Association of Aflatoxin Levels with Viral Load, Liver Function, Immune Impairments, Vitamins A and E Levels and Tuberculosis Infection in HIV Positive Ghanaians

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Abstract

Background: Aflatoxins are potent cytotoxic, carcinogenic, and immunosuppressive agents. Chronic aflatoxin exposure has been shown to interfere with metabolism of proteins, food conversion, growth, and a number of micronutrients that are critical to health and immune functioning. Both aflatoxin and HIV cause immune suppression, which can impair resistance to both infections and chronic diseases.

Objective: We conducted a cross-sectional study among HIV+ and HIV- people in Ghana in which the association between certain clinical factors, immune status, infectious diseases and vitamins A and E levels were examined in relation to aflatoxin B1 albumin-adduct (AF-ALB) levels.

Method: Plasma samples collected from 314 (159 HIV+ and 159 HIV-) people were tested for AF-ALB levels. CD4+ T cell count, liver function profile, malaria parasitemia, hepatitis B (HBV) virus infection, vitamins A and E concentrations, and HIV viral load. Peripheral blood mononuclear cells prepared from participants' blood were used to determine the percentages of leukocyte immunophenotypes and cytokine expression by flow cytometry.

HIV positive participants were divided into high and low groups based on their median AF-ALB level and multivariable logistic and linear regression methods used to assess relationships between clinical conditions and AF-ALB levels. Hazard ratios (HRs) with 95% confidence intervals (CI) and a test for trend for opportunistic infections among HIV positive Ghanaians based on quartiles of AF-ALB were calculated. The association of vitamins A and E with HIV status, aflatoxin levels, and HBV infection was examined.

Results: Multivariable logistic regression showed statistically significant increased odds of having higher HIV viral loads (OR, 2.66; 95% CI, 1.15-7.70) and higher direct bilirubin levels (OR, 5.47; 95% CI, 1.03-22.85) among HIV positive participants in the high AF-ALB group. There were also higher levels of viral hepatitis and lower levels of albumin in association with high AF-ALB. Among both HIV positive and negative participants, high AF-ALB was associated with lower perforin expression on CD8+ T-cells ($P = .012$). HIV positive participants with high AF-ALB had significantly lower percentages of CD4+ T regulatory cells (Tregs; $P = .005$) and naive CD4+ T cells ($P = .029$) and significantly reduced percentages of B cells ($P = .01$) compared to HIV positive participants with low AF-ALB. HRs were significantly higher for developing symptomatic TB (HR 3.30; 95% CI 1.24-8.21) for those in the highest AF-ALB quartile compared to the lowest. Significantly higher HRs were not observed for other infectious (malaria, HBV, or pneumonia) investigated. HIV-infected participants had significantly lower levels of vitamin A (<.16 Mcg/dL; $p<0.0001$) and vitamin E (<6.22 mg/dL; $p<0.001$). For the total study group, higher AF-ALB was associated with significantly lower vitamin A (<4.83 g/dL for every 0.2 pmol/mg increase in AF-ALB). HBV-infected people had significantly lower vitamin A (<5.94mg/dL; $p<0.01$). Vitamins A and E levels were inversely associated with HIV viral load ($p<0.02$ for each), and low vitamin E was associated with lower CD4 counts ($p<0.004$).

Conclusions: Our results indicate that aflatoxin exposure may contribute to high viral loads and abnormal liver function in HIV positive people and so promote disease progression. High AF-ALB appeared to accentuate some HIV associated changes in T-cell phenotypes and B-cells in HIV positive participants. Those with the highest levels AF-ALB have an increased hazard of symptomatic TB. Our finding of the significant decrease in vitamin A associated with AF-ALB suggests that aflatoxin exposure significantly compromises the micronutrient status of people who are already facing overwhelming health problems, including HIV infection.

Hypothesis

- Based on the literature, we hypothesize that aflatoxin exposure will result in immune suppression, decrease resistance to infectious diseases, decrease in micronutrients that are critical to health and immune functioning, and result in more severe disease and faster progression to AIDS.

Specific Aims

- Examine the association between certain clinical factors (HIV viral load, liver function profile) and AF-ALB levels in HIV positive Ghanaians.
- Determine the percentages of leukocyte immunophenotypes and cytokine expression of HIV + and HIV- people in relation to AF-ALB levels.
- Determine the association between AF-ALB levels and development of opportunistic infections (TB, malaria, HBV, pneumonia, herpes) among HIV positive people.
- Examine the association of vitamin A and E levels with HIV status, AF-ALB levels, and HBV infection.

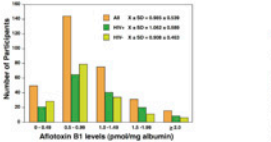
Method

- Recruited a convenience sample of clinic-based 155 HIV+ and 159 HIV- Ghanaians from Kumasi and its surroundings.
- Administered a questionnaire on demographic factors, health status and food consumption and collected a 20 mL blood sample. Clinical data were collected from medical records.
- HIV was separated from HIV+ plasma and peripheral blood mononuclear cells (PBMCs) and plasma tested for AF-ALB levels, CD4+ T cell count, liver function profile, malaria parasitemia, HBV infection, vitamin A and E concentrations, and HIV viral load. PBMCs were used to determine the percentages of leukocyte immunophenotypes and cytokine expression by flow cytometry.
- Data analyzed to address the specific aims (see abstract for details).

