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Aflatoxin and PAH exposure biomarkers in a U.S. population with a high incidence of hepatocellular carcinoma

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ABSTRACT

The incidence of hepatocellular carcinoma (HCC) is significantly elevated in a Hispanic community in Bexar County, Texas. Chronic exposure to dietary aflatoxins (AFs) is a major risk factor for HCC; increased risk has been linked to polycyclic aromatic hydrocarbon (PAH) co-exposure and hepatitis virus infection. The aims of this study were to assess AF and PAH exposures, investigate dietary factors that may contribute to increased AF exposure, and determine the prevalence of hepatitis virus infection in Bexar Co. Blood and urine samples were collected from 184 volunteers for biomarker analyses and hepatitis screening. Serum AFB_1-lysine adduct, urinary AFM_1 and 1-hydroxypyrene (1-OHP) levels were measured using high-performance liquid chromatography. The average AFB_1-lysine adduct level detected in 20.6% of serums was 3.84 ± 3.11 pg/mg albumin (range 1.01–16.57 pg/mg). AFM_1 was detected in 11.7% of urines, averaging 223.85 ± 250.56 pg/mg creatinine (range 1.89–935.49 pg/mg). AFM_1 detection was associated with increased consumption of corn tortillas ($p = 0.009$), nuts ($p = 0.033$) and rice ($p = 0.037$). A significant difference was observed between mean 1-OHP values of non-smokers (0.07 ± 0.13) and smokers (0.80 ± 0.68) μmol/mol creatinine ($p < 0.01$). A high hepatitis C virus positivity rate (7.1%) was observed. Findings suggest that the incidence and level of AF and PAH exposure were less than those observed in a high-risk population; however, participants consuming higher amounts of foods prone to AF contamination may be more vulnerable to exposure and interactions with other environmental/biological factors (i.e., HCV).

1. Introduction

The incidence of hepatocellular carcinoma (HCC) in the United States has steadily increased over the recent decades (El-Serag and Mason, 1999). The state of Texas in particular has been shown to have the highest HCC mortality rate in the U.S. (Devesa et al., 1999). A Hispanic population residing within several zip codes in a community in Bexar County, TX has been disproportionately affected by a high incidence of HCC (ATSDR, 2001). Age-adjusted cancer incidence rates from the Texas Cancer Registry averaged from 2002–2006 show that Hispanics in Bexar Co. have an increased HCC incidence rate of 16.5 (95% CI = 15.0–18.0) compared to Hispanics in Texas, with an incidence rate of 10.9 (95% CI = 10.4–11.4). Incidence rates (per 100,000) are age-adjusted to the 2000 U.S. standard population, and confidence intervals (CI) are 95% for rates (Texas Department of State Health Services, 2009). Notably, the HCC incidence rate for Hispanics living in Bexar Co. is considerably higher than all races in Bexar Co., with an incidence rate of 10.0 (95% CI = 9.2–10.8) and all races in Texas, with an incidence rate of 5.8 (95% CI = 5.7–6.0). Hispanic males in Bexar Co. had the highest incidence rate during this time period at 27.1 (95% CI = 24.2–30.2). Thus we were interested in exploring factors that may contribute to HCC in this community.

Multiple factors including diet, environment, lifestyle, health status, gender and genetic susceptibility play a role in the etiology of HCC. Chronic dietary exposure to aflatoxins (AFs), fungal contaminants commonly detected in grain and nut crops (i.e., corn and peanuts), has been established as a major risk factor for HCC development (CAST, 2003; Wogan, 1992). AFB_1, the most prevalent of the AFs, is a potent hepatocarcinogen in animals and humans (IARC, 2002). Biomarkers of AF exposure, e.g., AFB_1-lysine albumin adduct and AFM_1 metabolite, are reliable indicators of chronic and acute exposure, respectively, that have been correlated with elevated HCC risk in several human populations (Groopman et al., 2005). Hoque et al. (1999) previously demonstrated the presence of AFB_1-lysine

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adducts in a small number of HCC patients (5/5 sera samples) registered at the University of Texas (U.T.) M.D. Anderson Cancer Center, prompting the question 'does AFB_1 play a role in the etiology of HCC in the U.S.?' Populations may be at an increased risk for HCC due to additional biological factors, namely hepatitis virus, and/or environmental carcinogen exposures. Wu et al. (2007) documented an elevated HCC risk associated with exposure to a class of environmental contaminants known as polycyclic aromatic hydrocarbons (PAHs). In that report, the greatest risk was found among participants concurrently exposed to high levels of AFs and chronically infected with hepatitis B virus (HBV). While a variety of biological indicators of PAH exposure exist, the urinary biomarker 1-hydroxypyrene (1-OHP) has been validated in many human populations and is widely accepted due to the presence of pyrene in most PAH mixtures (Bouchard and Viau, 1999). In recent work, we reported that a population in Ghana (highly exposed to AFs) was co-exposed to PAHs based on the presence of 1-OHP in the majority of urines collected (Johnson et al., 2009). Cancer mortality patterns in Ghana show that liver cancer is the leading cause of cancer mortality in men and the third highest in women (Wiredu and Armah, 2006). In areas of high HCC incidence, such as sub-Saharan Africa, China, and Southeast Asia, HCC occurrence is closely related to HBV infection. Ross et al. (1992) formerly demonstrated a synergistic interaction between HBV and AFB_1 in the development of liver cancer. Subsequently, Sun et al. (1999) followed a cohort of Chinese men with chronic HBV for 10 years and found that the relative risk of HCC was significantly increased in subjects with detectable AFM_1 levels. In addition, co-infection with hepatitis C virus (HCV) further increased HCC risk, indicating that HBV and HCV interact as risk factors.

