Eight-Year Follow-Up of the 90,000-Person Haimen City Cohort: I. Hepatocellular Carcinoma Mortality, Risk Factors, and Gender Differences[1]

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Abstract

In an 8-year follow-up of a prospective cohort study in Haimen City, China, we sought to identify hepatocellular carcinoma (HCC) risk factors in addition to hepatitis B virus (HBV) infection. Two cohorts of adults between ages 25 and 64 years at study entry were followed from 1992–1993 to 2000. The male cohort included 58,545 men, 15.0% of whom were HBV carriers. The female cohort included 25,340 women, 10.7% of whom were HBV carriers. 434,718 person-years of follow-up were accumulated, and 1092 deaths from HCC occurred. The relationship of potential risk factors measured at study entry to HCC mortality was analyzed using Cox proportional hazards models. For males, HCC mortality was significantly associated with HBV infection [relative risk (RR) 18.8; 95% confidence interval (CI), 15.7–22.5], history of acute hepatitis (RR, 2.3; 95% CI, 2.0–2.7), family history of HCC (RR, 2.3; 95% CI, 1.9–2.7), and occupation as a peasant (RR, 1.5; 95% CI, 1.3–1.8). For females, HCC mortality was significantly associated with HBV infection (RR, 33.5; 95% CI, 17.1–65.5) and acute hepatitis history (RR, 4.7; 95% CI, 3.0–7.5). HCC risk was not significantly associated with alcohol consumption, water source, or staple foods in either sex. There was no association with smoking in males, but there was a positive association for females. Environmental and genetic risk factors besides HBV infection play a significant role in HCC mortality in this extremely high-risk population. Gender differences in HCC mortality and known risk factors are substantial and warrant further study. Identification of risk factors amenable to intervention should be a high priority in the prevention of HCC.

Introduction

Worldwide liver cancer is the fourth most common cause of cancer mortality, accounting for an estimated 427,000 deaths in 1990 (1). Most primary liver cancers in adults are HCC,[3] and incidence is highest in the developing world in areas where HBV is endemic (2). Although chronic HBV infection has long been known to be a major cause of HCC, incidence rates still vary widely, even within endemic populations. For instance, the nationwide age-standardized rate for liver cancer in China is estimated at 34.7 per 100,000 person-years in males, but regional variation within China is significant. It has long been known that the southern coastal region of China is an area of particularly high HCC mortality (3). The age-standardized rate for males in Qidong City, which is part of this region, was estimated as 72.1 per 100,000 in 1988–1992 (4).

In 1992, we initiated a large prospective cohort study of HCC mortality in adult residents of Haimen City in Jiangsu Province, China (5). Haimen City is located in the delta region of the Yangtze River, immediately adjacent to Qidong City, and within the area of highest HCC mortality in China. The primary goal of this study was to identify risk factors for HCC in addition to HBV infection. Here we report the findings from this cohort after ~8 years of follow-up.

Materials and Methods

Detailed cohort enrollment methods for this study have been published previously (5, 6). The protocol was reviewed and approved by the Institutional Review Board of Fox Chase Cancer Center, the Medical Ethics Review Group of Haimen City, and the Ethics Review Committee of Shanghai Medical University. Between February 1992 and December 1993, study teams from the HCAS traveled to 1008 villages in each of the 35 townships of Haimen City to enroll subjects in the prospective cohort study. At entry, each subject completed a one-page questionnaire and donated a 9.0-ml sample of blood by venipuncture. 90,836 adult residents of Haimen City underwent enrollment screening. Questionnaire data collected included name, residence, date of birth, occupation (peasant, factory worker, functionary, entrepreneur, private businessperson), cigarette use, alcohol consumption (≥4 drinks/week), tea drinking (≥4 × per week), past pesticide exposure, drinking water source by decade from 1960s–1990s (ditch, pond, shallow well, deep well), staple food by decade from 1960s to 1990s (corn, rice, wheat), history of acute hepatitis, history of jaundice, history of cirrhosis, and family history of HCC. If patients had

a family history of HCC, they were asked to indicate the degree of relation of the affected individual(s) (*e.g.*, father, mother, brother, sister, uncle, aunt). Questionnaires were completed by trained interviewers, employed by the HCAS.

