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Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma

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Aflatoxins have long been suspected to be human hepatic carcinogens but no direct study was feasible until assays to measure individual aflatoxin exposure became available. We have used assays for urinary aflatoxin B_1, its metabolites AFP_1 and AFM_1, and DNA-adducts (AFB_1-N^7-Gua) to assess the relation between aflatoxin exposure and liver cancer, as part of an ongoing prospective study of 18 244 middle-aged men in Shanghai, People's Republic of China.

After 35 299 person-years of follow-up, 22 cases of liver cancer had been identified. For each case, 5 or 10 controls were randomly selected from cohort members without liver cancer on the date the disorder was diagnosed in the case and matched to within 1 year for age, within 1 month for sample collection, and for neighbourhood of residence. Subjects with liver cancer were more likely than were controls to have detectable concentrations of any of the aflatoxin metabolites (relative risk 2·4, 95% confidence interval 1·0–5·9). The highest relative risk was for aflatoxin P_1 (6·2, 1·8–21·5). In an analysis adjusting for the effects of hepatitis B surface antigen seropositivity, level of education, cigarette smoking, and alcohol consumption, the relative risk for the presence of aflatoxin metabolites was 3·8 (1·2–12·2). composed outcome

There was a strong interaction between serological markers of chronic hepatitis B infection and aflatoxin exposure in liver-cancer risk. Reduction of aflatoxin exposure may be a useful intermediate goal in prevention of liver cancer, since the benefits of wide-scale hepatitis B vaccination will not be apparent for many years.

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Introduction

Aflatoxins, compounds produced by fungi, are established hepatic carcinogens in many animal species.[1-3] Although chronic infection with hepatitis B virus is now regarded as the major cause of hepatocellular carcinoma,[4] aflatoxin exposure may interact with hepatitis B virus in hepatocellular carcinogenesis in areas (eg, south China and subtropical Africa) where such exposure is common.[5,6] The absence of adequate methods to assess aflatoxin exposure and specific metabolites has hindered definitive epidemiological studies of this possible relation. By means of new assays to measure aflatoxin metabolites and DNA-adducts in urine,[7-11] we are prospectively evaluating the association of aflatoxin and liver cancer in Shanghai, People's Republic of China. Liver cancer is the third most common cause of death from cancer in China.[12] Almost half the estimated world-wide total number of liver-cancer cases each year (250 000) arise in China.[13]

Subjects and methods

Between January, 1986, and September, 1989, we invited all men aged 45–64 years living in four small geographically defined areas of metropolitan Shanghai to take part in a prospective epidemiological study of diet and cancer. Each subject completed a detailed food-frequency questionnaire and provided information on other lifestyle characteristics (eg, smoking and alcohol consumption) and a health history. These questionnaires were administered by nurses from the Shanghai Cancer Institute, usually at the subject's home. Each subject provided a 10 ml blood sample and a single urine sample. 25 ml urine was centrifuged, and the supernatant was loaded into a Sep-Pak C18 cartridge (Waters, Massachusetts, USA) and stored at −4°C.

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18 244 subjects took part in the study; follow-up is achieved in several ways. The Shanghai Municipality consists of twelve urban districts, each with its own vital statistics unit. Copies of death certificates, which include cause of death, for residents of the districts targeted for the cohort study are routinely ascertained and matched against the cohort master file. For deaths due to cancer that had not been previously identified, we contact the hospital of record (if any) to confirm the diagnosis.

An important method for identifying new cancer cases among cohort members is through linkage with the Shanghai Cancer Registry.[14] Hospitals in Shanghai where cancer is diagnosed or treated have a legal obligation to report cases to the Registry. Case-ascertainment by the Registry through the hospital system is estimated to be 85% complete, and most of the other cancers that occur in Shanghai are identified by the Registry through death certificates. Cancers among cohort members are identified through manual screening of reports, based on address corresponding to the districts included in this study.

As another way of identifying new cancers among cohort members and of reducing losses to follow-up, we attempt to contact each cohort member every year. Retired nurses employed by the Shanghai Cancer Institute visit the last known address of each living cohort member and record details of the interim health history. For subjects who have moved, the new address is sought from neighbours or from the local police department. Losses to follow-up until 1990 (ie, subjects for whom no death certificate has been found and who have not been located through routine follow-up) total 24.

