specific estimates are imprecise due to small numbers. Similar results were obtained when all 755 women were stratified by presence or absence of malaria parasitemia, intestinal infection and serum folate status.

Discussion

The study investigated the association between anemia and aflatoxin B_1 -lysine adduct levels among pregnant women attending antenatal clinic in two hospitals in Kumasi, Ghana.

Our findings suggest that the prevalence of anemia among these pregnant women is associated with the AF-ALB levels in their blood.

Aflatoxins should normally not be found in human blood. Our finding that all the participants studied had AF-ALB indicates how widespread aflatoxins are in the population. This finding is similar to that of a study conducted by Jolly et al. who found aflatoxins in the blood of all non-pregnant subjects evaluated in the Ejura Sekyedumase district of Ghana.[64] These results indicate the constancy with which populations from different regions are exposed to aflatoxins in their food.

About 31% of participants had hemoglobin levels less than 11g/dl. This proportion is comparable to the figures reported by Engmann and colleagues who found that 34% of pregnant women were anemic in a study conducted in Accra, Ghana.[100]

The finding of increased odds of anemia with increasing levels of AF-ALB levels in blood is novel given that till date, there has been no study in humans that shows this association. Studies conducted to investigate this association have used guinea pigs, rabbits and cattle.[88, 101] The mechanisms suggested by these studies is that aflatoxins may cause inhibition of hematopoiesis, promote defective hematopoiesis, increase destruction of RBC, or a combination of all three.[92] In humans, it is not clear how aflatoxins may cause anemia, however, the fact that it acts as a toxin suggests that it may, like most other toxins, cause anemia by a hemolytic process.[102] Besides, the accumulation of evidence suggests that aflatoxins may cause DNA damage, mutations, and suppress bone marrow functions.[51, 52] The mechanism for this toxicity is thought to occur by a pathway which involves the metabolism of aflatoxins into epoxide intermediates which go on to bind DNA and RNA. These intermediates interfere with DNA-dependent RNA polymerase, thereby inhibiting RNA and protein synthesis.[53] These effects of aflatoxins may partly explain their possible role in the etiology of anemia.

Since there are many causes of anemia which involve complex pathways, it was necessary to tease out the effect of other causes of anemia from any patho-physiologic pathway involving aflatoxins that impact the occurrence of anemia. The eligibility criteria for the study deliberately recruited participants who were young, did not suffer complications of delivery such as severe hemorrhage, and whose medical records confirmed the absence of chronic diseases such as HIV/AIDS, drugs use which may cause hemolyis, or any evidence to suggest the presence of sickle cell disease or other hemoglobinopathies.

The retention of significant associations between anemia and aflatoxins after exclusion of participants with laboratory results indicative of IDA suggests that the presence of iron may promote the activity of aflatoxin metabolites. Metabolic studies have shown that these aflatoxin metabolites are produced by enzymatic processes which involve hydroxylation, reduction and epoxidation.[53, 103] Iron is a key requirement in the body for oxidative and reductase reactions and is possibly involved in these metabolic pathways. Perhaps, in IDA, the decreased iron levels in the blood depress the conversion of aflatoxins to metabolites which may be involved in the patho-physiology of anemia. Thus, those participants with normal iron levels will preserve the enzymatic processes which lead to production of aflatoxin metabolites and consequently, contribute to anemia. Although IDA is the commonest cause of anemia, excluding the effect of malaria parasitemia is particularly important because malaria causes anemia by hemolysis of RBCs, an etiological pathway through which aflatoxins are thought to act. The stronger association between AF-ALB and anemia among participants with malaria parasitemia is consistent with malaria contributing to anemia.[67] Perhaps in the presence of malaria

(compared to its absence) the odds of having anemia are increased by the presence of aflatoxins. Furthermore, a plausible explanation for this observation could be that these results support the findings from other studies that iron deficiency tends to prevent malaria since this sub-group of participants was devoid of iron deficiency.[104, 105] While there were moderate changes in the effect estimates depending on whether or not helminths and folate deficiency was present, the retention of these associations in the absence of these known causes of anemia may indicate that the effect of aflatoxins is distinct. What is not known is how aflatoxins may interact with these known causes of anemia to modulate the occurrence of anemia. Further research is required to investigate how multivitamin and mineral metabolic processes may modulate the effect of aflatoxins on red blood cells.