Occupational categories were collapsed to peasant *versus* nonpeasant because of small numbers in each of the nonpeasant categories individually. In addition to current alcohol and cigarette smoking variables, duration and amount of alcohol or cigarettes consumed as well as consumption of individual types of alcohol (beer, grape wine, rice wine, grain wine, and liquor) were examined in univariate analysis. Because of the need to restrict the size of the screening questionnaire to one page, alcohol and tobacco questions were not detailed. Daily cigarette consumption and duration of smoking was asked only of current smokers. Subjects were considered alcohol drinkers only if they drank ≥4 drinks/week. Age of initiation of use was asked for each type of alcohol, but consumption was quantified only in terms of amounts per occasion of drinking, because all drinkers consumed at least 4 drinks/week.

For the drinking water and staple food questions, subjects were asked what percentage of their water/staple food came from the various sources on average during the 1960s, 1970s, 1980s, and 1990s separately. Public health measures to improve drinking water and reduce corn consumption (as a source of aflatoxins) began in Haimen City during the 1970s. Drinking water sources were collapsed into well (deep or shallow) *versus* non-well (ditch or pond), with the latter category thought to include drinking water of lower quality. Staple food sources were collapsed into corn *versus* wheat or rice. For the main analysis, both the drinking water and staple food consumption by decade items were coded as ever *versus* never. Other coding schemes, ≥50% *versus* <50%, ≥75% *versus* <25%, produced similar results.

History of acute hepatitis referred to a clinically diagnosed case of acute hepatitis for which, in the years prior to the inception of this cohort, public health infection control measures would have required hospitalization of the patient. For this reason, it was thought that self-reports of acute hepatitis were reliable and could be included in the analysis. Self reports of cirrhosis and jaundice, however, were rare and not considered reliable. These variables were not included in the analysis.

Subjects were followed from cohort entry until September 30, 2000 for vital status and HCC incidence. Each of the 35 township hospitals as well as city-wide referral hospitals, which include both Western and traditional Chinese medicine practitioners, are required to report all cancers diagnosed and deaths from any cause monthly to the HCAS. These reports include information from health centers in each village within the township. Although village health centers cannot diagnose cancers, doctors there would know of the diagnosis of a cancer or a death of a resident who was temporarily living elsewhere or who had sought treatment outside of Haimen City. On a yearly basis, each reporting group is evaluated by Haimen City public health authorities for the completeness and accuracy of reporting. For liver cancer, population level rates for Haimen City are similar to those reported in the neighboring Qidong City, whose cancer registry has met the quality standards necessary for inclusion in the International Agency for Research on Cancer's *Cancer Incidence on Five Continents* (4).

Twice per year, study personnel identify cohort members from village lists and verify death certificate information by contacting village and township doctors. For HCC cases, surviving family members were also contacted to provide missing information. Deaths of individuals who leave Haimen City for work purposes without being granted a permanent change of

residency are still reported to local authorities, although reporting is often delayed. Cohort members who were granted permanent changes of residency were considered lost to follow-up as of the date of the residency change.

During the follow-up period, 1092 subjects died of HCC. We verified diagnosis of HCC using existing medical records, which in some cases were incomplete, plus interviews with doctors or relatives of patients who had already died. We ascertained that diagnosis of HCC was established by histology in 96 cases (8.8%), by elevation of serum AFP (>400 ng/ml) together with a lesion in the liver detected by imaging (ultrasonography, computerized tomography, or magnetic resonance imaging) in 391 cases (35.8%), by imaging alone in 431 cases (39.5%), by AFP alone in 16 cases (1.5%), by clinical criteria alone in 19 cases (1.7%), and by death certificate and/or postmortem interviews with doctors and family in 235 cases (21.5%).

HCC incidence and mortality rates are virtually identical in Haimen City as well as other populations worldwide because median survival times are short, <6 months in Haimen City, because no effective therapy is available to most patients. For this cohort analysis, therefore, we used the date of death rather than date of diagnosis of HCC as the endpoint. This approach is less prone to biases that might have occurred with differential reporting of diagnosis information for persons who sought medical care outside of Haimen City (largely nonpeasants) or who might have had their tumors diagnosed before symptoms arose by taking part in periodic screening programs offered in Haimen City for HBV carriers.

