Hepatocellular Carcinoma and Aflatoxin Exposure in Zhuqing Village, Fusui County, People’s Republic of China

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Abstract

Hepatocellular carcinoma (HCC) is a common cause of cancer morbidity and mortality in Asia and Africa. Epidemiological studies have found that dietary exposure to aflatoxin B1 (AFB1) and chronic infection with hepatitis B virus are two major risk factors for HCC. We have collated the incidence and mortality data of malignant tumors from 1973 to 1999 in Zhuqing Village, Fusui County, an area with very high HCC rates, and found that this cancer accounted for 64% of the total cancer incidence. Dietary intake of AFB1 was monitored for 1 week in a study group consisting of 15 males and 14 females from different households in this village. Four of 29 participants (13.8%) and 3 of 15 (20%) male participants were hepatitis B virus surface antigen positive. AFB1 was detectable in 76.7% (23 of 30) of ground corn samples (range, 0.4-128.1 ppb), 66.7% (20 of 30) of cooking peanut oil samples (range, 0.1-52.5 ppb), and 23.3% (7 of 30) of rice samples (range, 0.3-2.0 ppb) collected from each household. Mean levels of serum AFB1-albumin adducts in this group were 1.24 ± 0.31 pmol/mg of albumin at the beginning of the study and 0.19 pmol/mg of albumin at the end of the period. Urinary AFB1 metabolites were detectable in 88.9% (24 of 27) samples (range, 0.9-3569.7 ng/24-h urine). These data provide the exposure and disease risk information for establishing intervention studies to diminish the impact of aflatoxin exposure in this high-risk population.

Introduction

HCC is one of the most common cancers in Asia, Africa, and in groups of Asian- and Hispanic-Americans, and is a leading cause of cancer death (1-3). In the People’s Republic of China, HCC is the second leading cause of cancer mortality, and in one high-risk region, Fusui County in Guangxi Zhuang Autonomous Region, the annual incidence rate is >50 cases per 100,000 people (4, 5). HCC usually strikes people at earlier ages in high-risk areas where the median age of onset of this malignancy is between 35 and 50 years. Previous epidemiological studies have found that chronic infection with HBV and dietary aflatoxin exposure are two major etiological risk factors for HCC in China (6-10). The synergistic interaction of HBV infection, aflatoxin exposure, and HCC has been observed in populations in Fusui County (11), Shanghai (12, 13), and other regions (14, 15). To date, there is still a low prevalence of hepatitis C virus infection in these areas of the People’s Republic of China (16, 17).

Primary prevention, such as vaccination for HBV in infants and food safety procedures to control aflatoxin contamination, offer strategies for lowering HCC rates in the world; however, positive outcomes will require many years. An immediate challenge in cancer prevention and control is to manage those who are already at high risk, such as individuals who are HBsAg carriers and have chronic aflatoxin exposure. The purpose of the following study was to characterize HCC incidence and mortality, status of dietary aflatoxin exposure, and HBV infection in the high-risk region of Fusui County for use in the design of future chemopreventive intervention studies (18, 19).

Materials and Methods

Study Population. Zhuqing Village, located 45 km southwest of Fusui County capital, is a rural farming community of ~3300 residents and is one of the three townships with the highest incidence and mortality of HCC in Fusui County. Data for analyses of incidence and mortality of malignant tumors were collected from village clinics and were confirmed by the Office of Malignant Tumor Reporting operated by the Fusui Liver Cancer Institute. The incidence and mortality were standardized according to the National Population Proportion of China in 1990.

Procedure for Molecular Biomarkers Study. This collaborative study by the Johns Hopkins University, Guangxi Cancer Institute, Shanghai Cancer Institute, and Fusui Liver Cancer Institute used methods and consent forms approved by the Institutional Review Boards for human studies at Johns Hop-
kins University and at the Guangxi Cancer Institute. The consent form in bilingual (Chinese and English) format was developed, approved, and explained in detail at village meetings among residents and investigators prior to recruitment. To be eligible, participants had to meet the following criteria: adults 25–60 years of age in good general health with no history of chronic illness, no personal history of cancer, no use of prescribed medications, no pregnancy or lactation for women residents of different households, agree to stay in the village for the 1-week study period, and able to provide necessary informed consent. In April 1999, weighed portions of each meal from the participants and 24-h urine in three cycles (morning, noon, and evening) were collected from participants for 7 consecutive days. The urine volume was measured and tested with chemsticks for renal function, and a 200-ml urine aliquot per day was stored at −20°C. In addition, 10 ml of blood were drawn into Vacutainers at the beginning and 5 ml at the end of the 7-day study from each participant. Serum samples were immediately separated by centrifugation at the village clinic and stored at −20°C until analysis.