Clearly, the cross-sectional nature of this study limits our ability of draw causal relationships between anemia and aflatoxins. Nevertheless, the observed mechanisms identified in experimental animals and our results provide a basis to commission studies to delineate this relationship by using more rigorous study designs. A further limitation of our study stems from our inability to measure plasma ferritin, plasma transferrin saturation or serum soluble transferrin receptor. These limited our ability to adequately measure and assess the prevalence of iron deficiency anemia, which is the most frequent cause of anemia in pregnancy. However, our use of MCV, MCH, and MCHC values did provide us with surrogate indices with which to categorize participants into those that were iron deficient or not for analysis. By so doing, we provided an opportunity to assess the effect of aflatoxins by the RBC hemolytic mechanism which has been suggested from animal studies. Nevertheless, it is noteworthy that there may be residual confounding as a

result of other causes of anemia such as Vitamin B_{12} deficiency, bone marrow depression, Vitamin B_6 deficiency, and other nutritional deficiencies which have not been controlled for by this study. In spite of this, we believe these effects if present, are minimal. The relatively young age of the patients and the absence of any indication of chronic diseases, assured us that the anemia of chronic diseases may have very little impact on our results. A strength of the study lies in the large sample size. This made it possible to categorize variables while retaining reasonable degrees of power to detect otherwise small effect sizes. Because our participants were drawn from a large teaching hospital and an equally large public maternity hospital which cater to the medical needs of a varied population of individuals, this study is generalizable to the larger population of pregnant women in Kumasi residents and indeed women in the Ashanti region of Ghana. The 100% participation rate of the participants further strengthens this external validity. Potentially, these results may be observable among populations in other African countries and Asia where aflatoxin contamination of food-stuff is common.

In conclusion, our study found an association between anemia and aflatoxins. The multi-factorial nature of the etiology of anemia makes it necessary to exercise caution in interpreting the significance of this association. The practical implications of this study lies in the need for policy makers to put in place information and educational tools to increase awareness about aflatoxin contamination of food crops and how this can be prevented. Additionally, health care providers need to educate pregnant women attending prenatal clinics about the potential health hazards associated with eating unwholesome foods which are likely to have been contaminated by aflatoxin producing fungi. By so doing, it is hoped that the health of many pregnant women in resource poor countries will

be safeguarded from known toxins and thereby contribute towards achieving the Millennium Development Goals which are targeted at reducing maternal morbidity and mortality.

Figure 1 legend

Data analytical scheme

Table 1: Socio-demographic variables by anemia status

Table 2: Bivariate and multivariable analysis for all (755) participants and participants without laboratory diagnosis suggestive of iron deficiency anemia (IDA).

Table 3: Sub-group Bivariate and multivariable analysis of participants without IDA.