For the purposes of this analysis, 58,454 male subjects and 25,430 female subjects are included for a total of 83,794. Among the 7,042 (7.8%) subjects excluded, 633 (9.0%) were outside the required age range of 25–64 at study entry and 6409 (91.0%) were from townships of <1000 population, where mortality reporting was considered inadequate. Subjects who were lost to follow-up during the course of the study because of dissolution of their work units or permanent moves to locations outside Haimen City were considered censored at the time of their exit from Haimen City. In total, 6,862 subjects (7.6%) were censored prior to September 30, 2000.

Serum was separated from whole blood within 24 h of collection in the laboratories of the HCAS. Samples were tested immediately for HBsAg by radioimmunoassay, serum alanine aminotransferase, and serum AFP. Research subjects were informed of the results of these tests. Aliquots of 9,360 serum samples were transported to Fox Chase Cancer Center laboratories in Philadelphia and tested for HBsAg by commercial enzyme immunoassay (AUSZYME; Abbott Laboratories, North Chicago, IL). The HCAS RIA was 88.9% sensitive and 95.2% specific when compared with AUSZYME as the gold standard. We selected serum from 65 HCC cases for testing for antibody to HCV (anti-HCV) by enzyme immunoassay (HCV EIA; Abbott Laboratories). Of these, 1 (1.5%) was positive. Because this low prevalence of anti-HCV was also consistent with data collected by public health authorities in Haimen City demonstrating a low prevalence in this population, we elected not to do further testing of our cohort samples to preserve the limited quantities of banked serum.

Statistical analysis was conducted using SAS (SAS Institute, Cary, NC) version 8. RR of HCC associated with various risk factors was calculated using Cox proportional hazards models for males and females separately and including a term for age and age^2 in the model. After univariate analysis, a multivariate Cox model was fit for males and females including all candidate risk factors that were significant at the $\alpha = 0.05$

Table 1 Characteristics of study subjects at baseline

Status at study entry	Male	Female
No. of subjects	58,454	25,340
Age (yr)		
25–34	10,934 (18.7%)	6148 (24.3%)
35–44	20,943 (35.8%)	9839 (38.8%)
45–54	16,888 (28.9%)	6384 (25.2%)
55–64	9689 (16.6%)	2969 (11.7%)
HBsAg		
Positive	8795 (15.0%)	2711 (10.7%)
Negative	49,659	22,629
Occupation		
Peasant	42,178 (72.2%)	21,148 (83.5%)
Nonpeasant	16,276	2192
Smoking		
Yes	37,856 (64.8%)	891 (3.5%)
No	20,598	24,449
Drinking alcohol ($\geq$4 drinks/week)		
Yes	35,171 (60.2%)	3429 (13.5%)
No	23,283	21,911
History of acute hepatitis		
Yes	11,932 (20.4%)	3657 (14.4%)
No	46,522	21,683
Family history of HCC		
Yes	3362 (5.8%)	1190 (4.7%)
No	55,092	24,150

level or for which there was evidence of substantial confounding of other risk factors. A risk factor was considered a potential confounder if its presence resulted in a change of >10% in at least one other parameter estimate in the model compared to when it was removed.

Adjustment for discrepancies between Fox Chase Cancer Center and HCAS HBsAg test results was achieved using induced relative risk models (7, 8). Measurement error probabilities were estimated from the validation sample of 9,398 individuals on whom both Fox Chase Cancer Center and HCAS test results were available. All of the RRs included in the final model were essentially unchanged after adjustment for the measurement error except for HBsAg itself, which was somewhat biased toward the null in the unadjusted model. The variance of the regression parameters was estimated using a bootstrap resampling procedure. Sensitivity analysis demonstrated that the error adjustment procedure produced stable estimates of RRs when the adjustment parameters were varied within 10% of their observed values.