By March 1, 1990, the 18 244 original participants in the cohort had contributed 35 299 person-years of follow-up. 176 cases of cancer had been identified, including 22 of primary liver cancer. In 5 of these cases the diagnosis was based on a confirmatory biopsy sample, in 8 on high serum alpha-fetoprotein concentrations with consistent clinical and radiographic history, and in 9 on positive computed tomography scan and/or ultrasonography with consistent clinical history.

140 controls were selected for the 22 cases, matched to cases within 1 year of age, within 1 month of sample collection, and for neighbourhood of residence. The controls were randomly selected from among all cohort members enrolled in the study who did not have a history of liver cancer when cancer was diagnosed in the index case. 10 controls were matched to each of the first 6 identified cases of liver cancer, and 5 each to the other 16 cases.

For analysis of aflatoxin metabolites, these compounds were eluted from the Sep-Pak cartridge with 10 ml 80% methanol/water. The eluate was evaporated to dryness under reduced pressure and reconstituted with 0·3 ml 0·1 mol/l hydrochloric acid by heating for 10 min. After cooling, 0·5 ml 1·0 mol/l ammonium formate, pH 5·0, was added, water added to 10 ml, and the sample applied to an immunoaffinity column containing a 4 ml bed volume of anti-aflatoxin monoclonal antibodies bound to Sepharose (Vicam, Somerville, Massachusetts, USA).[7–11] The column was washed with phosphate-buffered saline, and aflatoxin derivatives were released from the column with 7 ml 70% dimethylsulphoxide/water. After removal of dimethylsulphoxide, the sample was evaporated to dryness under vacuum. For high-performance liquid chromatography (HPLC) the sample was reconstituted in 0·1 ml 0·1 mol/l hydrochloric acid and 0·1 ml 1·0 mol/l ammonium formate, and the pH was adjusted to 3·0. Analytical reverse-phase HPLC analysis was done on an HPLC gradient liquid chromatograph with a Hewlett-Packard (Avondale, Pennsylvania, USA) model 1040A diode-array detector to measure aflatoxin metabolites. The HPLC column was a C18 5 μm × 25 cm Ultrasphere analytical column (Rainin Institute, Woburn, Massachusetts, USA), and chromatographic separation was achieved by elution for 20 min with 13% ethanol followed by a 13–25% ethanol/water linear gradient generated over 25 min, then elution at 25% ethanol. The aqueous mobile phase was buffered to pH 3·0 with triethylammonium formate. Column temperature is maintained at 35°C. Authentic standards were used to determine retention times for the derivatives of interest and to generate calibration curves for individual aflatoxins. Hepatitis B surface antigen (HBsAg) was measured by a standard radioimmunoassay method (Ausria II, Abbott, Chicago, Illinois, USA).

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TABLE I—DETECTABLE URINARY AFLATOXINS/DNA-ADDUCTS IN LIVER-CANCER CASES AND CONTROLS

—	No of Cases	No of Controls	RR (95% CI)
No aflatoxin biomarkers	9	87	1·0
AFB_1-N^7-Gua	6	11	4·9 (1·5–16·3)
AFP_1	6	9	6·2 (1·8–21·5)
AFM_1	6	20	3·0 (1·0–9·3)
AFB_1	10	41	2·3 (0·9–5·9)
Any of these compounds	13	53	2·4 (1·0–5·9)

RR = relative risk; CI = confidence interval

Statistical analyses were done by standard methods for matched case-control studies, with conditional logistic regression methods.[15] All p values are two-sided.

Results

96% of the 18 244 participants were aged 45–64 years and 55% were 55–64 years at entry to the study. 29% of participants had had no formal education or only primary-school education. However, 19% had had a university education. 41% were born in metropolitan Shanghai. 50% of the cohort were cigarette smokers at enrolment, and 7% were ex-smokers. Only 41% of participants reported consumption of alcoholic beverages at least once a week.

We measured urinary concentrations of the parent aflatoxin B_1 and two of its main metabolites (P_1 and M_1) and the AFB_1-N^7-guanine DNA-adduct. 13 (59%) of the 22 liver-cancer cases and 53 (38%) of the 140 controls were positive for at least one of these metabolites. We defined a positive result as the presence of at least 1 ng of an individual aflatoxin compound in the injected sample.

In univariate analysis, subjects with liver cancer were significantly more likely than were controls to have detectable concentrations of any of the aflatoxin compounds (table I). The strongest association was for aflatoxin P_1, although the risk of liver cancer was raised with a positive result for any individual compound.

