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Aflatoxin B_1 exposure and liver cirrhosis in Guatemala: a case–control study

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ABSTRACT

Objective In Guatemala, cirrhosis is among the 10 leading causes of death, and mortality rates have increased lately. The reasons for this heavy burden of disease are not clear as the prevalence of prominent risk factors, such as hepatitis B virus, hepatitis C virus and heavy alcohol consumption, appears to be low. Aflatoxin B_1 (AFB_1) exposure, however, appears to be high, and thus could be associated with the high burden of cirrhosis. Whether AFB_1 increases the risk of cirrhosis in the absence of viral infection, however, is not clear.

Design Cirrhosis cases (n=100) from two major referral hospitals in Guatemala City were compared with controls (n=200) from a cross-sectional study. Logistic regression was used to estimate the ORs and 95% CIs of cirrhosis and quintiles of AFB_1 in crude and adjusted models. A sex-stratified analysis was also conducted.

Results The median AFB_1 level was significantly higher among the cases (11.4 pg/mg) than controls (5.11 pg/mg). In logistic regression analyses, higher levels of AFB_1 was associated with cirrhosis (quintile 5 vs quintile 1, OR: 11.55; 95% CI 4.05 to 32.89). No attenuation was observed with adjustment by sex, ethnicity, hepatitis B virus status, and heavy alcohol consumption. A significantly increasing trend in association was observed in both models (p trend <0.01). Additionally, the cirrhosis–AFB_1 association was more prominent among men.

Conclusions The current study found a significant positive association between AFB_1 exposure and cirrhosis. Mitigation of AFB_1 exposure and a better understanding of additional risk factors may be important to reduce the burden of cirrhosis in Guatemala.

Summary box

What is already known about this subject?

- Previous studies have reported an association between aflatoxin B_1 (AFB_1) and cirrhosis, particularly in populations with high prevalence of chronic infection with hepatitis B virus (HBV).
- Whether AFB_1 increases the risk of cirrhosis in the absence of viral infection, however, has not been well examined.

What are the new findings?

- In this case–control study, the median AFB_1 level was significantly higher among the cases than controls.
- Furthermore, there was a significantly increasing trend in the association between AFB_1 and cirrhosis, even after adjustment with important covariates such as sex, ethnicity, HBV status, and heavy alcohol consumption.
- In addition, the cirrhosis–AFB_1 association was stronger among men than women.
- To our knowledge, this is the first study to report an association between AFB_1 and cirrhosis in Guatemala, a population with low prevalence of viral chronic hepatitis and low rate of heavy alcohol consumption.

How might it impact on clinical practice in the foreseeable future?

- Interventions to reduce exposure to AFB_1 as well as effort to understand the role of other risk factors for cirrhosis may be important to reduce the burden of the disease in Guatemala.

INTRODUCTION

Aflatoxin B_1 (AFB_1) is a known risk factor for hepatocellular carcinoma (HCC),[1] the dominant type of liver cancer. In Guatemala, the estimated incidence of HCC is the highest in the Western hemisphere.[2] The major risk factors for HCC in Guatemala are not well characterised, but the prevalence of AFB_1 exposure appears to be high.[3] The great majority of HCCs (≥80%) develop in persons with pre-existing cirrhosis.[4] Therefore, insights into the relationship between AFB_1 and cirrhosis could be informative.

With over one million deaths per year, cirrhosis is the 11th most common cause of death worldwide.[5] In combination with HCC, cirrhosis accounts for 3.5% of all deaths globally.[5] In Guatemala, cirrhosis is among the 10 leading causes of death and accounts for an estimated 3.4% of all premature deaths.[6] In addition, mortality rates of cirrhosis have

increased with an average annual per cent change of 14.4% over the past two decades,[7] thus representing an important public health issue in Guatemala.

