ORIGINAL ARTICLES

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

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SUMMARY

Objectives: 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.

Design: A cross-sectional study was conducted among 785 pregnant women in Kumasi. Aflatoxin B_1 lysine adduct levels (AF-ALB) were determined by High Performance Liquid Chromatography. Analysis of variance was used to determine mean log AF-ALB levels and significance of differences in these levels according to socio-demographic variables. Logistic regression was used to identify independent associations of socio-demographics with having AF-ALB levels (≥ 11.34pg/mg; upper quartile).

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 socioeconomic status: higher education and income, being employed and having a flush toilet. 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.

Conclusions: 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.

Keywords: 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.[1] In humans, aflatoxins have been implicated in the pathogenesis of primary liver cell carcinoma, immunosuppresion, malnutrition, infertility and growth retardation.[2]

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 foodstuff.

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 regulatory limit of 20 ppb.[3]

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.[4] Acute aflatoxin outbreaks are a 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%).[5] 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.[6] 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 puerperal women from developing countries indicate very high levels of aflatoxin in maternal serum, neonatal umbilical cord blood, and in breast milk.[7-14] 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).[9] These findings 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.[13-16] 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 transplacental transfer of aflatoxins and its accumulation in the fetus.[17] 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.[18] 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.[19] A study conducted by Wild and colleagues in France did not report any aflatoxin contamination of 42 samples of breast milk.[20] 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) [21] may be partly responsible for these observed disparities in aflatoxin contamination of breast milk in developed and less developed countries.

No studies to date have assessed the role of socio-demographic variables in aflatoxin exposure among pregnant women in sub-Saharan Africa, where aflatoxin contamination of food is common. 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.[22-23] 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.

MATERIALS AND METHODS

Study setting

The study was conducted in Kumasi, the capital city of the Ashanti region of Ghana in West Africa, which has a population of approximately 1.2 million.[24] Its climate is tropical with two rainy seasons occurring from April to June and from September to October.[25]

Study design and participants

As described in an earlier study[26], 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 trained interviewer administered a questionnaire. 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).[27] Obstetric information was obtained from the women's antenatal care (ANC) charts and is published elsewhere.[28] 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 [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 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 analysis. 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 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 logistic, 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).[30]

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. Approximately 25% of the women had AF-ALB levels of >5.0-≤10.0 pg/mg albumin and 15% had AF-ALB >25.0 pg/mg albumin.

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 socioeconomic status, 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.

Table 1 Demographic characteristics of 755 pregnant Ghanaian women and corresponding 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)
Col % = Column Percent

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 β 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 high aflatoxin B_1-lysine adduct levels (> =11.34pg/mg) and socio-demographic characteristics of 755 pregnant Ghanaian women.

Variables	Crude Odds Ratio	95% Confidence Interval	Adjusted Odds Ratio♣	95% Confidence Interval
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

Ref= Reference category

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.[31] 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.[32] 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)[32] 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 participants from large number households, this earning should place them above the poverty line. Since 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 comprised 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.[33] 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[34] and those reported by Wang et al. from areas with high liver-cancer risk in China.[35] It is however higher than levels reported by a study among Gambians.[36]

The results of multivariable analysis indicate that 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.[33] 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. Some of the commonly contaminated food products are maize-based such as "Kenkey". Kenkey is a staple food eaten by most residents and frequently contaminated with aflatoxins.[37]

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 influenced by identical factors as the income levels. 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 necessarily 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 associated with having lower 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 M_1 in breast milk and the parity of participants [11]. On the other hand, a study (in Qalyubiyah, Egypt) which also used breast milk as the source of aflatoxin M_1 found that detection of aflatoxin in breast milk was more likely if the participant had more than one child [38]. 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 foodstuff 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 generalizability of our results to the general population of women in Kumasi city, Ghana.

CONCLUSION

In conclusion, this 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.

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