While HBV is endemic to parts of the world with high HCC cases, the frequency of HBV infection in the U.S. is far lower. Conversely, an association between HCV infection and HCC incidence has been demonstrated in the U.S., particularly in Texas (Davila et al., 2004). Records from U.T.M.D. Anderson Cancer Center have shown that more than 50% of HCC cases observed in Texas could be attributed to HCV infection (Hassan et al., 2002). In recent work, Chen et al. (2007) showed AF biomarkers of exposure were associated with advanced liver disease in HCV patients in an endemic area in Taiwan. While it is well-established that a viral–chemical interaction exists between the hepatitis virus and AFs, the possible contribution of AFs in the human diet has not yet been assessed in Bexar Co. Due to the disproportionate occurrence of HCC observed in a minority community in Bexar Co., an environmental health study was conducted as a preliminary survey to 1) assess AF and PAH exposures; 2) investigate dietary factors that may contribute to increased AF exposure, and 3) determine the prevalence of HBV and HCV infection.

2. Materials and methods

2.1. Participant recruitment and sample collection

Study participants were recruited from three zip codes (where the incidence of liver cancer is significantly elevated) located within the San Antonio metropolitan area of Bexar Co. This area encompasses nearly 11% of Bexar Co.'s population, and residents are predominantly Hispanic (90.2%). Like other southern regions of Texas, corn and corn-based products represent a staple food source; hence, we were interested in assessing AF exposure in this community. A total of 186 participants were recruited at the San Antonio Metropolitan Health District (SAMHD) from October 2007 to May 2008. Volunteers (males and females) who qualified as study participants met the following criteria: 1) at least 18 years of age and 2) a minimum of two year residency (within the last 12 months) in one of the three specified study zip codes. The study protocol was approved by the Institutional Review Board at Texas A&M University, and all participants were provided written informed consent, as well as an oral explanation of the study protocol prior to beginning the study. Upon enrollment, SAMHD public health officials administered an environmental and personal health questionnaire (in English or Spanish) and collected demographic information through in-person interviews. Biological samples, including venous blood and urine, were collected and stored frozen (−20 °C) until transport to Texas A&M University and the University of Georgia. Following sample collection, it was noted that two participants did not meet the eligibility criteria concerning residency, and data collected from these subjects were not included. Thus, 184 participants comprised our study population.

2.2. Chemicals and laboratory analysis

Authentic AFB_1, AFM_1 and 1-OHP standards were purchased from Sigma Chemical Co. (St. Louis, MO). Blood specimens were analyzed for complete blood count, HBV surface antigen (HBsAg) and anti-HCV antibodies according to standard laboratory operating procedures at the SAMHD.

2.3. Serum aflatoxin B_1-lysine adduct analysis

Serum AFB_1-lysine adduct levels were measured by a modified high-performance liquid chromatography fluorescence (HPLC-f) method (Qian et al., 2009). In brief, serum samples (150 µl) were digested by Pronase (Calbiochem, San Diego, CA) and loaded onto a Waters Oasis Max cartridge (Milford, MA). Cartridges were sequentially washed and eluted with 2% formic acid in methanol. The eluents were evaporated to dryness and reconstituted in 150 µl of 10% methanol prior to HPLC injection. Analysis was carried out on a 1100 liquid chromatography system (Agilent Technologies, Wilmington, DE), and chromatographic separation was performed on a 250×4.6 mm Agilent C18 column, particle size 5 µm. 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 405 nm (excitation) and 470 nm (emission). Peaks for authentic AFB_1-lysine adduct standard and samples were co-eluted at retention times averaging 12.7 min. The detection limit of this method was 10 pg/ml. Results were adjusted for serum albumin levels.

2.4. Urinary aflatoxin M_1 and 1-hydroxypyrene analyses

Urinary AFM_1 levels were analyzed using immunoaffinity column purification followed by HPLC-f using methods previously described by Wang et al. (2008). Urinary 1-OHP levels were also measured with an HPLC-f method based on a procedure developed by Gardiner et al. (1992), as previously described by Johnson et al. (2009). Quantification of AFM_1 and 1-OHP were based on peak area and retention times as compared to external standards run daily. The limit of detection for urinary AFM_1 and 1-OHP using these methods was 0.5 pg/ml and 0.25 nmol/L of urine, respectively. Creatinine concentrations were measured at St. Joseph's Regional Health Center Laboratory in order to correct for variations in urine dilution.

2.5. Statistical analyses

Median, mean, standard deviation (SD) and detectable range were calculated for concentrations of all biomarkers measured. Statistical analyses were done using SPSS software version 15.0 (Chicago, IL). For comparisons, t-tests or Wilcoxon tests were used as appropriate to examine differences between biomarker data. Chi-square tests were performed to examine demographic data and variables assessed by the questionnaire. Crude odds ratio estimates for the relationship between various dietary factors and aflatoxin biomarkers were determined by generating 2×2 contingency tables. A p-value ≤ 0.05 (two-tailed) was considered significant.