Measurement of HBV Seromarkers and Liver Function. All serum samples were tested for HBsAg and anti-HBs by RIA using the AUSRIA II kit (Abbott Laboratories, North Chicago, IL). A test for the presence of anti-HBe and HBe antigen/anti-HBe was then performed using a commercially available Corab kit purchased from Abbott Laboratories. Liver function tests (aspartate aminotransferase, alanine aminotransferase, and alpha-fetoprotein) were performed in the clinical laboratory of Guangxi Tumor Hospital, which is affiliated with Guangxi Cancer Institute and Guangxi Medical University, according to the clinical diagnostic procedures.

Measurement of AFB1 in Food, Serum Albumin Biomarkers, and Urinary Biomarker. Food analysis for AFB1 was performed using a previously published immunoaffinity method (20). Urinary aflatoxin biomarkers and serum aflatoxin-albumin adducts were analyzed according to previously published methods (19, 21).

Statistical Analysis. All analytical data are expressed as mean ± SE, and levels of serum aflatoxin-albumin adducts were compared between the beginning and end of the study and statistically analyzed by Student’s t test. Raw data of cases and deaths were adjusted to standardized incidence and mortality using the National Population Proportion of China in 1990. Time trends analyses were performed according to the method described by Parkin et al. (2).

Results

Over the past 27 years, Zhuqing Village reported 110 cases and 105 deaths from cancer. Of this total, 70 cases were HCC, resulting in 68 deaths and a standardized HCC incidence of 83.3 cases per 100,000 residents per year and a mortality of 80.9 cases per 100,000 residents per year. Time trend analysis demonstrated no significant change in incidence and mortality over this period. HCC is the leading malignancy in this village, constituting nearly 64% of total cancers, followed by stomach cancer (13.6%). The ratio of HCC in males versus females was 3:1 (58 cases versus 12 cases). More strikingly, the age distribution of HCC cases revealed that most cases occurred in early middle age. The median of age of HCC onset was 34 years in males and 41 years in females.

To characterize AFB1, HBV exposure in this village, 15 males and 14 females from different households were recruited. Two of the study subjects dropped out during the 1-week period. In total, 56 blood samples, 90 raw food samples, 279 cooked food samples, which were aggregated by subject, and 567 urine samples were collected. Additionally, a consecutive 7-day dietary survey for each participant household was obtained. The daily diet of residents consisted mainly of corn and rice with side dishes including vegetables and, occasionally, pork. Almost all of the residents of the village stored ground corn for their daily food. Ground corn either in corn rice or corn porridge was consumed by all study participants, and the average daily corn consumption was 575 g for male subjects and 322 g for female subjects. Rice was consumed only at dinner; the average daily rice intake was 185 g in men and 171 g in women. Locally produced peanut oil was the sole source for cooking oil; daily intake was ~18 g per participant.

The results of the AFB1 analysis in mixed food samples obtained from study participants are listed in Table 1. Corn was the major source of dietary aflatoxin exposure. Twenty-three of the 30 (76.7%) mixed ground corn samples had detectable levels of AFB1 with an average of 23.7 ± 6.6 ppb (range, 0.4–128.1 ppb). Nineteen of the 30 ground corn samples had AFB1 levels >20 ppb, and 5 of these were >50 ppb. Peanut oil was a second major source of dietary aflatoxin exposure. Twenty of the 30 peanut oil samples had detectable levels of AFB1 with an average of 7.8 ppb (range, 0.1–52.5 ppb). Four of these 30 samples contained >10 ppb. Rice, when compared with corn and peanut oil, was a minor source of dietary aflatoxin exposure. Although 7 of 30 (23.3%) samples had detectable levels of AFB1, the average level was 1.1 ppb (range, 0.3–2.0 ppb).

