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Association of Aflatoxin With Gallbladder Cancer in Chile

Leticia Nogueira, MPH, PhD,
Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland

Claudia Foerster, PhD,
School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

John Groopman, PhD,
Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Patricia Egner, MS,
Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Jill Koshiol, PhD,
Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland

Catterina Ferreccio, MD, MPH on behalf of Gallbladder Cancer Chile Working Group
School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

In Chile, gallbladder cancer is a leading cause of cancer death in women. Other than gallstones, gallbladder cancer etiology remains largely unclear. Exposure to aflatoxin, a liver carcinogen, is associated with bile duct epithelium proliferation in both animals and humans,[1] and with gallbladder cancer in primates.[1] Aflatoxin contamination has been identified in Chile, including in *ají rojo* (red chili peppers). *Ají rojo* is associated with gallbladder cancer[2]; however, the association of aflatoxin with gallbladder cancer in humans has not been directly evaluated.

Corresponding Author: Catterina Ferreccio, MD, MPH, Department of Public Health/Faculty of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 434, Santiago, Chile (catferre@gmail.com).

Author Contributions: Dr Koshiol had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Nogueira, Foerster, Koshiol, and Ferreccio contributed equally to this work.
Study concept and design: Nogueira, Koshiol, Ferreccio.
Acquisition, analysis, or interpretation of data: Nogueira, Foerster, Groopman, Egner, Koshiol, Ferreccio.
Drafting of the manuscript: Nogueira, Foerster, Groopman, Koshiol.
Critical revision of the manuscript for important intellectual content: Nogueira, Foerster, Egner, Koshiol, Ferreccio.
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Additional Information: The members of the Gallbladder Cancer Chile Working Group appear in the eList in the Supplement.

Methods

We evaluated plasma aflatoxin-albumin adducts and gallbladder cancer in a pilot study conducted from April 2012 through August 2013. We recruited incident gallbladder cancer cases identified through rapid ascertainment at cancer referral hospitals in Santiago, Concepción, and Temuco, Chile. We initially recruited all cases consecutively and later recruited only surgical cases to provide tissue samples for future studies. We aimed at 1:1:1 matching by age and sex, as well as hospital for controls with gallstones (to ensure associations with gallbladder cancer were not solely due to gallstones) or study site for community controls. Pairing of controls with gallstones was limited by the small number of patients older than 50 years who underwent gallbladder surgery.

Community controls were selected from a random listing of the population enrolled in the same health center registry as the cases or through neighborhood sampling. Participants had to be previously cancer-free and covered by public health insurance (>90% of population). Chile and US institutional review board-approved written consent was obtained for data and blood collection at enrollment.

Aflatoxin forms adducts with albumin in peripheral blood that accumulate up to 30-fold higher with chronic vs single exposure.[3] Using isotope dilution mass spectrometry,[3] we assessed aflatoxin B_1-lysine adduct (AFB_1 adduct) detection (≥0.5 pg/mg of albumin) and level. Conditional and unconditional logistic regression models produced similar results. Therefore, we used polytomous logistic regression as the most powerful analytic approach. We evaluated variables in Table 1 as potential confounders. We retained questionnaire-derived *ají rojo* consumption (at least weekly), which changed the magnitude of the odds ratio (OR) for AFB_1 adduct detection by greater than 10%. We assessed statistical significance at $P < .05$ using 2-sided tests. Analyses were conducted in SAS version 9.3 (SAS Institute Inc).

Results

Participation rates were similar for cases with gallbladder cancer (85%, 52/61) and controls with gallstones (86%, 37/43) but lower for community controls (57%, 50/88); male and younger potential community controls refused more often. However, age did not differ among community controls by aflatoxin status (median age, 66 vs 65 years) and none of the 8 males had detectable levels. We included all participants with available plasma: 36 cases (69%), 29 controls with gallstones (78%), and 47 community controls (94%). Cases and controls had similar characteristics except for *ají rojo* consumption (Table 1).

The AFB_1-adducts were detected in 23 cases (64%), 7 controls with gallstones (18%), and 9 community controls (23%). Levels were highest in cases (median, 7.6 pg/mg; Table 1), who were more likely to have detectable AFB_1 adducts than controls with gallstones (OR, 9.4; 95% CI, 2.8-37.2) or community controls (OR, 13.2; 95% CI, 4.3-47.9) (Table 2). Restricted to participants with AFB_1 adducts, cases with gallbladder cancer had higher levels per change of 10 pg/mg of albumin than controls with gallstones (OR, 4.0; 95% CI, 1.0-78.0) or community controls (OR, 2.5; 95% CI, 1.0-16.7).

