Hepatitis B Virus, Aflatoxins, and Hepatocellular Carcinoma in Southern Guangxi, China

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ABSTRACT

We examined the roles of the hepatitis B virus and aflatoxin B1 in the development of primary hepatocellular carcinoma (PHC) in a cohort of 7917 men aged 25 to 64 yr old in southern Guangxi, China, where the incidence of PHC is among the highest in the world. After accumulating 30,188 man-yr of observation, 149 deaths were observed, 76 (51%) of which were due to PHC. Ninety-one% (69 of 76) of PHC deaths were hepatitis B surface antigen (HBsAg) positive at enrollment into the study in contrast to 23% of all members of the cohort (RR=38.6). Three of the four patients who died of liver cirrhosis also were HBsAg positive at enrollment. There was no association between HBsAg positivity and other causes of death. Within the cohort, there was a 3.5-fold difference in PHC mortality by place of residence. When estimated aflatoxin B1 levels in the subpopulations were plotted against the corresponding mortality rates of PHC, a positive and almost perfectly linear relationship was observed. On the other hand, no significant association was observed when the prevalence of HBsAg positivity in the subpopulations was compared with their corresponding rates of PHC mortality.

INTRODUCTION

The incidence of PHC varies widely throughout the world. While it is a relatively rare malignancy in the United States and most of Western Europe (1), it is the third and fourth commonest cancer in Chinese men and women, respectively (2). There is considerable geographical variation in PHC incidence within China; the high-risk regions are located along the country’s southeastern coastline, in the provinces of Jiangsu, Zhejiang, Fujian, Guangdong, and Guangxi Autonomous Region (Ref. 2; Fig. 1). In Fusui County in southern Guangxi, the standardized rate of primary liver cancer among men is 120 per 100,000 population per yr, which is more than 35 times the corresponding rate in men in the United States (Table 1). In this paper, we describe a cohort study initiated in 1982 to examine the roles of the HBV and AFB1 in this PHC endemic area.

MATERIALS AND METHODS

Over a period of 12 mo from July 1982 to June 1983, we assembled a cohort of 7917 men who were permanent residents of Fusui or Wuming counties in southern Guangxi. These men were all male residents of five communities (four agricultural communes and one mining community) who were between the ages of 25 and 64 yr and were judged to be free of PHC (as evidenced from a normal physical examination and negative serum α-fetoprotein level) at the time of recruitment when demographic information and a blood specimen were collected from each participant. All blood specimens were spun down shortly after collection, and the serum was transported to the Cancer Research Institute, Guangxi Medical College in Nanning, for storage at −20°C.

Three follow-up surveys were initiated in July 1984, November 1985, and September 1986 to ascertain the vital status of members of this cohort. All deaths that had occurred by July 31, 1986, were noted. In rural China, the smallest administrative unit of county government is the production brigade. Each production brigade maintains a complete census file of its residents, which is continually updated. During the three follow-up surveys, a team of trained field workers from the Department of Epidemiology, Guangxi Medical College, reviewed the census files of the production brigades from which the cohort was drawn to record all deaths and to identify individuals who had moved out of the study area. For each death, a systematic investigation was launched to ascertain the date and cause of death, and the mode and place of diagnosis.

Between May 29 and June 10, 1987, an experienced technician from the Liver Center, Huntington Memorial Hospital, Pasadena, travelled to Nanning and tested the blood specimens of 2072 members of the cohort for HBsAg by radioimmunoassay with standardized commercial kits (AUSRIA II; Abbott Laboratories, North Chicago, IL). These 2072 subjects included (e) all deaths that had occurred among cohort members through July 31, 1986, and (b) an approximate 25% random sample of the whole cohort stratified by age (10-yr groupings) and county of residence (Fusui, Wuming). Hereafter, this 25% stratified random sample of the whole cohort will be referred to as the subcohort. Sampling of this subcohort was accomplished through a set of computer-generated random numbers with values between 1 and 7917.