Serum samples collected from study participants were analyzed for levels of aflatoxin-albumin adduct, and all 56 serum samples were positive. The average level of aflatoxin-albumin adducts for the 29 serum samples collected at the beginning of the study was 1.24 ± 0.31 pmol/mg of albumin (range, 0.81–2.41 pmol/mg of albumin). The mean level of aflatoxin-albumin adducts for 27 serum samples collected at the end of the study was 1.21 ± 0.19 pmol/mg of albumin (range, 0.92–1.67 pmol/mg of albumin). Thus, there was no statistically significant change for the adduct levels across these two time points.

Pooled urine samples collected from 27 study participants at day 7 were analyzed for urinary aflatoxin biomarkers, and the results are shown in Table 2. AFM1 was detectable in 24 of 27 (88.9%) samples with an average level of 192.3 ± 62.5 ng/24-h urine (range, 0.9–1258 ng/24-h urine). AFB-mercapturic acid was also found in 24 of 27 (88.9%) samples with an average level of 103.6 ± 25.3 ng/24-h urine (range, 0.1–525.9 ng/24-h urine). AFB-N-guaine was detectable in 22 of 27 (81%) samples with an average level of 407.3 ± 158.7 ng/24-h urine (range, 64.9–1789.8 ng/24-h urine). Aflatoxin P1 was detectable in 7 of 27 (25.9%) samples with an average level of 92.2 ± 8.3 ng/24-h urine (range, 77.3–137.5 ng/24-h urine).

The results of serum HBV markers analyses for the 29

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Positive %</th>
<th>Positive Mean ± SE (range), ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>30</td>
<td>23</td>
<td>76.7</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>30</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td>Rice</td>
<td>30</td>
<td>7</td>
<td>23.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Positive %</th>
<th>Positive Mean ± SE (range), pmol/mg albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>30</td>
<td>23</td>
<td>0.31 ± 0.31 (range, 0.81–2.41)</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>30</td>
<td>20</td>
<td>0.19 ± 0.19 (range, 0.92–1.67)</td>
</tr>
<tr>
<td>Rice</td>
<td>30</td>
<td>7</td>
<td>1.1 ± 0.3 (range, 0.3–2.0)</td>
</tr>
</tbody>
</table>
Table 2 Detection of aflatoxin urinary biomarkers in study participants of Zhuqing Village, Fusui, Guangxi, China

<table>
<thead>
<tr>
<th>AFB 1 biomarker</th>
<th>No. of samples</th>
<th>Positive</th>
<th>% Positive</th>
<th>AF, mean ± SE, ng/24-h urine (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM 6</td>
<td>27</td>
<td>24</td>
<td>88.9</td>
<td>192.3 ± 65.2 (0.9–1258.0)</td>
</tr>
<tr>
<td>AFB-NAC</td>
<td>27</td>
<td>24</td>
<td>88.9</td>
<td>103.6 ± 25.3 (6.6–494.9)</td>
</tr>
<tr>
<td>AFB-N7-Gua</td>
<td>27</td>
<td>11</td>
<td>40.7</td>
<td>407.3 ± 158.7 (64.9–1789.8)</td>
</tr>
<tr>
<td>AFQ 1</td>
<td>27</td>
<td>7</td>
<td>25.9</td>
<td>92.2 ± 8.3 (77.3–137.5)</td>
</tr>
<tr>
<td>AFB P 1</td>
<td>27</td>
<td>8</td>
<td>29.6</td>
<td>649.4 ± 425.4 (80.4–3569.7)</td>
</tr>
</tbody>
</table>

* AF, aflatoxin; AFB-NAC, AFB 1-mercapturic acid; AFB-N7-Gua, AFB 1-N7-guanine; AFQ 1, aflatoxin Q 1 ; AFB P 1 , aflatoxin P 1 .