Cirrhosis is a severe chronic liver disease which occurs in response to liver injury, featuring encapsulation or replacement of the damaged liver tissue by scar tissue with distortion of the hepatic vasculature and architecture.[8] The disease is often asymptomatic until complications such as variceal bleeding, ascites and jaundice occur.[9] In the USA, Europe and some countries in Latin America, cirrhosis is a leading indication for liver transplantation.[10 11] HCC and cirrhosis are known to share common risk factors, including heavy alcohol consumption, hepatitis B virus (HBV), hepatitis C virus (HCV), and the related metabolic abnormalities of obesity and non-alcoholic fatty liver disease (NAFLD).[12] It has also been reported that AFB_1 is associated with cirrhosis among persons infected with HBV or HCV.[13–15] Whether AFB_1 increases the risk of cirrhosis in the absence of viral infection, however, remains unclear.

In 2017, our group reported high levels of serum AFB_1-albumin adducts and low prevalences of HBV (0.9%) and HCV (0.5%) infections in a cross-sectional study of Guatemalan adults.[3] In addition, our group has found that the most important source of AFB_1 exposure in the population was consumption of tortillas, a primary staple in the Guatemalan diet.[16] This finding was consistent with prior evidence of high AFB_1 levels in maize samples across the country.[17] The current study was designed to assess the association between AFB_1 and cirrhosis in Guatemala.

METHODS

Study population

One hundred cirrhosis cases were ascertained between February and November 2015 at two large public hospitals in Guatemala City (Hospital General San Juan de Dios and Hospital Roosevelt). The cases were outpatients recruited at the hospitals' outpatient clinics and emergency rooms. Cirrhosis was diagnosed by abdominal ultrasonography using a quantitative scoring system, including morphological appearance of the liver surface, liver parenchymal texture, intrahepatic vascular structure and spleen size.

Controls were selected from a cross-sectional study of Guatemalan adults, aged 40 years and older, that was conducted in 2016. The cross-sectional study enrolled 461 individuals from five departments of Guatemala in order to determine the prevalence of risk factors for liver cancer. The study recruitment was based on a non-random household visit using maps of the community when available. Details of the study have been previously described.[3] The selection of 200 controls for the current study was based on the residence of the cirrhosis cases, 83% of whom resided in the department of Guatemala or vicinity. Hence, 85% of the controls were chosen from the departments of Guatemala and Escuintla (approximately 64 kilometers from the capital city). Individuals in the cross-sectional study who reported a history of cirrhosis were not eligible to be controls in the current analysis (n=7). A flow chart of the inclusion and exclusion criteria for cases and controls is presented in online supplementary figure S1.

All cases and controls provided informed consent to participate.

Data collection

Study participants were interviewed by trained staff using a structured questionnaire that included information on sociodemographic characteristics (eg, age, sex, residence, ethnicity and occupation), alcohol and maize consumption, as well as use of medications. Study participants also donated blood samples which were used to determine hepatitis B surface antigen (HBsAg), antibody to hepatitis C virus (anti-HCV) and AFB_1-lysine (AFB_1-lys) adducts.

AFB_1-lysine adduct assessments

The determination of AFB_1-lys adduct levels was performed by isotope-dilution mass spectrometry[18] at Dr. John D Groopman's laboratory at the Johns Hopkins University Bloomberg School of Public Health. Adduct concentrations (pg/AFB_1-lys/mL serum) were normalised to total serum albumin and expressed as pg AFB_1-lys adduct/mg albumin. Details of the laboratory methods have been previously described.[3]

Study covariates

The covariates used in the analysis included age, sex, ethnicity (indigenous vs not indigenous), residence (department of Guatemala and vicinity vs other departments), occupation (farmer vs other), heavy alcohol consumption (alcohol consumption ≥2 drinks for men or ≥1 drink for women per day in the last year, or report of a period in life where five or more drinks every day were consumed), HBsAg and anti-HCV.

