Sociodemographic, Health & Immune Factors Associated with Aflatoxin Ingestion in Ghanaians

Pauline Jolly, PhD, MPH[1], Jennifer Appawu[1], and William Ellis, PhD[2]

[1]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, Alabama, [2]Department of Biochemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Abstract

Background: Aflatoxins are potent carcinogens found in crops, such as, peanuts, rice, maize and other cereals that form the staple foods of people in developing countries. Epidemiological studies have linked dietary aflatoxin exposure with hepatocellular carcinoma (HCC) in humans and have shown that the risk of aflatoxin-induced HCC is about 30 times greater in people infected with hepatitis B virus (HBV). Numerous animal studies have shown that aflatoxin is immune suppressive and results in increased susceptibility to infections, reactivation of chronic infection and reduction of antibody responses to vaccines. Chronic aflatoxin exposure has also been shown to interfere with metabolism of proteins and a number of micronutrients that are critical to health and immune functioning. However, few studies have investigated the effect of aflatoxin on the health and immune status in humans.

Methods: We conducted a cross-sectional study in four villages in the Ejura Sekyedumase district of Ghana to measure the aflatoxin albumin adduct biomarker (AF-ALB) levels in the blood of the people and examine the association between AF-ALB levels and several socio-demographic and health factors and immune status.

Results: AF-ALB were found in the plasma of all participants. By multivariate analyses, ethnic group, the village in which participants lived, the number of individuals in the household, and having at least one child in secondary school were significant predictors of AF-ALB. Participants who reported symptoms of acute aflatoxicosis, such as, a history of yellow mouth, history of sore swollen stomach, and history of painful vomiting were more likely to have high AF-ALB levels. High proportions of the study group had abnormal levels of total protein (30.9%), low levels of albumin (40%) and high alanine aminotransferase (ALT, 41%). Approximately 31% of participants were positive for HBV (16.4%) or hepatitis C virus (HCV, 14.3%) infections. HBV infection, high total protein and high ALT were significantly associated with high AF-ALB. Thirty-one percent of participants were deficient in vitamin A and 71% deficient in vitamin E. Those with high AF-ALB had significantly lower vitamin A and E levels. Participants with high AF-ALB had significantly lower percentages of activated T and B cells (CD3+CD69+ and CD19+CD69+) and lower levels of CD8+T cells that expressed perforin, or both perforin and granzyme A compared to those with low AF-ALB.

Conclusion: These findings indicate strongly that the health of people in this region of Ghana is adversely affected by aflatoxin exposure. The alterations in immunological parameters in those with high AF-ALB levels could result in impairments in cellular immunity that could decrease resistance to infections. There is need for specifically targeted interventions to reduce aflatoxin exposure in Ghana.

Aflatoxins

- Aflatoxins are produced by fungi (*Aspergillus sp.*) that grow on crops such as corn, peanuts & other oil seeds
- They are potent hepatotoxic, genotoxic, carcinogenic and immunosuppressive agents
  - The major aflatoxins are B1, B2, G1, and G2
  - B1 is the most biologically active aflatoxin
  - AFM1 is a metabolite of B1 found in milk and urine

Outbreaks of Acute Aflatoxicosis - Worldwide

- Outbreak of Aflatoxin Poisoning --- Eastern and Central Provinces, Kenya, January--July 2004. MMWR 53:790-793, Sept. 2, 2004. (317 cases, 125 deaths; CFR=??)
- Spoilt Maize Threatens Schools Food Programme, Barasa, L. – Daily Nation, Nairobi, May 20, 2005. (15 deaths)
- Outbreak of Acute Hepatitis Caused by Aflatoxin Poisoning in Kenya, A. Ngindu, et al. - Lancet, June 12, 1982, pp. 1346-1348.
- Other large outbreaks have occurred in India (1975) and Malaysia (1995).

Regional map of Ghana

Maize Peanuts

Maize and peanuts in Ejura, Ghana

Maize after harvest in Ejura, Ghana

Study objectives

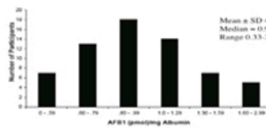
- Establish a baseline for aflatoxin exposure in the people
- Examine sociodemographic and health factors associated with AF-ALB levels
- Examine cellular immune status in relation to AF-ALB levels
  - Determine percentages and cytokine expression of different white blood cell phenotypes (CD3, CD4, CD4CD69, CD8, CD19, CD19CD69, CD14, CD56)
- Examine vitamin A and E levels in relation to AF-ALB