Figure 1: Statistical analysis scheme

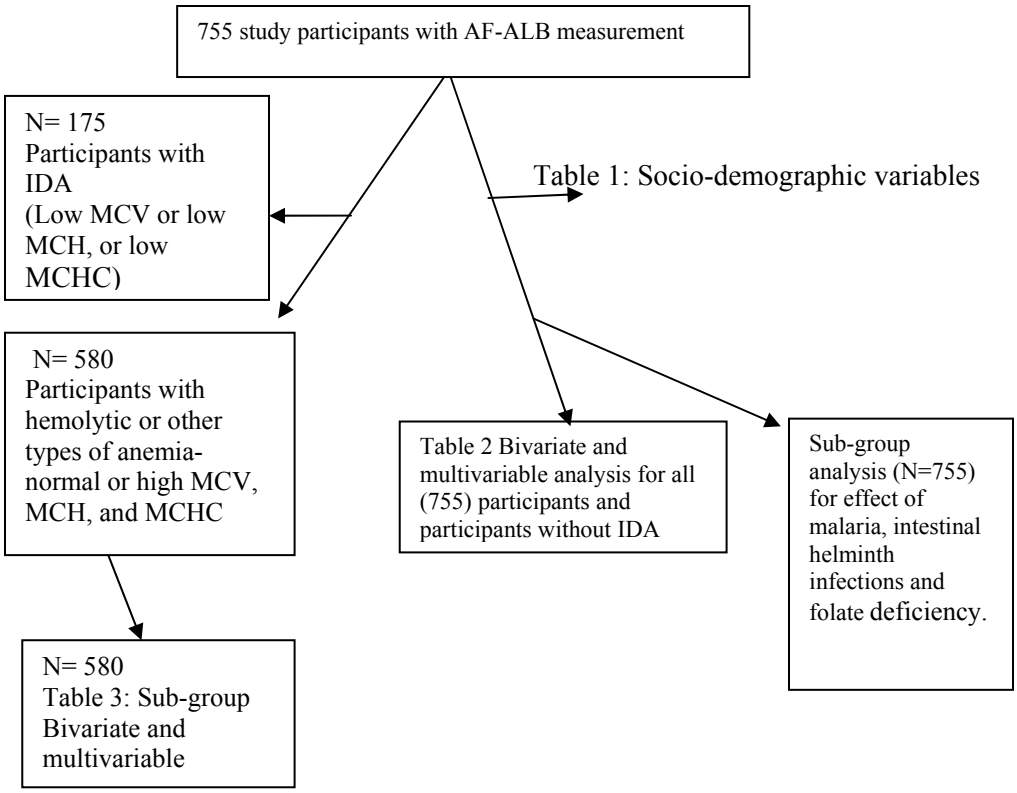

Table 1. Demographic characteristics of 755 pregnant Ghanaian women by anemia status.

Variable	Anemia YES[a]		Anemia NO		Total		P-value
	No.	%	No.	%	No.	%	
Age group in years							0.17
< 20	39	17.0	64	12.4	103	13.8	
20-24	60	26.2	127	24.6	187	25.1	
25-29	67	29.3	148	28.6	215	28.8	
≥ 30	63	32.1	178	34.4	241	32.3	
Formal Education							0.32
None	66	26.3	109	21.3	169	22.8	
Primary or middle school	61	26.8	147	28.7	208	28.1	
Junior high school	78	34.2	172	33.5	250	33.7	
≥ Senior high school	29	12.7	85	16.6	114	15.4	
Weekly income (Ghana cedis)							0.29
< 10	63	27.9	141	27.5	204	27.6	
10-19.9	13	5.8	42	8.2	55	7.4	
20-35.4	96	42.5	187	36.5	283	38.3	
≥ 35.5	54	23.9	143	27.9	197	26.7	
Employment							0.29
Unemployed	70	30.7	154	30.0	224	30.2	
Self-employed	139	60.9	332	64.6	471	63.5	
Employed[b]	19	8.3	28	5.5	47	6.3	
Marital status							
Single	57	25.0	101	19.7	158	21.3	0.08
Living in union	49	21.5	93	18.1	142	19.2	
Married	122	53.5	319	62.2	441	59.5	
No. of children							0.43
0	91	41.0	174	34.8	265	36.7	
1	87	39.2	214	42.8	301	41.7	
2	34	15.3	90	18.0	124	17.2	
3	10	4.5	22	4.4	32	4.4	
Ethnicity							0.06
Akan	146	63.8	365	70.6	511	68.5	
Others	83	36.2	152	29.4	235	31.5	

Table 1 continued…

Maternal aflatoxin B_1-albumin adduct level (Quartiles)	Anemia Yes No.	%	Anemia No No.	%	Total No.	%	P-value
Low (≤ 2.67pg/mg)	46	20.1	140	27.1	186	24.9	**0.05***
Moderate (> 2.67 to ≤ 4.97 pg/mg)	53	23.1	134	25.9	187	25.1	
High (≥ 4.97 to ≤ 11.34 pg/mg)	60	26.2	126	24.4	186	24.9	
Very High (> 11.34 pg/mg)	70	30.6	117	22.6	187	25.1	

[a]= Anemia defined as Hemoglobin < 11.0g/dl

[b]= Public service employee

*=Significant

Numbers may not add up to 755 due to missing values

Table 2: Odds ratios and 95% confidence intervals for the association between anemia and aflatoxin B_1-lysine adduct levels in maternal blood among pregnant women in Kumasi, Ghana.

