Environmental exposure and clinical correlates of hepatocellular carcinoma in New York City: A case only study

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Short Report

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Abstract

In the U.S., Hepatocellular carcinoma (HCC) incidence rates have increased. We aimed to determine whether environmental exposure plays a role in the high incidence of HCC observed in New York City. We conducted a hospital-based case only study to examine the prevalence of aflatoxin B1 (AFB1)- and polycyclic aromatic hydrocarbon (PAH)-albumin adducts and the distribution of adducts by different characteristics of HCC patients. Blood samples were collected from 155 HCC patients for biomarker analyses. We observed that about 46% and 49% of cases had detectable AFB1- and PAH-albumin adducts, respectively. There were significant differences between AFB1-albumin adducts and selected factors such as HCV infection status ($p = 0.04$), diabetes ($p = 0.03$) and Barcelona Clinic Liver Cancer (BCLC) stage ($p = 0.02$). Cases with detectable PAH-albumin adducts had a smoking history compared with those with nondetectable levels ($p = 0.04$). The level of AFB1-albumin adducts was positively correlated with plasma bilirubin ($r_s = 0.32$, $p < 0.0001$) and adiponectin concentrations ($r_s = 0.28$, $p = 0.0005$). The level of aflatoxin B1-albumin adducts was negatively associated with blood albumin concentration ($r_s = -0.28$, $p = 0.0009$) and plasma DNA LINE-1 methylation ($r_s = -0.16$, $p = 0.04$). Our study provides additional evidence that environmental exposures including to aflatoxin might partially explain the increase in the incidence of HCC in the US.

Introduction

Hepatocellular carcinoma (HCC) death rates are rising, even as most other cancers in the US are declining.¹ In New York City (NYC), HCC is one of the leading causes of cancer-related death. The risk of dying from HCC is much greater for people who live in the Bronx and nearby neighborhoods that are predominantly populated by Blacks and Hispanics, compared to other parts of NYC and the US.² Differences in traditional risk factors likely contribute to HCC disparity,² but less is known about the role of environmental exposure in both geographic and racial disparities.

Aflatoxins, genotoxic hepatocarcinogens, are toxic fungal metabolites that commonly contaminate crops. Polycyclic aromatic hydrocarbons (PAHs), possible human carcinogens, are ubiquitous pollutants caused by incomplete combustion of various materials, including diesel fuel and tobacco.³ Using a prospective study design, we reported that higher levels of aflatoxin B1- (AFB1) and PAH-albumin adducts were associated with HCC risk in Taiwan where the incidence of HCC is high.⁴ A recent survey of 184 volunteers recruited in a Hispanic community in South Texas where the incidence of HCC is high reported aflatoxin adducts were detected in 21% of the participants with a mean level 5-fold higher than that detected in the US general population.⁵ These observation suggest that aflatoxin might explain some of the epidemiology of US HCC in term of the significant geographic and ethnic variations in incidence.¹

Here, we conducted a hospital-based case only study to examine the prevalence of AFB1- and PAH-albumin adducts and the distribution of adducts by different characteristics of HCC patients that were

treated in a medical center located in the heart of the Washington Heights neighborhood of northern Manhattan.

Materials And Methods

Patients

This study used information from a total of 155 adult patients (≥ 18 years old) with newly diagnosed HCC and treated in the Hepatobiliary Oncology Clinics at the Columbia University Irving Medical Center (CUIMC) during October 2008 to July 2014. Subjects completed an epidemiologic questionnaire, underwent a physical examination (including weight and height measurements) and provided a fasting morning blood sample at the time of enrollment. Information on biochemical blood analyses, including viral hepatitis, albumin, and total bilirubin, were obtained from the medical records. Simultaneously, an additional 30 mL of blood was processed to isolate plasma and buffy coat which were frozen at -80°C . This study was approved by the CUIMC Institutional Review Board.

Aflatoxin B1- and PAH-albumin adduct measurement

We used ELISA assay for aflatoxin-and PAH-albumin adducts.⁴ Briefly, 50 μL of albumin extracts, equivalent to 200 μg of albumin, were added to 96-well plates (Easywash, Corning, NY) previously coated with 5 ng of 7 β , 8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE-I)-DNA. The standard curve was generated by adding serial dilutions of BPDE-I-tetrols in PBS from 5 to 2500 fmol in 50 μL into the coated wells followed by 50 μL of antibody. The values for each subject were expressed as fmol of PAH per mg albumin. Samples with less than 15% inhibition were considered undetectable and assigned a value of 50 fmol/mg albumin.

For AFB₁-albumin adduct were assayed by ELISA: enzyme digested albumin was extracted and the equivalent of 200 μg of albumin added to 96-well plates previously coated with 3 ng AFB₁ epoxidized-modified human serum albumin. Polyclonal antiserum 7 was used and samples with $< 20\%$ inhibition were considered non-detectable and assigned a value of 4.5 fmol/mg.