Statistical analysis

Medians and IQRs were calculated for continuous variables, and percentages were used for categorical variables. To examine differences in the characteristics between cases and controls, t-tests or Wilcoxon rank-sum tests were used for continuous variables, and χ^2 or exact tests were used for categorical variables. Additionally, median and IQR of AFB_1 for each covariate were computed among the controls, and the differences in the median were assessed by the Wilcoxon rank-sum test. Unconditional logistic regression was used to calculate the ORs and 95% CIs for the association between cirrhosis and the serum AFB_1-albumin adduct levels by quintiles. A dose–response relationship between cirrhosis and AFB_1 was examined, and p trends were calculated by scoring (1–5) the quintiles and including the score as a continuous variable in unadjusted and adjusted models. The logistic model selection was based on two different approaches: a stepwise variable selection procedure and examining the change in the estimated ORs by adding

Table 1 Sociodemographic, clinical and other characteristics of individuals by cirrhosis status

Characteristics	Total (N=300)	Cases (n=100)	Controls (n=200)	P value*
Age, median (IQR)	55 (48–63)	54 (47–64)	56 (48–62)	0.07
Sex, n (%)				0.22
Male	129 (43.0)	48 (48.0)	81 (40.5)	
Female	171 (57.0)	52 (52.0)	119 (59.5)	
Indigenous ethnicity, n (%)				0.84
Yes	64 (21.3)	22 (22.0)	42 (21.0)	
No	236 (78.7)	78 (78.0)	158 (79.0)	
Department of residence, n (%)				0.57
Guatemala and vicinity	254 (84.7)	83 (83.0)	171 (85.5)	
Other	46 (15.3)	17 (17.0)	29 (14.5)	
Occupation, n (%)				0.06
Farmer	9 (3.0)	6 (6.0)	3 (1.5)	
Others	291 (97.0)	94 (94.0)	197 (98.5)	
Heavy alcohol consumption, n (%)†				<0.01
Yes	59 (19.8)	51 (52.0)	8 (4.0)	
No	239 (80.2)	47 (48.0)	192 (96.0)	
HBsAg (seropositivity), n (%)†				<0.01
Yes	8 (2.7)	7 (7.0)	1 (0.5)	
No	290 (97.3)	93 (93.0)	197 (99.5)	
Anti-HCV (seropositivity), n (%)				0.11
Yes	4 (1.3)	3 (3.0)	1 (0.5)	
No	296 (98.7)	97 (97.0)	199 (99.5)	
AFB_1-albumin adduct levels, median (IQR)	7.3 (3.5–14.6)	11.4 (5.7–25.7)	5.11 (2.4–12.0)	<0.01

*P values for categorical variables were obtained from χ^2 test (sex, indigenous ethnicity, residence and heavy alcohol consumption) or exact test (occupation, HBsAg and HCV status), and for the continuous variables Wilcoxon test (AFB_1-lysine) or t-test (age).
†Categories do not sum to the total due to missing data.
AFB_1, aflatoxin B_1; anti-HCV, antibody to hepatitis C virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

covariates, yielding the following covariates for the final model: sex, ethnicity, HBV status, and heavy alcohol consumption. Interaction terms were added to the final model and significance was evaluated using the log rank test. Finally, stratified analysis by sex was performed because of a statistically significant interaction with sex. All statistical analyses were conducted using SAS V.9.4 software, and two-sided p values <0.05 were regarded as statistically significant without adjustment for multiple comparisons.

RESULTS

Table 1 shows the characteristics of the study participants. The median age of the participants was 55 years (IQR: 48–63). Of the participants, 57% were women. The majority (85%) of individuals resided in the departments of Guatemala and vicinity. Cirrhosis cases were more likely to report heavy alcohol consumption (52%) than were the controls (4%) (p<0.01). The prevalence of HBsAg was low (2.7%) but was statistically higher in cases (7%) than controls (0.5%) (p<0.01). The prevalence of anti-HCV was low (1.3%), and there was no significant difference in prevalence between the cases (3%) and controls (0.5%) (p=0.11). The median AFB_1 level was significantly higher among the cases (11.4 pg/mg) than among the controls (5.11 pg/mg) (p<0.01).