	All 755 women				Women without laboratory diagnosis of iron deficiency anemia (N = 580)[a]			
Maternal aflatoxin B_1-albumin adduct level (Quartiles)[b]	**Crude OR**	**(95% CI)**	**Adjusted OR[c]**	**(95% CI)**	**Crude OR**	**(95% CI)**	**Adjusted OR[c]**	**(95% CI)**
Low* (≤ 2.67pg/mg)	Ref		Ref		Ref		Ref	
Moderate (> 2.67 to ≤ 4.97 pg/mg)	1.2	(0.76-1.91)	1.34	(0.83-2.16)	1.52	(0.90-2.57)	1.68	(0.90-2.59)
High (≥ 4.97 to ≤ 11.34 pg/mg)	1.4	(0.92-2.28)	1.56	(0.98-2.50)	1.29	(0.75-2.22)	1.57	(0.74-2.26)
Very High (> 11.34 pg/mg)	**1.82**	**(1.17-2.84)**	**1.85**	**(1.16-2.95)**	**2.06**	**(1.23-3.44)**	**2.02**	**(1.19-3.41)**

[a]Laboratory diagnosis of Iron deficiency anemia based on participants with values below the reference ranges for any one of the following: MCV (Mean Corpuscular Volume), MCHC (Mean Corpuscular Hemoglobin Concentration) , or MCH (Mean Corpuscular Hemoglobin). There were 175 participants with this laboratory diagnosis.

[b]Alflatoxin levels categorized into quartiles

OR = Odds ratio, CI = Confidence interval

[c]Odd ratio adjusted for age, educational level, income, marital status, and ethnicity

Figures in bold are significant

Table 3: Adjusted odds ratios with 95% confidence intervals for anemia and maternal aflatoxin levels stratified by malaria infection, helminth infection and folate deficiency status among 580 pregnant women without laboratory evidence of iron deficiency anemia,[a] in Kumasi, Ghana.

Maternal aflatoxin B_1-albumin adduct level (Quartiles)[b]	Malaria		Helminthic Infection[c]		Folate deficiency	
	Yes (N = 89)[e]	No (N = 488)	Yes (N = 144)	No (N = 436)	Yes (N = 227)	No (N = 353)
	OR (95%CI)[d]	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Low (≤ 2.67pg/mg)	Ref	Ref	Ref	Ref	Ref	Ref
Moderate (> 2.67 to ≤ 4.97 pg/mg)	0.62 (0.13-2.94)	1.90 (1.05-3.44)	1.37 (0.44-4.29)	1.54 (0.84-2.83)	0.93 (0.39-2.23)	2.12 (1.07-0.42)
High (≥ 4.97 to ≤ 11.34 pg/mg)	0.49 (0.10-2.37)	1.43 (0.77-2.66)	2.02 (0.65-6.29)	1.06 (0.55-2.05)	2.41 (1.07-5.44)	0.63 (0.26-1.54)
Very High (> 11.34 pg/mg)	**4.48** (0.87-23.18)	**1.91 (1.06-3.45)**	1.84 (0.61-5.5)	**2.06 (1.13-3.78)**	2.23 (0.99-5.06)	1.94 (0.97-3.90)

[a]Laboratory diagnosis of iron deficiency anemia based on participants with values below the reference ranges for any one of the following: MCV (Mean Corpuscular Volume), MCHC (Mean Corpuscular Hemoglobin Concentration), or MCH (Mean Corpuscular Hemoglobin).
[b]= Alflatoxin levels categorized into quartiles
[c]= Helminths investigated were *Hookworm, Ascaris lumbricoides, Strongyloides stercoralis, and Trichuris trichura*
OR= Odds ratio, CI=Confidence Interval
[d]All odds ratios adjusted for age, educational level, income, marital status, and ethnicity
[e]Numbers may not add up to 580 due to missing values
Figures in bold are significant

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ASSOCIATION BETWEEN BIRTH OUTCOMES AND AFLATOXIN B_1-LYSINE ADDUCT LEVELS IN PREGNANT WOMEN IN KUMASI, GHANA

FAISAL M.B SHUAIB, JOHN E. EHIRI, ELLEN FUNKHOUSER, CRAIG WILSON, SHARINA D. PERSON, WILLIAM O. ELLIS

PAULINE E. JOLLY

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Abstract

Objective: To investigate the association between birth outcomes and blood levels of aflatoxin B_1 (AFB_1)-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 birth weight, 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' AFB1-lysine level (> 11.34 pg/mg) were more likely to have low birth weight babies (OR, 2.09; 95% CI, 1.19–3.68), and showed a trend of increasing risk for low birth weight (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.