Measurement of LINE-1 methylation

Plasma DNAs were isolated using QIAmp UltraSense Virus Kits (Qiagen, Valencia, CA). Aliquots of DNA (500 ng) were bisulfite-treated with the EZ DNA methylation kit (Zymo Research, Orange, CA) following the manufacturer's protocol. Pyrosequencing for LINE-1 methylation levels was carried out using PCR and sequencing primers as described previously.⁵ Briefly, PCR was carried out in a 25 μL reaction mix containing 50 ng bisulfite-converted DNA, 1X Pyromark PCR Master Mix (Qiagen), 1X Coral Load Concentrate (Qiagen) and 0.2 μM forward and reverse primers. Following amplification, the biotinylated PCR products were purified and incubated with the sequencing primer designed to bind adjacent to the CpG sites of interest. Pyrosequencing was conducted using a PyroMark Q96 instrument (Qiagen), with subsequent quantitation of methylation levels determined with the PyroMark CpG 1.010 software.

Relative peak height differences were used to calculate the percentage of methylated cytosines at each given site. Percent methylation within a sample was subsequently determined by averaging across all three interrogated CpG sites in the analysis.

Plasma adiponectin measurement.

Human adiponectin levels was determined in duplicate using commercial ELISA Kits (ThermoFisher Scientific, Camarillo, CA, USA). A serial dilution of adiponectin (from 0.5 to 32 ng/ml) was used to generate a standard curve.

Statistical Analysis

We used the χ^2 test for categorical variables and Student's *t*-test for continuous variables to assess the difference in selected characteristics between exposure groups (detectable vs undetectable adducts). We calculated the Spearman rank correlation coefficients (r_s) to determine the correlation of adducts and plasma LINE-1 methylation. All analyses were performed with SAS software 9.4 (SAS Institute, Cary, NC).

Results

The racial distribution of our HCC cases is diverse and includes 48% whites, 25% of Hispanics, 13% Asian and 10% Blacks. There were about 46% and 49% of cases with detectable AFB1- and PAH-albumin adducts, respectively. The mean level was 10.8 fmol/mg for AFB1-albumin adducts and 116.9 fmol/mg for PAH-albumin adducts.

There were significant differences between AFB1-albumin adducts and selected factors such as HCV infection status ($p = 0.04$), diabetes ($p = 0.03$) and Barcelona Clinic Liver Cancer (BCLC) stage ($p = 0.02$) (Table 1). Cases with detectable PAH-albumin adducts were older ($p = 0.02$) and had smoking history compared with those with nondetectable levels ($p = 0.04$).

Table 1

The distribution of aflatoxin B1 (AFB1)- and polycyclic aromatic hydrocarbon (PAH)-albumin adducts by selected characteristics among hepatocellular carcinoma cases

	AFB1-albumin adducts			PAH-albumin adducts		
	Detectable	Undetectable	P value	Detectable	Undetectable	P value
N (%)	N = 71 (46%)	N = 84 (54%)		N = 73 (49%)	N = 76 (51%)	
Age, mean (SD)	61.5 (10.8)	64.3 (9.8)	0.11	65.4 (10.6)	61.7 (8.3)	0.02
Gender						
Female	18 (26)	16 (19)	0.34	14 (19)	19 (25)	0.39
Male	53 (74)	68 (81)		59 (81)	58 (75)	
Race						
White	38 (53)	37 (46)	0.1	41 (56)	30 (39)	0.01
Hispanics	14 (20)	25 (30)		20 (27)	18 (24)	
Blacks	11 (15)	5 (6)		4 (5)	11 (15)	
Asian	6 (8)	14 (17)		7 (10)	13 (17)	
Other	2 (2)	3 (4)		1 (1)	4 (5)	
Foreign Born						
No	38 (56)	41 (55)	0.63	41 (58)	36 (51)	0.45
Yes	30 (44)	34 (45)		30 (42)	34 (49)	
Education						
Less than high school	34 (50)	31 (39)	0.19	29 (41)	34 (49)	0.36
High school and above	34 (50)	48 (61)		42 (59)	36 (51)	
Annual income						
<\$30 000	21 (37)	26 (39)	0.78	23 (39)	23 (38)	0.71
\$30 000–60 000	14 (25)	13 (19)		15 (25)	12 (20)	
>\$60 000	22 (39)	28 (42)		21 (36)	25 (42)	
HCV						
Negative	24 (34)	42 (50)	0.04	35 (48)	30 (39)	0.3

	AFB1-albumin adducts		PAH-albumin adducts			
Positive	47 (66)	42 (50)		38 (52)	46 (61)	
HBV						
Negative	62 (87)	65 (77)	0.11	63 (86)	58 (76)	0.12
Positive	9 (13)	19 (23)		10 (14)	18 (24)	
Alcohol Use						
Never	27 (38)	29 (34)	0.65	23 (31)	31 (41)	0.24
Ever	44 (62)	55 (66)		50 (69)	45 (59)	
Smoking history						
Never	22 (32)	36 (46)	0.1	23 (33)	35 (49)	0.04
Ever	46 (68)	43 (54)		47 (67)	36 (51)	
Diabetes						
No	47 (66)	41 (49)	0.03	38 (52)	48 (63)	0.17
Yes	24 (34)	43 (51)		35 (48)	28 (37)	
BCLC stage						
0 or A	20 (29)	38 (46)	0.02	29 (40)	28 (37)	0.76
B or C	50 (71)	45 (54)		44 (60)	47 (63)	