To examine the relationship between HBV and the various causes of mortality in this cohort, we selected four controls from members of the subcohort for every case that had died. First, we ranked the cases according to their dates of death $t_i$, later than or the same as $t_j$. Then, for each Case $i$ that died on date $t_i$, we identified all members of the subcohort who were of the same race (Han, non-Han), age (within 2 yr), and county of residence (Fusui, Wuming) as Case $i$, and who were enrolled in the study and were alive on date $t_i$. From this pool of eligible subjects, we chose as controls the four men whose enrollment dates into the study were closest to that of Case $i$. Whenever there were more than four men with equally close dates of enrollment relative to Case $i$, four were randomly chosen from the set. These four men were excluded from consideration as potential controls for later cases ($i + 1, i + 2, \ldots$), or as a later case. Four cases of death (one case each of stomach ulcer, accident, brain cancer, and pulmonary tuberculosis) were excluded from the analysis due to this criterion. Eighty-eight and 98% of controls were enrolled within 1 and 10 days, respectively, of their matched cases. We used standard matched set methods (3) to analyze the HBsAg results of these case-control sets. The computations were performed using the statistical package EPILOG.

We also used standard methods of cohort analysis to examine cause of death in relation to HBsAg status in members of the subcohort. Mortality rates were adjusted by age (25 to 34, 35 to 44, 45 to 54, 55+), race (Han, non-Han), and county of residence (Fusui, Wuming) by direct standardization using the person-yr distribution of the entire subcohort as an internal standard (4). The method of Breslow et al. (5) was used to compute RRs and associated 95% CL. The statistical package GLIM was used to perform these calculations.

Between 1978 and 1984, the Fusui Liver Cancer Institute regularly sampled and tested staple foods consumed in the counties of southern Guangxi for contamination by AFB1. Twice a year, samples of raw foods were collected from all over the region and analyzed for their...
RESULTS

Table 2 describes selected demographic features of the cohort. Three-fourths (75.8%) were residents of Fusui, 72.3% were between the ages of 25 and 44 yr, 70.2% were Zhuang (a minority race in China, 94% of Chinese citizens are Han), and 47.0% were farmers. As expected, the distributions of demographic factors in the subcohort and whole cohort were very similar (data not shown).

We accumulated a total of 30,188 man-yr of observation, an average of 3.8 yr per person by July 31, 1986. During this period, only 94 subjects moved out of the study area. One hundred forty-nine deaths were observed among the 7917 members of the cohort, and 76 (51.0%) deaths were due to PHC. The expected number of PHC deaths based on age-specific population rates (Table 1) and accumulated man-yr of obser-

vation is 75. The next most common causes of death were accident (8.7%), circulatory disease (7.3%), and pulmonary tuberculosis (7.3%). There were 4 deaths attributable to liver cirrhosis (Table 3).

Two cases of PHC were diagnosed by histopathology. Sixty-seven cases exhibited persistently raised serum levels of α-fetoprotein (greater than 500 ng/ml for more than 1 mo) and changes on a liver scan interpreted as PHC. Two cases showed clinical evidence of PHC, liver scan results interpreted as PHC, and grossly raised levels of alkaline phosphatase, gamma glutyl transpeptidase, and lactate dehydrogenase. The remaining five cases of PHC were diagnosed by clinical evidence only.

Table 4 presents the distribution of HBsAg status among PHC cases and their age-race-residence-matched controls. There was a significant association between HBsAg positivity and death from PHC. Ninety-one % (69 of 76) of PHC cases were HBsAg positive at enrollment into the study relative to 22% (68 of 304) of controls (RR = 38.6, 95% CL = 16.0, 117.1). This highly significant association between HBsAg-positive state and PHC was independent of race, county of residence, age at death, and duration of follow-up. Similar results were obtained when the 5 patients who were diagnosed clinically were excluded from the analysis. Death from liver cirrhosis also was related to the HBsAg-positive state; three of the four subjects that died of liver cirrhosis were HBsAg positive at entry to the study. On the other hand, deaths from causes
other than PHC and liver cirrhosis did not differ from controls in their HBsAg status (RR = 1.4, 95% CL = 0.8, 2.5).

