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# Association between Anemia and Aflatoxin $B_1$ Biomarker Levels among Pregnant Women in Kumasi, Ghana

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*Abstract.* Aflatoxins are fungal metabolites that contaminate staple food crops in many developing countries. Up to 40% of women attending a prenatal clinic in Africa may be anemic. In a cross-sectional study of 755 pregnant women, Aflatoxin $B_1$-lysine adducts (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 < 11 g/dL. Logistic regression was used to examine the association of anemia with AF-ALB. 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% (odds ratio [OR], 1.21, $P = 0.01$) with each quartile of AF-ALB reaching an 85% increased odds in the "very high" compared with the "low" category (OR, 1.85; confidence interval [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. 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.[1] 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 with 1 in 4,100 in industrialized countries.[1] The Millennium Development goal #5 is to reduce the maternal mortality ratio by 75% between 1990 and 2015,[2] 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.[2]

Anemia afflicts about two billion people worldwide.[3] It is a clinical condition characterized by reduced ability of the red blood cells (RBCs) to carry oxygen to body tissues, usually as a result of their decreased hemoglobin content. The World Health Organization (WHO) defines anemia as a hemoglobin level of < 11 g/dL during the first and third trimester, whereas the cutoff is 10.5 g/dL in the second trimester.[4]

Anemia in pregnancy is one of the leading causes of maternal mortality. 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.[5] Only 23% of pregnant women in industrialized countries are anemic compared with 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.[6] Anemia in pregnancy is ranked among the top four causes of morbidity and mortality among pregnant women in Africa and is the second leading cause in Asia.[7] About 40% of women attending prenatal clinics in Africa suffer from anemia at different stages of pregnancy.[8] Sixty-two reports compiled by WHO on the proportion of maternal deaths associated with anemia indicate that in 74% of these reports, anemia was a contributory factor to the documented maternal mortality.[9] Although 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.[10] Consequently, many women who die during pregnancy or delivery from causes related to anemia die at home and are thus 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, vitamin $B_{12}$, and vitamin A deficiencies, infective causes such as malaria, and intestinal helminth infections, ineffective erythropoiesis, severe hemorrhage, hemoglobinopathies, chronic diseases, G6PD deficiency, and hemolytic anemia from drugs or toxins.[8,11–13] Consequently, any assessment of the cause of anemia must put these into consideration 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 foodstuff 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.[14] Aflatoxins are toxic metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. They are classified on the basis of 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.[15,16] Staple foods such as cereals, groundnuts, and other oil seeds are most prone to aflatoxin contamination.[17,18] Aflatoxin contamination is influenced by high humidity, high temperatures, insect and rodent activity, and inadequate drying of the crops. Contamination occurs

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frequently in countries of Africa and Asia. This contamination can occur at any stage of food production from pre-harvest to storage.[19] Measurements of aflatoxin levels in food crops have been conducted in some West African countries such as Ghana where eight samples of maize from 15 (53%) processing sites revealed contamination with fumonisins and aflatoxins.[19,20] One study, which collected samples from major processing sites in Accra, Ghana, reported aflatoxin levels that ranged from 2–662 μg/kg.[20] The latter quantities far exceed the United States Department of Agriculture's (USDA) regulatory limit of 20 ppb.[21] 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.[22] Aflatoxins have been implicated in the pathogenesis of conditions such as primary liver cell carcinoma, malnutrition, immunosuppresion, and growth retardation.[17]

Aflatoxins have been associated with hemolytic anemia in mammals.[23] Studies with these mammals (cattle, guinea pigs, and rabbits) have shown that aflatoxins cause hemolysis of RBCs and may interact with RNA and DNA to cause a depression of hemopoiesis in primates.[24] Although this has not been documented to occur in humans, it is not unreasonable 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 ~1.2 million[25]; Kumasi is the second largest city in Ghana. The climate is hot and humid with two rainy seasons occurring from April to June and from September to October.[26] The climate and poor pre- and post-harvest handling of crops contributes to fungal growth and aflatoxin contamination of staple crops such as maize, corn, and groundnuts.[21,27] In Ghana, up to 44% of women still deliver at home.[28] However, because Kumasi is relatively urban, many more women in this city tend to use hospitals for delivery services.