Figure 1 presents the statistically significant correlation of AFB1-albumin adducts and selected blood markers. There was a positive association between AFB1-albumin adducts and total bilirubin concentration, with a correlation coefficient of 0.32. The level of AFB1-albumin adducts was negatively associated with blood albumin concentration ($r_s = -0.28$, $p = 0.0009$) and plasma DNA LINE-1 methylation ($r_s = -0.16$, $p = 0.04$). The correlation coefficient between AFB1-albumin adducts and plasma adiponectin concentration was 0.28 ($p = 0.0005$). No associations were found between PAH-albumin adducts and blood markers.

Discussion

Our results indicate that both aflatoxin and PAH exposures are common in US HCC cases. Although it is estimated that 0.7% of US HCC show evidence of aflatoxin exposure from the Cancer Genome Atlas analysis,⁶ we observed that ~ 46% of HCC cases had detectable aflatoxin adducts. Our study provides additional evidence that environmental exposure including to aflatoxin might partially explain the increase in the incidence of HCC in the US.⁵

Aflatoxin, a genotoxic hepatocarcinogen, can induce G→T mutations, in particular at codon 249 *p53*.⁷ A significant racial difference in *P53R249S* mutations, a hallmark of aflatoxin-related HCC, in tumors from HCC cases in South Texas was also observed; The prevalence of *TP53R249S* mutations was highest in Asians (72.7%), followed by Blacks (18.2%), and Hispanics (9.1%).⁸ In our study, we did not see any racial difference in AFB1-albumin adducts. However, measuring AFB1-DNA adducts and *p53* mutations in HCC tumors, we previously observed that AFB1-DNA adducts were associated with *p53* DNA mutation.⁷ As adducts serve as a biomarker of exposure, examining the mutational spectrum of tumors might assist in the better understanding of racial differences in mechanisms of AFB1 carcinogenesis.

A strong interaction between aflatoxin and HBV was reported.⁹ In our case-control study nested in a community-based cohort, we found that HCC risks was 6-fold higher for individuals with chronic HCV infection and low AFB1-albumin adduct, and 20-fold for individuals with both chronic HCV infection and high adducts compared to those with low adducts and no HCV infection.¹⁰ In the present study, we observed that individuals with detectable AFB1-albumin adduct were more likely to be HCV positive. Further study is needed to better understand the molecular mechanism involved in AFB1 promoting hepatocarcinogenesis in HCV-infected liver.

We found AFB1-albumin adducts were associated with blood markers including total bilirubin, albumin, plasma LINE-1 methylation and adiponectin. Adiponectin is known to regulate inflammation, tumor microenvironment and behavior.¹¹ A study of HCC tissues found that increased adiponectin significantly correlated with tumor size, indicating its possible role in prediction of poor prognosis.¹² We previously reported lower plasma levels of LINE-1 methylation was associated with worse survival in HCC patients.¹³ Both animal and human studies found aflatoxin is immunosuppressive by impairing and suppressing normal immune function.¹⁴ Further study is need to understand the potential effects of aflatoxin exposure on HCC prognosis.

Analyzing plasma banked up to 12 years before diagnosis, we demonstrated a 2-fold increased risk for development of HCC with increased PAH-albumin adducts independent of viral status.⁴ In the present study, we found that about half of the HCC cases had detectable PAH-albumin adducts and those with adducts were more likely to be ever smokers. Previous studies have suggested a role for smoking in development of HCC.¹⁵ That the correlation of PAH-albumin adduct and smoking suggest that PAH-albumin adducts can be used a biomarker for validating smoking cessation in HCC prevention.

Study strengths include using biomarker measurement to quantify environmental exposure at the individual level. The limitations of this study are small sample size and case only study design. A larger study will be needed to identify individuals at high risk of aflatoxin exposure which will useful for HCC prevention.

Declarations

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Data sharing: Contact the corresponding author for any inquiries regarding data or analytical code.

Disclosure of Interest:

The authors report no conflict of interest.

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Figures

Figure 1

The correlation of aflatoxin B1-albumin adducts and blood markers. Top left panel: A. The correlation of aflatoxin B1-albumin adducts and total bilirubin concentration ($r_s=0.32$, $p<0.0001$). Bottom left panel: B. The correlation of aflatoxin B1-albumin adducts and blood albumin concentration ($r_s=-0.28$, $p=0.0009$). Top right panel: C. The correlation of aflatoxin B1-albumin adducts and plasma DNA LINE-1 methylation ($r_s=-0.16$, $p=0.04$). Bottom right panel: D. The correlation of aflatoxin B1-albumin adducts and plasma adiponectin concentration ($r_s=0.28$, $p=0.0005$)