Introduction

It is estimated that more than 20 million infants worldwide, representing 15.5 percent of all births, are born with low birth weight. Among these cases, 96% occur in developing countries.[3] A baby's low weight at birth (defined as birth weight less than 2500g) is either the result of preterm birth (before 37 weeks of gestation) or due to small for gestational age (sex specific birth weight at or below the 10th percentile for the weight-for-gestational-age of an international reference population).[106] Babies who are small for gestational age are predisposed to adverse birth outcome including stillbirth which occurs when a fetus is born dead after 20 weeks of gestation.[107] Low birth weight is closely associated with fetal and neonatal mortality and morbidity, inhibited growth and cognitive development, and chronic diseases later in life.[3] Many factors affect the duration of gestation, fetal growth, birth weight and thus, the future health of the infant. These factors relate to the fetus itself and the nutrition and health of the mother.[3] It is known that maternal nutrition and health have an impact on the welfare of the fetus and is a predictor of the infant's health.[108] 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.[109, 110] These environmental toxins may find their way to the fetus 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 fetus could lead to intrauterine growth retardation and low birth weight.[24, 29, 31] 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.[111]

At least 4.5 billion people, mostly in resource poor countries, are at risk of chronic exposure to aflatoxins from contaminated food crops.[5] Aflatoxins are a family of toxic metabolites which are derived from the fungi *Aspergillus parasiticus* and *Aspergillus flavus*. These fungi are ubiquitous in hot (temperatures between 25 and 35 degrees centigrade), 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.[112] 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 in quantities and aflatoxins levels in quantities far exceeding the USDAs regulatory limit of 20 ppb.[58, 113]

The carcinogenic, immunosuppressive, and growth retarding effect of long term ingestion of aflatoxins in diet have been documented.[53] 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 which reported on the relationship between birth weight and aflatoxin levels, four indicated significant negative correlations with P values ranging from $P<0.001$ to $P<0.05$.[1, 24, 29, 31] Two studies indicated this negative correlation only among female live births[26, 32] while one study found no association between birth weight and aflatoxins in serum.[27] Furthermore, one study found a significant association ($P<0.01$) between height at birth and aflatoxins.[22]

To date, only two reports have speculated on a possible association between stillbirths and aflatoxin: two stillbirth cases were reported where high levels of aflatoxins were found in both maternal peripheral blood and cord blood.[26] Similarly, one still birth was reported by Lamplugh et al. in their study which was based on aflatoxin in maternal blood.[33]. Only Yousef et al. working in the United Arab Emirate (UAE) has reported the absence of a significant correlation between aflatoxin M_1, and gestational age.[30]

Other investigators have evaluated the association between clinical conditions such as neonatal jaundice, and aflatoxins in neonatal serum. While two studies conducted in UAE[31] and Zaria Nigeria,[25] found no correlation, one study conducted in Ibadan, Nigeria[28] 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, anemia and gut helminth infestation which have been associated with birth outcomes.[114, 115]

Although the above mentioned 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 birth weight, 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 4 of reducing childhood mortality, more directed research is needed to

unravel unknown or suspected causes of low birth weight 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 birth weight, 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 the Kumasi the capital city of the Ashanti region of Ghana in West Africa, which has a population of approximately 1.2 million. [65] Kumasi is the second largest city in Ghana with an area of about 254 square kilometers. It lies about 270Km North of Accra, the capital city of Ghana. Its climate is tropical with two rainy seasons occurring from April to June and from September to October.[66]

Study design and participants

As described in an earlier study,[67] this was a cross-sectional study of women presenting for delivery at two hospitals in Kumasi, the Komfo Anokye Teaching Hospital (KATH) and the 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 a 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 prior to its commencement.

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 (stillbirth, ectopic pregnancy, preterm delivery, and 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).[68] 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, malaria prophylaxis, antihelminthic medication, illnesses, and treatment during pregnancy. A single blood sample was collected in EDTA by venepuncture for determination of malaria antigen, hemoglobin, folate, and aflatoxin levels. Stool samples were obtained for determination of intestinal helminths.

Laboratory procedures

Determination of aflatoxin B_1 albumin adducts. Serum AFB_1-lysine adduct, the major form of AFB_1-albumin adducts which reflects aflatoxin exposure in the previous 2–3 months, was measured by a modified HPLC-fluorescence method.[70] In brief, 150 µl 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 injected to HPLC.

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 X 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 adduct's concentration was adjusted by serum albumin level.