When we examined the subcohort by standard methods of cohort analysis, we obtained results consistent with those based on the nested case-control scheme. Twenty-three % of members of the subcohort were HBsAg positive on the day of enrollment. There was little difference in prevalence of HBsAg between residents of Fusui (23.3%) and Wuming (22.0%). There were 18 PHC deaths in the subcohort, 17 of whom were HBsAg positive on the day of enrollment. The mortality rates of PHC adjusted for age, race, and county of residence in HBsAg-positive and HBsAg-negative subjects were 952.8 and 17.5 per 100,000 person-yr, respectively. The RR for the HBsAg-positive relative to the HBsAg-negative state was 55.1 (95% CL = 7.3, 414.0). There were 15 deaths other than PHC and cirrhosis in the subcohort, and no association was observed with the HBsAg-positive state (RR = 0.9, 95% CL = 0.3, 3.2).

Table 5 presents the mortality rates of PHC among members of our cohort by the five communities from which they were drawn. There was a gradient in PHC risk across the five subpopulations; the difference was 3.5-fold between the two communities with the highest and the lowest rate of PHC mortality. When the mortality rates were plotted against the estimated levels of AFB2 exposure in the communities based on food surveys conducted during 1978 to 1984 (6, 7), a positive and almost perfectly linear relationship was observed (Pearson correlation coefficient = 1.00, P = 0.004; Fig. 2). On the other hand, no significant association was observed (Pearson correlation coefficient = 0.28, P = 0.65) when we compared the prevalence of HBsAg positivity with the corresponding rates of PHC mortality in the five communities (Table 5).

**DISCUSSION**

The evidence in support of a direct role of HBV in the etiology of PHC has been reviewed (9, 10). Previous studies using the reverse passive hemagglutination method to measure serum HBsAg had suggested that the HBV carrier state is related to PHC risk in Guangxi (6, 11), although the magnitude of the observed associations was less than that reported from Taiwan by Beasley et al. (12) who used the more sensitive radioimmunoassay method to detect serum HBsAg. Beasley et al. (12) had reported that, among 22,707 Chinese men (mean age at recruitment was 49 yr) in Taiwan after an average follow-up period of 6.2 yr per man, 113 of the 116 observed PHC deaths occurred in members who were HBsAg positive at enrollment into the study. The authors calculated that the PHC incidence in HBsAg-positive carriers was 527/100,000 person-yr compared to 3/100,000 person-yr in noncarriers. By using the same sensitive method of measurement, our study shows that the risk of PHC in HBsAg-positive men in southern Guangxi is at least as high as that in their counterparts in Taiwan. Among our subjects (mean age at recruitment was 39 yr), the incidence of PHC in HBsAg-positive men was 954/100,000 person-yr relative to 17/100,000 person-yr in HBsAg-negative men.

Our study population is also exposed to relatively high levels of aflatoxins through ingestion of moldy foods. Corn, a staple food in many parts of southern Guangxi, is frequently contaminated with AFB1 (6, 7, 11). AFB1 acts as a hepatic carcinogen in many animal species, ranging from fish and rodents to monkeys (13). Evidence for its carcinogenic effect in humans is suggestive, although less than definitive. Levels of dietary aflatoxins in a number of communities in southern Africa and southeast Asia (a range of more than 20-fold) were found to correlate closely with local rates of PHC incidence (the Kendall rank correlation coefficient = 0.95 on a log-log scale) (14). A case-control study conducted in the Philippines (15) observed an RR of 17.0 in individuals exposed to very high levels of dietary aflatoxins (7+ mg per day), and an RR of 13.9 in those exposed to moderate levels (4 to 6 mg per day) when compared to those who were exposed to relatively low levels (0 to 3 mg per day). Since the study did not measure HBV serology of the

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**Table 4 HBsAg status of PHC cases and their matched controls**