**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,[12] all women who had a singleton, uncomplicated pregnancy were identified from admission records and invited to participate. Women who had a 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 before its implementation.

**Study instruments and data collection.** After informed consent was obtained, questionnaires were administered by two trained interviewers and the principal investigator. 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).[29] Obstetric information was obtained from the women's antenatal care (ANC) charts, which were well kept in both institutions. The 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. The principal investigator of the project closely supervised the data collection process and provided immediate data auditing feedbacks. Data entry involved double data entry and measures of agreement such as coefficient of variation and regression plots were used to verify integrity of data being collected. Laboratory technicians from KATH collected a single blood sample in EDTA by venepuncture for determination of complete blood count (CBC) and differentials, RBC 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 high-performance liquid chromatography (HPLC)-fluorescence method.[30] In brief, 150 μL serum samples were digested by Pronase and loaded onto an Oasis Max cartridge from Waters Co. (Milford, MA). 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.

The HPLC analysis was carried out on an 1100 liquid chromatography system (Agilent Technologies, Wilmington, DE). Chromatographic separation was performed on an Agilent C18 column (5-μm particle size, 250 × 4.6 mm). The mobile phase consisted of 20 mM ammonium phosphate monobasic (pH 7.2) and methanol in a linear gradient profile. The concentration of $AFB_1$-lysine adducts was monitored at wavelengths of 405 nm (excitation) and 470 nm (emission). The peak of authentic 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; hence, these conditions were also evaluated. Determination of malaria antigen in plasma was done using the Malaria Antigen CELISA (Cellabs, Sydney, Australia) assay with sensitivity and specificity of 98% and 96%, respectively.[31] 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, *A. lumbricoides* and *T. trichura* ova in stool samples was done using the Kato-Katz thick smear technique.[32] Samples for detection of *S. stercoralis* were processed using the Baermann method.[33] Hemoglobin level was measured in an automatic cell counter (Sysmex M-2000; Digitana AG, Hamburg, Germany). Anemia was defined as hemoglobin level < 11 g/dL.[3] Serum folate concentrations were measured by radioimmunoassay Quantaphase II (Bio-Rad, Hercules, CA). The reference interval for folate in women is 3.9–18.1 ng/mL.[34] Folate deficiency was based on serum levels of < 3.9 ng/mL.

Because we were interested in examining hemolytic anemia, possibly caused by aflatoxins, we attempted to separate participants with IDA from those with hemolytic anemia using measures of hemoglobin in RBCs. In the absence of plasma iron measurements, these measures were used as surrogates of IDA. Moreover, Phiri and others[35] have shown that measures such as the mean corpuscular hemoglobin concentration (MCHC) are valid measures for assessing iron deficiency. These tests were conducted by the KATH hospital laboratory. They included the following: mean corpuscular volume (MCV) with a reference range of 80–96 fL, MCHC with reference range 32–36 g/dL, and mean corpuscular hemoglobin (MCH) with reference range of 27 to 33 pg.[36] On the basis of 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 are either unchanged or increased above the upper limit of 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 on the basis of the distribution of AF-ALB in blood ("low": ≤ 2.67 pg/mg, "moderate": > 2.67 to ≤ 4.97 pg/mg, "high": > 4.97 to ≤ 11.34 pg/mg, and "very high": > 11.34 pg/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 stepwise technique.[37] 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, to minimize the confounding 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.

## 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 the woman

TABLE 1
Demographic characteristics of 755 pregnant Ghanaian women by anemia status

| Variable | Anemia YES* no. | % | Anemia NO no. | % | Total no. | % | *P* value |
|---|---|---|---|---|---|---|---|
| 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-used | 139 | 60.9 | 332 | 64.6 | 471 | 63.5 | |
| Used† | 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 | |

*Anemia defined as hemoglobin < 11.0 g/dL.
†Public service employee.
Numbers may not add up to 755 because of missing values.

presented with anemia. The AF-ALB was 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 < 11 g/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. Furthermore, there appeared to be a stronger association between anemia and aflatoxin in the absence of iron deficiency: OR and 95% CI in the absence of IDA is 2.02 (1.19–3.41), whereas it is 0.95 (0.34–2.64) in the presence of IDA (not shown in Table 2).