Table 3 Detection of serum HBV markers in study participants of Zhuqing Village, Fusui, Guangxi, China

<table>
<thead>
<tr>
<th>HBV marker</th>
<th>No. of samples</th>
<th>Positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>29</td>
<td>4</td>
<td>13.8</td>
</tr>
<tr>
<td>HBsAb a</td>
<td>29</td>
<td>14</td>
<td>48.3</td>
</tr>
<tr>
<td>HBcAb</td>
<td>29</td>
<td>24</td>
<td>82.8</td>
</tr>
<tr>
<td>HBeAg</td>
<td>29</td>
<td>2</td>
<td>6.9</td>
</tr>
<tr>
<td>HBeAb</td>
<td>29</td>
<td>24</td>
<td>82.8</td>
</tr>
</tbody>
</table>

a HBsAb, anti-HBsAg; HBcAb, anti-HBc; HBeAg, HBe antigen; and HBeAb, anti-HBe antigen.

people recruited to the study are shown in Table 3. All participants had normal values for alanine aminotransferase and aspartate aminotransferase and were negative for α-fetoprotein, although 4 of 29 (13.8%) were HBsAg positive. Three of these four people were men, constituting 20% (3 of 15) of the male participants in the study. Two of these male participants were positive for HBe antigen. Higher percentages (48.3–82.8%) of anti-HBs, anti-HBc, and anti-HBe marker were detected, further demonstrating a high rate of HBV infection in this population.

Discussion

Over the past three decades, 64% of malignant tumor cases in Zhuqing Village, Fusui County were HCC, and exposure to aflatoxin and HBV was also prevalent. In this study, the rate of HBsAg positivity was found to be comparable to previous studies in the same region, in which ~12–15% of the general population was HBsAg positive. More than 90% of subsequent HCC cases tested HBsAg positive, and incidence of HCC among HBsAg carriers was close to 1% per year (11, 16, 22). Studies by Yeh and Shen (8) in the early 1980s in Fusui described a strong interaction between HBV infection and dietary aflatoxin exposure. Furthermore, a nested case-control study in Shanghai (12, 13) revealed a statistically significant synergistic increase in the relative risk of HCC with exposure to aflatoxin and HBV. This synergistic interaction between HBV and AFB 1 was further confirmed by several studies that used similar biomarkers for aflatoxins and HBV in other populations (14, 15).

Data from the present study showed that dietary AFB 1 exposure is still predominant in this population as demonstrated by the high positive rate and level of aflatoxin contamination in corn and peanut oil samples (Table 1), which were consumed daily by the study participants. The absolute AFB 1 daily intake, as calculated by the average contamination in the diet multiplied by the amount of food consumed, was ~14 µg/day in men and 8 µg/day in women. These levels are less than that reported for the region 15 years earlier (23), which may reflect a switch from almost 100% corn to some portions of rice as the primary dietary item within the past decade. However, this determination may underestimate the real amount of aflatoxin exposure because the heterogeneity of aflatoxin contamination of foodstuffs can lead to inter- and intra-individual variations in the population.

Human urine and serum specimens collected from the Fusui area have been used in the development and validation of molecular dosimetry and biomarkers for aflatoxin exposure. Groopman et al. (23) analyzed urine samples collected from a study group of 42 people in Fusui County in 1984 and found associations between urinary excretion of AFB 1-N7-guanine adduct and AFM 6, and dietary AFB 1 intake. Gan et al. (24) examined formation of aflatoxin-albumin adducts in serum samples from the same group. A significant correlation (r = 0.69) of aflatoxin-albumin adduct level with AFB 1 intake was observed. When serum aflatoxin-albumin adduct data were compared with urine AFB-N7-guanine adduct level, a significant correlation was found (r = 0.73). The results of the present study are consistent with these previous findings.

Finally, a recent study showed that 57% (20 of 35) of HCC cases from Fusui and neighboring areas of Guangxi Region had a G→T transversion at codon 249 of the p53 tumor suppressor gene (25), a frequency of p53 mutations comparable to other parts of the world with high levels of aflatoxin contamination (26, 27). Thus, the high-risk region around Fusui County is a strong candidate for implementation of primary and chemopreventive interventions.

Acknowledgments

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