Table 2 depicts the median values of AFB_1-lys adducts by sociodemographic and other characteristics among the controls. Indigenous persons had a significantly higher median AFB_1-lys adduct levels than did non-indigenous persons (15.2 pg/mg vs 4.8 pg/mg, p<0.01). Similarly, individuals who resided outside the department of Guatemala and vicinity had a significantly higher median AFB_1 -lys adduct level than did the individuals who lived in the department of Guatemala and vicinity (17.8 pg/mg vs 4.9 pg/mg, p<0.01). No differences in median AFB_1-lys adduct levels were observed by age, sex, occupation, excessive alcohol consumption, body mass index or HBsAg and anti-HCV status.

Table 3 shows the results of the logistic regression analysis for the association between cirrhosis and AFB_1-lys adduct levels estimated as ORs. Higher levels of AFB_1 -lys

Table 2 Median and IQR of AFB_1 by covariates in the control group

Characteristics	AFB_1-albumin adduct levels* (n=200)	P value†
Age‡		0.45
<56 years	4.7 (2.5–11.7)	
≥56 years	5.5 (2.4–12.3)	
Sex		0.24
Male	6.3 (2.7–12.6)	
Female	4.7 (2.4–11.5)	
Indigenous ethnicity		<0.01
Yes	15.2 (4.3–36.0)	
No	4.8 (2.3–9.5)	
Department of residence		<0.01
Guatemala and vicinity	4.9 (2.4–10.4)	
Other	17.8 (3.4–33.4)	
Occupation		0.95
Farmer	4.6 (1.5–33.4)	
Other	5.2 (2.5–11.9)	
Heavy alcohol consumption		0.20
Yes	4.0 (1.6–6.3)	
No	5.1 (2.5–12.2)	
Body mass index		
<25.0 kg/m^2	5.5 (3.2–9.5)	0.40
25.0–29.9 kg/m^2	4.7 (2.3–11.3)	
≥30 kg/m^2	4.7 (2.3–8.8)	
HBsAg (+)		0.53
Yes	9.5 (9.5–9.5)	
No	5.1 (2.5–11.9)	
Anti-HCV (+)		
Yes	11.5 (11.5–11.5)	0.44
No	5.1 (2.4–12.1)	

*Unit=pg AFB_1-lysine/mg albumin.
†P values were obtained from Wilcoxon test.
‡The median age among controls is 56.
AFB_1, aflatoxin B_1; anti-HCV, antibody to hepatitis C virus; HBsAg, hepatitis B surface antigen.

was statistically significant associated with cirrhosis. In the unadjusted analysis, the OR of quintile 5 versus quintile 1 of AFB_1-lys adduct was 11.55 (95% CI 4.05 to 32.89), while in the adjusted analysis the OR comparing the highest quintile of AFB_1-lys adduct with the lowest quintile was 12.41 (95% CI 3.23 to 47.74). In both models, there was a significantly increasing trend in the relationship with increasing quintile (p trend=0.001). Using a three-knot restricted linear cubic regression spline, ORs were similar to those of the quintile analysis (data not shown). In addition, adding interaction terms between AFB_1-lys adducts and the covariates in the final model yielded only one statistically significant interaction, which was between AFB_1-lys adducts and sex (p=0.01).

The sex-specific analysis of the association between cirrhosis and AFB_1-lys adducts is presented in table 4. For instance, among women, the adjusted OR comparing the highest quintile of AFB_1 with the lowest quintile was 5.61 (95% CI 1.24 to 25.38), while among men the equivalent comparison had an adjusted OR of 9.64 (95% CI 1.21 to 76.94).

DISCUSSION

In the current study, cases had significantly higher levels of AFB_1-lys adducts than did the controls. In addition, there was a statistically significantly increasing trend in the association (OR) between AFB_1-lys adduct levels and cirrhosis that remained after adjustment for sex, ethnicity, HBV status and heavy alcohol consumption. In addition, evidence of effect modification by sex was observed, with the association between AFB_1-lys adduct levels and cirrhosis being more pronounced among men than women.