Discussion |

Several lines of evidence support the biological plausibility of the association of gallbladder cancer with aflatoxin, including experimental, animal, and occupational data[4]; low hepatitis B virus prevalence in Chile[2,5]; and genetic variation that may affect xenobiotic excretion.[4] In addition, AFB_1 adduct plasma levels are similar to those associated with increased risk of hepatocellular carcinoma.[6]

Despite the small number of participants, the associations between aflatoxin exposure and gallbladder cancer were statistically significant. Recall bias may affect self-reported variables, but not exposure measurement. We cannot rule out reverse causation (ie, cancer may affect AFB_1 adduct detection) using cross-sectional data. Larger and longitudinal efforts are needed to substantiate these preliminary findings (eg, by identifying aflatoxin-related *TP53* mutations), obtain more precise effect estimates, and identify sources of aflatoxin. These findings, if confirmed, may have implications for cancer prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics of Patients With Gallbladder Cancer Compared With 2 Control Groups

		Controls		
	Gallbladder Cancer (n = 36)	**With Gallstones (n = 29)**	**Community (n = 47)**	***P* Value**[a]
Age, median (range), y	61 (41-77)	57 (44-76)	65 (3779)	.60
Female sex, No. (%)[b]	32 (88.9)	21 (77.8)	39 (83.0)	.50
Location in Chile, No. (%)				
Santiago	17 (47.2)	14 (48.3)	27 (57.4)	
Concepción	10 (27.8)	8 (27.6)	10 (21.3)	.90
Temuco	9 (25.0)	7 (24.1)	10 (21.3)	
History or presence of gallstones, No. (%)[b,c]	34 (94.4)	29 (100.0)	8 (17.8)	<.001
Education, median (range), y	6 (2-16)	8 (2-15)	6 (0-25)	>.99
Ever smoking, No. (%)[b]	12 (33.3)	14 (50.0)	21 (44.7)	.40
Typical weight as adult, No. (%)[b]				
Normal weight	13 (41.9)	7 (29.2)	13 (30.2)	
Overweight	10 (32.3)	11 (45.8)	19 (44.2)	.90
Class I obesity	6 (19.4)	4 (16.7)	6 (14.0)	
Class II/III obesity	2 (6.5)	2 (8.3)	5 (11.6)	
Self-reported diabetes, No. (%)[b]	9 (25.0)	5 (17.9)	8 (17.4)	.70
Family history of gallbladder cancer, No. (%)[b]	2 (7.4)	1 (4.8)	1 (2.9)	.80
Consumption at least weekly, No. (%)[b,d]				
Ají rojo paste	17 (47.2)	5 (17.9)	9 (19.1)	.009
Fresh *ají verde*	20 (55.6)	12 (42.9)	18 (38.3)	.30
Aflatoxin B_1-lysine adduct				
No. detectable	23	7	9	
Level, median (IQR), pg/mg of albumin[e]	7.6 (11.1)	3.5 (3.5)	2.4 (2.5)	<.001

Abbreviation: IQR, interquartile range.

[a]Calculated using the Kruskal-Wallis test for difference in medians for continuous variables and the Fisher exact χ^2 test for categorical variables.

[b] Percentages exclude individuals with missing data.

[c] Gallstone status assessed through questionnaire, medical record abstraction, ultrasound, specimen collection or histology, or a combination of these.

[d] Participants were asked, "Before the last 3 years, how many times per day, week, or month did you eat…"

[e] Among those with detectable levels.

Table 2.

Association of Aflatoxin B_1-Lysine Adducts in Patients With Gallbladder Cancer Compared With 2 Control Groups[a]

	Aflatoxin B_1-Lysine Adduct	
	Detection	Change in Level per 10 pg/mg of Albumin[b]
Controls with gallstones vs community controls	1.4 (0.4-4.4)	0.6 (0.03-5.7)
Patients with gallbladder cancer vs controls with gallstones	9.4 (2.8-37.2)	4.0 (1.0-78.0)
Patients with gallbladder cancer vs community controls	13.2 (4.3-47.9)	2.5 (1.0-16.7)

[a]Values are expressed as odds ratios and 95% confidence intervals from polytomous logistic regression models adjusted for at least weekly consumption of *ají rojo* paste. Estimates obtained using community controls as the referent group in one model and controls with gallstones as the referent group in the other model.

[b]Restricted to individuals with detectable levels (≥0.5 pg/mg of albumin).