Other factors that influence the risk of liver cancer in this cohort are shown in table II. Positivity for HBsAg was strongly associated with risk of liver cancer. Present or former cigarette smokers also had an increased though not significant risk, but there was no evidence of a dose-response

TABLE II—OTHER RISK FACTORS FOR LIVER CANCER

—	No of Cases	No of Controls	RR (95% CI)
Education			
⩽9 yr	16	85	1·0
⩾10 yr	6	55	0·6 (0·2–1·6)
HBsAg			
Negative	10	125	1·0
Positive	12	15	7·8 (3·0–20·6)
Cigarette smoking			
Never	5	62	1·0
Ever	17	78	2·6 (0·9–7·2)
*No of cigarettes daily**			
1–19	10	41	3·1 (1·0–10·3)
⩾20	7	37	2·1 (0·6–6·9)
*No of years smoking**			
1–29	5	23	2·6 (0·7–9·4)
⩾30	12	55	2·5 (0·9–7·6)
Alcohol consumption			
Never	12	81	1·0
Ever	10	59	1·1 (0·5–2·8)
Daily amount of ethanol (g)†			
1–29	5	43	0·8 (0·3–2·3)
⩾30	5	16	2·2 (0·7–7·1)

*With respect to never smokers
†With respect to never drinkers.

TABLE III—MULTIPLE CONDITIONAL LOGISTIC REGRESSION ANALYSIS

—	Adjusted RR* (95% CI)
Level of education†	0·5 (0·1–1·5)
HBsAg positivity	8·5 (2·8–26·3)
Presence of urinary aflatoxins/DNA-adducts	3·8 (1·2–12·2)
Cigarette smoking (ever)	1·8 (0·6–5·6)
Heavy (≥30 g/day) alcohol consumption	1·8 (0·4–7·4)

*Adjusted for all other variables in table.
†≤9 yr or ≥10 yr

category * category interaction

TABLE IV—HBsAg/AFLATOXIN INTERACTION ON LIVER-CANCER RISK

—	Aflatoxin negative			Aflatoxin positive		
	Cases	Controls	RR (95% CI)	Cases	Controls	RR (95% CI)
HBsAg						
Negative	4	74	1·0	6	51	1·9 (0·5–7·5)
Positive	5	13	4·8 (1·2–19·7)	7	2	60·1 (6·4–561·8)

relation between liver-cancer risk and either duration of smoking or number of cigarettes smoked daily. Alcohol consumption overall was not associated with an increased risk of liver cancer, although there was a suggestion of an increased risk with heavy consumption. Level of education was inversely, but not significantly, associated with risk.

In a multiple conditional logistic regression analysis with adjustment for level of education, cigarette smoking, alcohol consumption, and HBsAg serology, the relative risk of liver cancer associated with detectable urinary aflatoxins was 3·8 (95% CI 1·2–12·2; table III).

Although the analysis was based on small numbers, there seemed to be a strong interaction between HBsAg positivity and aflatoxin exposure in risk of liver cancer. Among individuals who were HBsAg negative, the increase in risk of liver cancer associated with urinary aflatoxin positivity was only 1·9-fold; for those who were HBsAg positive, detection of aflatoxin in urine increased the risk 12·5-fold (table IV).

Discussion

Preliminary results from this prospective study provide the most direct evidence so far for an aetiological role of aflatoxin in hepatocellular carcinogenesis. Such a role has long been suspected, largely because aflatoxins produce liver tumours when given to animals.[16] Nonetheless, there is still debate about the specific role of aflatoxin in human liver cancer.[17,18]

Aflatoxin contamination of foods correlates with the incidence of liver cancer in high-risk areas, such as southeast Asia and tropical Africa.[5,6] In a study in Guangxi, China, average consumption of foods known to be commonly contaminated with aflatoxins (peanuts, peanut oil, corn, and beans) were calculated for individual villages. The mortality rate from liver cancer among HBsAg-positive individuals in the "high"-consumption villages was 10 times higher than that among HBsAg-positive individuals in the "low"-consumption villages.[5] With estimates of aflatoxin consumption based on the regular collection and testing of staple foods consumed in southern Guangxi over 6 years, we found a positive and almost linear relation between estimated dietary aflatoxin intake and liver-cancer mortality in four communes.[19] Some studies have used food-frequency questionnaires and such aflatoxin food surveys to show that high consumption of foods likely to have aflatoxin contamination is associated with higher risk of liver cancer in individuals.[20,21] With similar methods we found no such relation in a case-control study of liver cancer in Hong Kong, but aflatoxin contamination of foods in Hong Kong is very low.[22]