The results of the current study are consistent with other studies from Africa and Asia, where AFB_1 exposure has historically been high. In The Gambia, a study found that probable exposure to AFB_1 significantly increased the risk of cirrhosis and that HBV infection had a synergistic effect on the AFB_1–cirrhosis association.[19] Similarly, a study in Egypt reported a significantly higher proportion of AFB_1 signature mutation in *TP53* among persons with chronic liver disease compared with controls.[20] A Turkish study also reported a significantly higher mean level of AFB_1 among individuals with cirrhosis compared with controls .[13] Similarly, a study in Taiwan found that high serum AFB_1 levels were associated with advanced liver disease.[14] In addition, a recent nested case–control study in Taiwan reported a dose–response association between AFB_1-albumin adduct levels and cirrhosis.[15]

Fewer studies have been reported from the Americas, and the results have not been consistent. A study in Mexico found that persons with cirrhosis had high urinary levels of AFB_1 adducts.[21] In Brazil, an autopsy study found an association between AFB_1 residues and chronic liver diseases, including cirrhosis.[22] In contrast, a US study reported that the AFB_1 signature mutation in *TP53* was not evident in the tissue of individuals with cirrhosis.[23]

In the current study, the AFB_1 biomarker used reflects the formation of mutagenic AFB_1-DNA adducts, and the risk of liver carcinogenesis has been demonstrated to increase with the level of aflatoxin exposure.[24] A mechanism underlying the possible development of cirrhosis induced by AFB_1 is not clear. In animal studies, parenchymal changes in the liver caused by steatosis, such as liver cell damage, mononuclear cell infiltration and fibrosis, have been observed after administration of AFB_1.[25–31] Furthermore, a recent study has suggested that myofibroblast-like cells may be involved in fibrosis due to AFB_1 exposure.[31] Other studies have postulated similar

Table 3 Association of cirrhosis status by quintile of AFB_1-lysine adduct levels

AFB_1-albumin adducts	Range (pg/mg albumin)	Cases	Controls	Crude model OR	Crude model 95% CI	Adjusted model* OR	Adjusted model* 95% CI
Quintile 1	0.49–2.68	5	54	1.00	–	1.00	–
Quintile 2	2.75–4.98	15	45	3.60	1.21 to 10.67	4.92	1.32 to 18.35
Quintile 3	5.07–9.58	21	39	5.82	2.02 to 16.76	4.85	1.31 to 17.88
Quintile 4	9.66–19.66	27	33	8.84	3.10 to 25.20	12.01	3.34 to 43.14
Quintile 5	19.68–171.58	31	29	11.55	4.05 to 32.89	12.41	3.23 to 47.74
P value for trend					0.001		0.001

Interaction terms were included for the covariates; only AFB_1 and sex were statistically significant (p=0.01).
*Adjusted for sex, ethnicity, HBV status, and heavy alcohol consumption.
AFB_1, aflatoxin B_1; HBV, hepatitis B virus.

mechanisms, including formation of DNA adducts, protein adducts, and lipid peroxidation.[32] In addition, it has been suggested that AFB_1 may act both as a procarcinogen to induce DNA damage and as a liver-damaging agent.[15] Liver injury has also been shown in experimental animal studies to increase cytochrome p450 enzyme activity, which increases the activation of AFB_1 and results in greater injury to the liver.[33–35]

Sex difference in the prevalence of cirrhosis has been described in several studies. For example, a US population-based survey reported that cirrhosis was nearly seven times more common among men than women.[36] The study also reported that 54% of the cases with cirrhosis were attributable to viral hepatitis, excessive alcohol consumption and diabetes,[36] all of which have been reported to be more common in men than in women.[37–39] In general, the prevalence and severity of NAFLD also appear to be higher in men compared with women.[40] Sex differences in AFB_1 levels and in the metabolism of AFB_1 have also been observed in some studies. Our previous work in Guatemala found that men had significantly higher circulating levels of AFB_1-lys adducts than women.[3] Animal studies have shown that castration of male rats reduced the hepatic metabolism of AFB_1 (approximately 50%),[41] and have reported that male rats are more likely to develop AFB-induced glutathione-S-transferase-P-positive hepatocytes (a marker of preneoplastic foci) than do female rats.[42] This evidence may help to explain the current finding of the AFB_1–cirrhosis association being more pronounced among men than women.