Demographic characteristics of HIV+ and HIV- participants

Characteristics		HIV positive	Healthy negative
Number of participants		155	159
Age (mean ± SD) years		38.25 ± 9.43	40.77 ± 17.52
Gender	Male	52 (33.6%)	83 (52.2%)
	Female	104 (66.4%)	76 (47.8%)
HIV viral load (copies/mL)		84,097 ± 22,072	0
	Mean ± SD		
CD4 count (cells/ul) Mean ± SD		308 ± 105	1101 ± 106

AF-ALB levels in HIV positive and HIV negative study participants



Clinical Factors Associated with AF-ALB among HIV+ Ghanaians

Variable		AF-ALD ≤ median N	AF-ALD ≤ median %	AF-ALD > median N	AF-ALD > median %	Adjusted point estimate (95% CI)	P-value
CD4+ count							
<200		23	35.4	29	41.4	Ref.	
200-400		32	49.2	31	44.3	0.96 (0.37, 2.52)	0.74
> 500		10	15.4	10	14.3	1.27 (0.30, 5.34)	0.70
HIV viral load							
≤ 10,000		30	39.0	30	38.5	**2.84 (1.17-7.70)**	**0.03**
> 10,000		47	61.0	48	61.5	Ref.	
Direct bilirubin							
Low (<0.3 mg/dL)		11	14.1	11	14.3	0.91 (0.25, 3.21)	0.35
Normal (0.1-0.3 mg/dL)		59	79.6	65	84.4	Ref.	
High (>0.3 mg/dL)		8	10.3	1	1.3	**5.47 (1.03, 22.85)**	**0.046**

Final Linear Regression Model for Selected Clinical Characteristics and AF-ALB for HIV Positive Patients

Variable	Parameter estimate	Standard error	Pr>
Intercept	0.01553	0.38521	0.02
CD4 count	0.0042862	0.38521	0.05
Viral load	2.7175581 E-7	w125393 E-7	0.37
Total Protein	0.3644	0.02218	0.26
Albumin	-0.04850	0.05579	**0.01**
Total Bilirubin	0.36126	0.10665	**<0.01**
Direct Bilirubin	0.91790	0.20157	**<0.01**
Alkaline Phosphatase	0.58444	1.52004	0.37
ALT	0.00144	0.00373	0.70
AST	-0.00114	0.00120	0.34

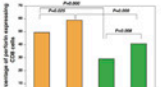
Percentages of CD4+CD25+CD45RO+ regulatory T cells

Percentage of CD3 - CD19+ B Cells

Summary: Immune impairments in HIV+ participants in relation to high AF-ALB levels

- Decrease in percentage of B cells indicates decrease in antibody response.
- Decrease in perforin expressing CD8 cells indicates decrease in cytotoxic activity.
- Decrease in regulatory T cells suggests increased immune hyperactivation and increased immunopathology.

Percentages of perforin expressing CD8+ T cells



Vitamins A and E and AF-ALB levels in HIV negative and positive participants

Characteristics	HIV negative	HIV positive	P-value
Vitamin A (µg/dL)			
Low (<20)	31.7%	83.2%	<0.001
High (>20)	68.3%	16.8%	
Vitamin E (mg/dL)			
Low (<0.5)	73.1%	87.9%	0.001
High (≥0.5)	26.9%	12.1%	
Aflatoxin B1 (pmol/mg albumin)			
Low (<0.8)	58.2%	43.6%	0.01
High (≥0.8)	41.8%	56.4%	

Vitamins A and E concentrations in relation to HIV viral load and CD4+ T cell counts

	Vitamin A concentration (mg/dL)			Vitamin E concentration (mg/dL)		
	Mean ± SD	Median	P-value	Mean ± SD	Median	P-value
Low viral load (<7.7 log)	12.36 ± 7.42	13.99	0.02	0.22 ± 0.20	0.29	0.02
High viral load (≥7.7 log)	15.22 ± 7.37	11.58		0.29 ± 0.20	0.18	
CD4 <200 cells/mm³	12.60 ± 8.50	11.60	0.40	0.18 ± 0.16	0.11	0.004
CD4 200-499 cells/mm³	14.00 ± 6.50	13.30		0.28 ± 0.18	0.27	
CD4 >499 cells/mm³	15.75 ± 8.66	16.37		0.34 ± 0.27	0.31	

Parameter estimate of predictors associated with Vitamins A and E concentrations

Parameter	Vitamin A Estimate (std Err)	Vitamin E Estimate (std Err)
Intercept	37.34 (3.06)***	0.33 (-0.07)***
Aflatoxin B1	-4.83 (2.16)**	-0.02 (0.04)
HIV infection	-16.94 (3.29)***	-0.22 (0.06)**
Hepatitis B	-5.66 (2.46)**	-0.007 (0.05)
R^2	0.36	0.12
F-value	13.80	5.20
P-value	<0.0001	0.0007

Note: * P≤0.05, ** P≤0.01, *** P≤0.0001

Adjusted for demographic, clinical and health factors

Percent of people developing diseases

Disease	Percent of People Developing Disease
Tuberculosis	31.2
Malaria	48.9
Herpes	31.9
Hepatitis-B	20.6
Pneumonia	10.6

Univariate and multivariate hazard ratios for symptomatic TB by AFB1 quartile

	Unadjusted analysis			Adjusted analysis[a]		
	Hazard Ratio	95% CI	P-value	Hazard Ratio	95% CI	P-value
1st Quarter	Ref.	—	—	Ref.	—	—
2nd Quarter	0.63	0.16-2.51	0.51	0.40	0.06-2.74	0.34
3rd Quarter	1.40	0.56-3.48	0.47	1.35	0.52-3.47	0.54
4th Quarter	2.55	0.99-6.58	0.05	3.30	1.34-8.11	0.01

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