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>HBsAg positive/ total no.</th>
<th>Controls</th>
<th>RR</th>
<th>95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>County</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusui</td>
<td>57/64</td>
<td>58/256</td>
<td>31.5</td>
<td>12.9, 96.3</td>
</tr>
<tr>
<td>Wuming</td>
<td>12/12</td>
<td>10/48</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Race “</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Han</td>
<td>12/12</td>
<td>10/48</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Non-Han</td>
<td>57/64</td>
<td>58/256</td>
<td>31.5</td>
<td>12.9, 96.3</td>
</tr>
<tr>
<td>Age at death (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–34</td>
<td>24/25</td>
<td>24/100</td>
<td>50.4</td>
<td>9.6, 1038.3</td>
</tr>
<tr>
<td>35–44</td>
<td>25/29</td>
<td>25/116</td>
<td>27.2</td>
<td>7.7, 164.3</td>
</tr>
<tr>
<td>45–64</td>
<td>20/22</td>
<td>19/88</td>
<td>49.7</td>
<td>9.2, 1034.8</td>
</tr>
<tr>
<td>Duration of follow-up (mo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–12</td>
<td>21/23</td>
<td>21/92</td>
<td>45.8</td>
<td>8.5, 950.5</td>
</tr>
<tr>
<td>13–24</td>
<td>19/20</td>
<td>22/80</td>
<td>38.6</td>
<td>7.1, 810.8</td>
</tr>
<tr>
<td>25+</td>
<td>29/33</td>
<td>25/132</td>
<td>35.0</td>
<td>10.1, 209.0</td>
</tr>
<tr>
<td>Total</td>
<td>69/76</td>
<td>68/304</td>
<td>38.6</td>
<td>16.0, 117.1</td>
</tr>
</tbody>
</table>

* Not all Han and non-Han cases resided in Wuming and Fusui counties, respectively, although stratifications by county and race show identical results.

**Table 5 Prevalence of HBsAg in serum and age-adjusted mortality rate of PHC among cohort members by place of residence**

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>Estimated mean level of AFB (mg/person/yr)</th>
<th>No. of study subjects</th>
<th>No. of PHC cases</th>
<th>Age-adjusted prevalence of HBsAg (%)</th>
<th>Age-adjusted mortality rate (per 100,000 person-yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>51.8</td>
<td>1070</td>
<td>23.1</td>
<td>25</td>
<td>613.5</td>
</tr>
<tr>
<td>B</td>
<td>18.0</td>
<td>1417</td>
<td>24.7</td>
<td>16</td>
<td>288.5</td>
</tr>
<tr>
<td>C</td>
<td>2.3</td>
<td>953</td>
<td>24.8</td>
<td>8</td>
<td>182.2</td>
</tr>
<tr>
<td>D</td>
<td>0.3</td>
<td>1915</td>
<td>21.6</td>
<td>12</td>
<td>175.4</td>
</tr>
<tr>
<td>E</td>
<td>—</td>
<td>2562</td>
<td>19.5</td>
<td>15</td>
<td>149.1</td>
</tr>
</tbody>
</table>

* Based on food surveys conducted during 1978 to 1984 (6, 7).

The person-yr distribution of the entire cohort was used as an internal standard.

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subjects and the Philippines is an HBV endemic region (16), it is uncertain how much of this significant association could be explained by the HBV carrier state.

Our study demonstrates considerable variation in PHC risk between communities in southern Guangxi. We observe a 3.5-fold difference in PHC mortality rate between the five communities from which our study subjects were drawn. We show that this variation in risk of PHC across communities correlates positively with the local levels of AFB1 exposure. On the other hand, there is little association between prevalence of HBsAg positivity and PHC mortality in these same communities. Despite the crudeness of our measurements on aflatoxins exposure (i.e., population-based instead of personal exposure assessments), it is reasonable to conclude that AFB1 seems to play a role in the unusually high rates of PHC in southern Guangxi.

The population prevalence of HBsAg is extraordinarily high in our study population. Close to one in every four adult men is a HBsAg-positive carrier of HBV. A cross-sectional survey (17) of 2021 children ages 1 to 9 yr in Long An County (adjacent to Fusui) revealed little difference in the prevalence of serum HBsAg by single years of age; the prevalence was 23.5% among the 1-yr olds, and the average prevalence for all survey participants was 22.3%. Thus, primary infection occurs very early in this high-risk population, possibly through vertical transmission from carrier mothers to infants during the perinatal period (18).

Even though most cases of liver cancer in this study did not have histopathological confirmation, there is evidence to indicate that virtually all were PHCs. A review of 48 consecutive biopsy cases of liver cancer from Fusui at the Affiliated Hospital of Guangxi Medical College in Nanning showed all to be PHCs (6). A separate autopsy series of 85 consecutive cases of liver cancer at the same hospital (which is in southern Guangxi) showed 80 PHCs, four cases of combined PHC and cholangiocarcinoma, and only one case of cholangiocarcinoma (6).

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REFERENCES

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