Nkwanta

Demographic characteristics of participants

	Males n = 80 n (%)	Females n = 82 n (%)
Age		
19-29	20 (25.0)	28 (34.1)
30-39	21 (26.3)	21 (25.6)
40-49	19 (23.8)	13 (15.9)
50-69	13 (16.3)	11 (13.4)
≥ 70	7 (8.8)	9 (11.0)
Education		
No formal	31 (39.2)	51 (63.0)
Formal	48 (60.8)	30 (37.0)
Occupation		
Farmer	54 (68.4)	39 (48.2)
Trader	3 (3.8)	22 (27.2)
Farmer/trader/other	7 (8.8)	9(11.1)
Other	15 (19.0)	11 (13.6)

AF-ALB in plasma of study participants



Sociodemographic factors associated with high AF-ALB

- Level of education (risk greater for those with primary or no education)
- Certain ethnic groups (cereal & peanuts as staples)
- Live in particular villages
- Larger number of household members (risk increased with > 5 household members)
- Children in secondary school (risk greater with 1 or more in secondary school)

Health indicators – study population

Abnormal liver function, hepatitis, malaria & HIV infections

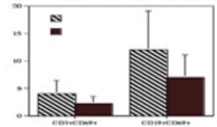
Total protein (high/low)	30%
Albumin (low)	33%
Alanine transaminase (high)	40%
Hepatitis B	16.4%
Hepatitis C	14.3%*
Hepatitis B & C	30.7%
Malaria antigen	20.3%
HIV+	2%

Health factors significantly associated with high AF-ALB

- Experienced yellowing of the mouth, painful vomiting,sore swollen stomach
- Hepatitis B positive
- Total protein (increased 0.27 units for every unit increase in AF-ALB)
- ALT (increased 0.20 units for every unit increase in AF-ALB)
- Vitamin A level (deficient = 31%; mean ± SD 35.47 ± 19.78 μg/dL; range= 8.3-117.52)
- Vitamin E level (deficient = 71% (mean ± SD 0.44 ± 0.28 mg/dL; range = 0.02-1.82 mg/dL).

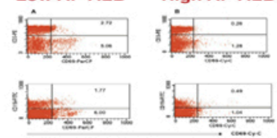
Those with high AF-ALB had significantly lower vitamin A & E levels
- Normal range for vitamin A = 25-75 μg/dL.
- Normal range for vitamin E = 0.6-1.4 μg/dL.

Percentages of activated T and B cells in relation to AF-ALB levels



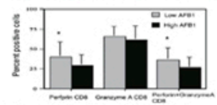
The percentages of CD3CD69 (2.164 ± 1.24 vs 3.905 ± 2.47) and CD19CD69 (6.92 ± 4.15 vs. 11.945 ± 7.07) were significantly lower (p=0.002) for those with high AF-ALB levels compared to those with low AF-ALB.

Low AF-ALB High AF-ALB



The percentages of CD3CD69 and CD19CD69 cells in a participant with high AF-ALB are much lower (0.26% and 0.49% respectively) than in a participant with low AF-ALB (2.72% and 1.77% respectively).

Perforin and Granzyme A positive CD8+ T cells and AF-ALB levels



The percentages of CD8+ T cells that contained perforin, and both perforin and granzyme were significantly lower in participants with high AF-ALB levels (29.402 ± 13.75 and 26.913 ± 12.96 respectively) compared to those with low AF-ALB levels (40.334±18.55 and 36.26±15.04 respectively, p=0.002 for both).

Summary: Immune status and high AF-ALB levels

Participants with high AF-ALB levels had:
- Significantly lower levels of T (CD3) and B (CD19) cells expressing the CD69 activation marker (prevent cells from mounting appropriate immune responses)
- Significantly lower levels of perforin and perforin + granzyme A secreting CD8+ T cells (prevent cell-killing function that prevents the spread of infection)
- Lower percentages of CD3-CD56+CD16- NK cells and perforin+ NK cells (not significant)
- Slightly lower percentage of CD14+ monocytes and lower monocyte phagocytic rate (not significant)

These alterations indicate impairment in cellular immunity in those with high AF-ALB.

Collaborators and Support

Pauline E. Jolly & Yi Jiang, MD, MPH, Research Associate , Department of Epidemiology, School of Public Health, University of Alabama at Birmingham, Birmingham, Alabama.

William Ellis, PhD, Professor, Department of Biochemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Jia Sheng Wang, PhD, Head, Department of Environmental Health Science, College of Public Health, University of Georgia, Athens, Georgia.

Curtis M. Jolly, PhD, Professor and Head, Department fo Agricultural Economics & Rural Sociology Auburn University, Auburn, Alabama.

Timothy Phillips, PhD, Professor, Faculty of Toxicology, veterinary Integrative Biosciences, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Texas.

Jonathan Williams, PhD, Director, Peanut Collaborative Research Support Program & Professor, College of Agricultural and Environmental Sciences, University of Georgia, Griffin, Georgia.

Supported by USAID Peanut Collaborative Research Support Program grant LAG-G-00-96-90013-00, University of Georgia, and the Minority Health International Research Training Program grant # MD001448-09, National Center on Minority Health and Health Disparities, NIH.