Given that anemia, malaria, and gut infestations have been associated with birth outcomes, the following laboratory procedures were conducted to evaluate these conditions and results were fit into models as confounding variables: (i) determination of malaria antigens*:* Determination of malaria antigen in maternal plasma was done using the Malaria Antigen Celisa assay with a sensitivity and specificity of 98% and 96% respectively.[95] (ii) determination of hookworms, *Ascaris lumbricoides*, and *Trichuris trichura* was done using the Kato-Katz thick smear technique.[96] Samples for detection of *Strongyloides stercoralis* were processed using the Baermann method[97] and (iii) measurement of hemoglobin level in an automatic cell counter (Sysmex M-2000; Digitana AG, Hamburg, Germany). The cut-off level for the third trimester was set at 11g/dl.

Definition of variables

Preterm delivery (PTD) refers to births which occurred before 37 completed weeks of gestation. Low birth weight (LBW) was defined as babies born with birth weight which is less than 2500g. A Small for gestational age (SGA) delivery was defined as sex-specific birth weight at or below the 10th percentile for the weight-for-gestational-age of an international reference population. A still born delivery was defined as a baby born dead after 20 weeks of gestation.[107]

Malaria status was described as the presence or absence of maternal peripheral malaria antigens at delivery. Worm infestation (intestinal) connotes the presence of helminth eggs or larvae in stool samples obtained from the participants. Hemoglobin level was based on three categories: Participants were said to have severe anemia when hemoglobin level is < 8 g/dl, moderately anemic when hemoglobin is between 8g/dl and 11g/dl. Participants with hemoglobin of greater than 11g/dl were considered to be without anemia.

Statistical analysis

Data analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC). 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 albumin adducts in pg/mg ("low": ≤ 2.67pg/mg, "moderate": > 2.67 to ≤ 4.97pg/mg, "high" : > 4.97 to ≤ 11.34pg/mg, "very high" : > 11.34pg/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 step-wise technique.[71] Separate models were run for each adverse birth outcome. 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 (range=0.44-268.73 pg/mg albumin). There were 103 (13.6%) infants who were small for gestational age, 153 (20.3%) who were of low birth weight, 144 (19.1%) who were born preterm and 33 (4.4%) who were stillborn.

The average age of those interviewed was 26.8 ± 6.3 years (s.d) {range: 14 to 48}. Most participants had attended either junior high school (34%) or primary school 28%. Fifteen percent had at least senior high school education. Compared to participants who had higher levels of education beyond high school level (11%), those with no formal education (30.7%) and junior high school education (33%) had higher levels of aflatoxins. The income bracket represented by 20-35.4 Ghana cedis (GHc) (one U.S Dollar=1.4 Cedis) had the most participants with 38.4%. A higher proportion of participants in the lowest income levels (P=0.07) and who were nulliparous (P=0.03) were each in the highest quartile level of aflatoxins (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 anemic, 26% of them were moderately anemic while four percent were severely anemic. 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 birth weight, preterm or still born with aflatoxin levels in the highest quartile (>11.34pg/mg albumin; Table 2. However, this increased odd was statistically significant only with respect to having a low birth weight baby (OR, 2.00; 95% CI, 1.22-3.28). Multivariable analysis showed that, while there was an increased odd with all outcomes when aflatoxin levels were in the highest quartile, this association was significant only with low birth weight 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 birth weight (P_{trend}=0.007).

Discussion

We investigated the possible association between birth outcomes and the serum levels of aflatoxins among pregnant women at delivery. Our findings suggest an association between "very high" levels of aflatoxins and low birth weight.

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 and her colleagues in Accra Ghana. They found that the average age of first delivery for women in their study was 22 years.[116] 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 birth weight infants (OR, 2.09; 95% CI, 1.19-3.68) when compared to women in the lowest quartile, there was a trend of increasing risk for low birth weight with increasing aflatoxin levels (P_{trend}=0.007). This association remained after adjusting for known confounders, including malaria parasitemia, anemia and worm infestations. This result

supports the hypothesis that exposure to aflatoxins may play a role in the incidence of low birth weight. Additionally, this result is similar to that obtained by other investigators in Edo, Nigeria[24] and the United Arab Emirates.[29, 31] While our results also corroborates the findings by two other studies conducted in Kenya[26] and Sierra Leone,[32] 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 levels ranging from 0.68pg/mg-229.49pg/mg albumin (mean=24.37pg/mg albumin). It is possible that the small number of events (33 stillbirths) 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 fetal life are required to produce stillbirths.