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 a 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 (30 mg) and folate (800 mcg).

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). The 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 *A. lumbricoides*, 5.8% who had *T. trichura*, and 3.7% who had *S. 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 because of 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 an 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 and others[22] who found aflatoxins in the blood of all non-pregnant subjects evaluated in the Ejura Sekyedumase district of Ghana. 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 < 11 g/dL. This proportion is comparable to the figures reported by Engmann and colleagues[38] who found that 34% of pregnant women were anemic in a study conducted in Accra, Ghana.

The finding of increased odds of anemia with increasing levels of AF-ALB levels in blood is novel. To the best of our knowledge, there has been no study in humans that shows this association. Studies conducted to investigate this association have used guinea pigs, rabbits, and cattle.[15,39] The mechanisms suggested by these studies include those that suggest that aflatoxins may cause inhibition of hematopoiesis, promote defective hematopoiesis, increase destruction of RBCs, or a combination of all three.[23] 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.[40] The accumulation of evidence suggests that aflatoxins may cause DNA damage, mutations, and suppress bone marrow functions.[41,42] The mechanism for this toxicity is thought to occur by a pathway, which involves the metabolism of aflatoxins into epoxide intermediates that go on to bind DNA and RNA. These intermediates interfere with

DNA-dependent RNA polymerase, thereby inhibiting RNA and protein synthesis.[17] These effects of aflatoxins may partly explain their possible role in the etiology of anemia in this population.

Because 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

Table 2

Odds ratios (OR) and 95% confidence intervals (CI) for the association between anemia and aflatoxin $B_1$ albumin adduct levels in maternal blood among pregnant women in Kumasi, Ghana

| | All 755 women | | | | Women without laboratory diagnosis of iron deficiency anemia ($N$ = 580)* | | | |
|---|---|---|---|---|---|---|---|---|
| Maternal aflatoxin $B_1$-albumin adduct level (quartiles)† | Crude OR | (95% CI) | Adjusted OR‡ | (95% CI) | Crude OR | (95% CI) | Adjusted OR‡ | (95% CI) |
| Low* (≤ 2.67 pg/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) |

* 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.
† Alflatoxin levels categorized into quartiles.
‡ Odds ratio adjusted for age, educational level, income, marital status, and ethnicity.
Ref = reference group.

TABLE 3

Adjusted odds ratios (OR) with 95% confidence intervals (CI) 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,* in Kumasi, Ghana

| Maternal aflatoxin $B_1$-albumin adduct level (quartiles)† | Malaria | | Helminthic infection‡ | | Folate deficiency | |
|---|---|---|---|---|---|---|
| | Yes ($N$ = 89)§ | No ($N$ = 488) | Yes ($N$ = 144) | No ($N$ = 436) | Yes ($N$ = 227) | No ($N$ = 353) |
| | OR (95% CI)¶ | OR (95% CI) | OR (95% CI) | OR (95% CI) | OR (95% CI) | OR (95% CI) |
| Low (≤ 2.67 pg/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) |

* 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).
† Alflatoxin levels categorized into quartiles.
‡ Helminths investigated were Hookworm, *Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Trichuris trichura*.
§ Numbers may not add up to 580 because of missing values.
¶ All odds ratios adjusted for age, educational level, income, marital status, and ethnicity.
Ref = reference group.

confirmed the absence of chronic diseases such as human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), drug use that may cause hemolyis, or any evidence to suggest the presence of sickle cell disease or other hemoglobinopathies.

The increase in the strength and 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.[17,43] 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 pathophysiology 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. While there were moderate changes in the effect estimates depending on whether 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 RBCs.

Clearly, the cross-sectional nature of this study limits our ability to draw causal relationships between anemia and aflatoxins. Nevertheless, the observed mechanisms identified in mammals 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, vitamin A deficiency, and other nutritional deficiencies, which have not been controlled by this study. Nevertheless, 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. 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 foodstuff 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 lie 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 toward reduction of anemia prevalence, particularly among pregnant women, thereby reducing maternal mortality and contributing to the achievement of the Millennium Development Goals, which are targeted at reducing maternal morbidity and mortality.

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