To our knowledge, this is the first study to assess the association between AFB_1 and cirrhosis in Guatemala, a population with low prevalence of viral chronic hepatitis and a low rate of heavy alcohol consumption. The strengths of the current study include the use of a robust biomarker of AFB_1

Table 4 Sex-specific association of cirrhosis status by quintile of AFB_1-lysine adduct levels

AFB_1-albumin adducts	Range (pg/mg albumin)	Cases	Controls	Crude model OR	Crude model 95% CI	Adjusted model* OR	Adjusted model* 95% CI
Female							
Quintile 1	0.77–2.40	4	30	1.00	–	1.00	–
Quintile 2	2.42–4.36	9	25	2.70	0.74 to 9.82	2.31	0.52 to 10.27
Quintile 3	4.47–7.77	11	24	3.44	0.97 to 12.16	2.02	0.44 to 9.31
Quintile 4	7.83–13.97	12	22	4.09	1.16 to 14.39	3.95	0.96 to 16.35
Quintile 5	14.62–137.42	16	18	6.66	1.93 to 23.07	5.61	1.24 to 25.38
P value for trend					0.002		0.014
Male							
Quintile 1	0.49–3.15	3	22	1.00	–	1.00	–
Quintile 2	3.42–6.59	4	22	1.33	0.27 to 6.67	2.85	0.36 to 22.41
Quintile 3	6.76–12.20	12	14	6.29	1.50 to 26.31	24.85	3.10 to 199.00
Quintile 4	12.49–29.60	16	10	11.73	2.77 to 49.62	25.44	3.26 to 198.64
Quintile 5	29.98–171.58	12	13	6.77	1.61 to 28.54	9.64	1.21 to 76.94
P value for trend					<0.001		0.010

*Adjusted for ethnicity, HBV status, and heavy alcohol consumption.
AFB_1, aflatoxin B_1; HBV, hepatitis B virus.

exposure and the use of a community-based control group that is representative of the underlying general population. In addition, the diagnoses of cirrhosis were determined by ultrasound. Although ultrasound is not the gold standard for diagnosing cirrhosis, it has been reported that the diagnostic accuracy of ultrasound in the detection of cirrhosis is clinically acceptable,[43] with a sensitivity of 52%–69% and a specificity of 74%–89%.[44]

Limitations of the current study include that the AFB_1-lys biomarker levels were determined at a single point in time, which may not accurately reflect the cumulative AFB_1 exposure over time. However, as maize is the most important staple in the Guatemalan diet, it is unlikely that dietary exposure varied greatly over time. In addition, lack of information on other factors among the cases, such as body size and clinical parameters, precluded the ability to examine their effects on the AFB_1–cirrhosis relationship. As there was no significant relationship between body size and AFB_1 among the controls, however, it is unlikely that body size would have an effect on the AFB_1–cirrhosis relationship.

In conclusion, the current study found that cirrhosis was associated with AFB_1 in Guatemala, a country with a high burden of liver disease. Interventions to mitigate exposure to AFB_1 as well as efforts to understand the role of other risk factors for cirrhosis may be important to reduce the burden of the disease in Guatemala.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The cirrhosis study was approved by the institutional review boards of both public hospitals, and the cross-sectional study was approved by the institutional review boards of Johns Hopkins University Bloomberg School of Public Health and the Institute of Nutrition of Central America and Panama (INCAP).

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Data availability statement Data that support the findings of this study are available upon request from the authors.

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