Our findings should be interpreted with caution in view of the fact that it was a cross-sectional study which limits 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 levels, while other investigators have used cord blood or even maternal breast milk to assess the exposure of the fetus 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 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 stuff 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 rates of these participants to aflatoxins. Our relatively large sample size was a strong feature of this study since it may have increased the ability to detect some associations that may have been missed by similar studies which generally had smaller sample sizes. [26, 31, 32]

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 birth weight 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 birth

weight predisposes these infants to poor growth and development and adverse health outcomes.

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

Variables	ALL		Quartile 1# (≤ 2.67pg/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)		P-value
	No.	%	No.	%	No.	%	No.	%	No.	%	
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 (Ghana cedis)†											
<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	

Footnotes:# = Aflatoxin B1-lysine adduct levels categorized into quartiles *=Statistically significant. Mean age ± STD =27±6.3 †= 1U.S Dollar is equivalent to 1.4 Ghana cedis (GHc)

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

Variable	Small for Gestational Age (n=103)		Low Birth Weight (n=153)		Preterm Delivery (n=144)		Stillbirth (n=33)	
	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)

Footnotes:

- **OR**=Odds ratio; **CI**=Confidence interval.
- α = adjusted for baby's gender, maternal income level, No of children, maternal educational level, anemia status, malaria status, and worm infection.
- "Low" aflatoxin level: ≤2.67 pg/mg
- "Moderate" aflatoxin level: >2.67 to ≤4.97 pg/mg
- "High" aflatoxin level: >4.97 to ≤ 11.34 pg/mg
- "Very high" aflatoxin level: >11.34 pg/mg
- Figures in bold are significant

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SUMMARY AND CONCLUSION

We found from a cross-sectional study of 755 pregnant women, that all participants had aflatoxin B_1-lysine adduct (AF-ALB) levels in blood, with a mean AF-ALB of 10.9 ± 19.00 pg/mg albumin (range=0.44-268.73 pg/mg/ albumin). The results indicate that socio-economic status has a bearing on the level of aflatoxin contamination of body fluid. Participants with higher income, being employed, having one child (verses no children) and having a flush toilet (verses no toilet facilities) were each independently associated with a 30-40% reduced odds of high AF-ALB levels..

Similarly, the study found that 30.3% of participants were anemic. The odds of being anemic increased 21% (OR, 1.21, p=0.01) with each quartile of AF-ALB reaching an 85% increased odds in the "very high" compared to the "low" category (OR, 1.85; CI, 1.16-2.95). This association was stronger among women with malaria and findings were robust when women with evidence of iron deficiency anemia were excluded.

Furthermore, participants in the highest AFB_1-lysine quartile with 'very high' AFB1-lysine level (>11.34 pg/mg) were more likely to have low birth weight babies (OR, 2.09; 95% CI, 1.19–3.68), and showed a trend of increasing risk for low birth weight (P_{trend} = 0.007) compared to participants in the lowest quartile.

In conclusion, our studies indicate that AF-ALB level in blood is related to socio-economic status of individuals. The associations found in the relationship between the levels of AF-ALB and the various outcomes suggest that AF-ALB adversely affects the pregnant woman and negatively impacts the outcome of births. These findings are of relevance to both patient education and formulation of policies that will improve crop handling, storage and processing such that vulnerable groups will be protected from

aflatoxin contaminated food. On a larger scale, implementing these public health measures may contribute towards achieving some of the Millennium Development Goals which are targeted at reducing maternal and infant morbidity and mortality.

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APPENDIX

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56 and ICH GCP Guidelines. The Assurance became effective on November 24, 2003 and expires on January 23, 2012. The Assurance number is FWA00005960.

Principal Investigator:	SHUAIB, FAISAL M
Co-Investigator(s):	JOLLY, PAULINE EVADNE
Protocol Number:	**X090607009**
Protocol Title:	*Association between aflatoxin B1 biomarker levels in pregnant women and birth outcomes in Kumasi, Ghana*

The IRB reviewed and approved the above named project on 6/8/09. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 6-8-09

Date IRB Approval Issued: 6/8/09

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

470 Administration Building
701 20th Street South
205.934.3789
Fax 205.934.1301
irb@uab.edu

The University of
Alabama at Birmingham
Mailing Address:
AB 470
1530 3RD AVE S
BIRMINGHAM AL 35294-0104