Chronic infection with hepatitis B virus is recognised as the major cause of liver cancer world wide. In some areas of China, more than 90% of liver-cancer cases involve such infections.[19] Although hepatitis B viral infection can be prevented through immunisation, even after large-scale vaccination programmes have been implemented the effect on liver-cancer mortality will not be apparent for many years.[23] Although it is based on a small number of cases and thus cannot give a reliable estimate of attributable risk, our study suggests that up to 50% of liver-cancer cases in Shanghai are related to aflatoxin exposure and that immediate protection from aflatoxin exposure might be a useful intermediate goal in prevention of primary liver cancer.

There is no obvious source of bias to explain our results. Individuals with early symptoms of liver cancer could have changed their diet and somehow increased consumption of aflatoxin-contaminated foods. In a detailed survey of aflatoxin contamination of foodstuffs in Shanghai, we found that aflatoxin is detectable in various foods, but the level of contamination is usually low. Peanuts, peanut-containing foods, and soy sauce are important exceptions. It is unlikely that consumption of these foods would have increased. Adjustment for other known risk factors for liver cancer (HBsAg, alcohol consumption, and smoking)[24] did not explain our findings.

Urinary aflatoxin metabolites and DNA-adducts reflect only recent dietary exposure to aflatoxins. Assays for serum albumin adducts to aflatoxin are now available[25] and may assess dietary intake in the longer term. Such assays would therefore be expected to show even stronger associations with liver-cancer risk. As our study continues, we will make such serum measurements, compare them with urinary findings, and use them as independent and combined indicators of liver-cancer risk. We will continue to assess the extent to which aflatoxin markers interact with other risk factors, especially markers of chronic infection with hepatitis B virus, in defining liver-cancer risk.

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Prophylactic ciprofloxacin for catheter-associated urinary-tract infection

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Patients receiving antibiotics during bladder drainage have a lower incidence of urinary-tract infections compared with similar patients not on antibiotics. However, antibiotic prophylaxis in patients with a urinary catheter is opposed because of the fear of inducing resistant bacterial strains. We have done a double-blind, placebo-controlled trial of prophylactic ciprofloxacin in selected groups of surgical patients who had postoperative bladder drainage scheduled to last for 3 to 14 days. Patients were randomly assigned to receive placebo (n = 61), 250 mg ciprofloxacin per day (n = 59), or 500 mg ciprofloxacin twice daily (n = 64) from postoperative day 2 until catheter removal.

75% of placebo patients were bacteriuric at catheter removal compared with 16% of ciprofloxacin-treated patients (relative risk [RR] [95% CI] 4·7 [3·0–7·4]). The prevalence of pyuria among placebo patients increased from 11% to 42% while the catheter was in place; by contrast, the rate of pyuria was 11% or less in patients receiving ciprofloxacin (RR 4·0 [2·1–7·3]). 20% of placebo patients had symptomatic urinary-tract infections, including 3 with septicaemia, compared with 5% of the ciprofloxacin groups (RR 4·0 [1·6–10·2]). Bacteria isolated from urines of placebo patients at catheter removal were mostly species of enterobacteriaceae (37%), staphylococci (26%), and *Entercoccus faecalis* (20%), whereas species isolated from urines of ciprofloxacin patients were virtually all gram-positive. Ciprofloxacin-resistant mutants of normally sensitive gram-negative bacteria were not observed.

Ciprofloxacin prophylaxis is effective and safe in the prevention of catheter-associated urinary tract infection and related morbidity in selected groups of patients requiring 3 to 14 days of bladder drainage.

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Introduction

Urinary-tract infection is the most common type of hospital-acquired infection, accounting for more than 30% of all cases.[1] Presence of a urinary catheter is an important risk factor for acquisition of nosocomial urinary-tract infection.[2-6] Of the measures that have been proposed to reduce the incidence of catheter-associated urinary-tract infection only the sterile closed drainage system has gained wide acceptance.[7,8] Even with a closed drainage system the risk of urinary-tract infection remains high at an estimated 5–10% for each day the catheter is in place.[2,9]

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