Results

Characteristics of study subjects at baseline are summarized in Table 1. A total of 434,718 person-years of follow-up was accumulated in the male cohort and 181,362 in the female cohort by September 30, 2000. There were 3,487 deaths among cohort members, with a crude mortality rate of $697.9/10^5$ person-years for men and $249.8/10^5$ for women. There were 900 HCC deaths among men (crude mortality rate per 100,000 person-years, 207.0; 29.7% of all deaths) and 77 among the women (crude rate, $42.5/10^5$ person-years; 17.0% of all deaths). HCC was the major cause of death among both men and women in the cohort, a finding that is consistent with population-level vital statistics for Haimen City. The cumulative risk of death from HCC during the 8 year follow-up was 0.5% for HBsAg($-$) males, 8.0% for HBsAg($+$) males, 0.1% for HBsAg($-$) females, and 2.0% for HBsAg($+$) females. Other major causes of death for men were chronic liver disease ($78.0/10^5$),

chronic lung disease ($68.1/10^5$), and lung cancer ($58.4/10^5$). For women, other major causes of death were stroke ($29.8/10^5$), chronic liver disease ($23.2/10^5$), and lung cancer ($17.6/10^5$). In addition to HCC and lung cancer, the most frequent causes of cancer death in men were stomach and esophageal cancer for men and stomach and cervical cancer in women.

Male Cohort, Main Analysis. HCC mortality data were fit to Cox proportional hazards models separately for the male and female cohorts. Estimates of the RR for individual risk factors with adjustment for age alone are shown in Table 2 for the male cohort. In addition to the variables shown, the relationship of HCC risk to drinking water and staple food sources in the 1960s, 1970s, and 1990s was not significantly associated with HCC risk. In Table 3, the results of the final multivariate model are shown. In this model, HBsAg positivity, a history of acute hepatitis, a family history of HCC, and occupation are significant risk factors for HCC in male adults. The drinking of alcohol, tea, or well water in the 1980s was not significantly associated with HCC risk.

Female Cohort, Main Analysis. Results of univariate and multivariate analysis for the female cohort are shown in Tables 4 and 5, respectively. As for the male cohort, drinking water and staple food were not significantly associated with HCC risk in females. In the multivariate analysis, only HBsAg status and history of acute hepatitis were significantly associated with HCC risk in the female cohort.

Alcohol Analyses. In the male cohort, 35,171 (60.2%) reported drinking alcohol $\geq$4 times per week. Among alcohol drinkers, 67.1% drank distilled spirits, 24.3% rice wine, 8.7% other grain wines, 15.8% grape wine, and 7.2% beer. (Total is >100% because individuals might drink more than one type of alcohol.) It was not possible to quantify weekly alcohol consumption amounts, but 9,348 drinkers (27.8%) reported that they drank the equivalent of $\geq$100 g of alcohol (5 ounces of distilled spirits or equivalent) per occasion of drinking. These were classified as heavy drinkers.

In the main analysis, alcohol drinking showed a small, nonsignificant protective effect against HCC in males. When drinkers were classified as moderate or heavy drinkers, there was a stronger protective effect for moderate drinkers in the multivariate model. The RR for moderate drinkers was 0.83 (95% CI, 0.72–0.95) and for heavy drinkers 0.99 (95% CI, 0.82–1.2). It was thought, however, that the apparent protective effect of moderate drinking may have been attributable to changes in drinking behavior by subjects with impaired liver function because of early HCC. Indeed, when the first 2 years of follow-up were excluded from the analysis, the apparent protective effect of alcohol was no longer significant. The RR for moderate drinkers was 0.89 (95% CI, 0.75–1.1) and heavy drinkers 1.0 (95% CI, 0.84–1.3), whereas the RRs for the other significant risk factors (age, HBsAg status, occupation, acute hepatitis history, and family history) were unchanged. Results were similar when the analysis was restricted the last 3 years of follow-up alone. The use of different cutoffs for definition of heavy drinking, 140 g or 200 g, which corresponded approximately to the 95th and 99th percentiles of the distributions, did not change these results. Similarly, inclusion or exclusion of the current smoking variable from these analyses did not affect the results. Furthermore, there was no significant association of HCC risk with consumption of any particular type of alcoholic beverage in the male cohort.

In the female cohort, only 3,429 individuals (13.5%) reported drinking alcohol $\geq$4 times/week. Of these, 323 (9.4%) were classified as heavy drinkers using the same criterion

Table 2 Male cohort: Age-adjusted association of HCC mortality rates with suspected risk factors

Status at study entry	Person-years	No. of HCC	RR	95% CI
HBsAg				
Positive	62,447	643	22.3	18.7–26.6
Negative	372,270	257		
History of acute hepatitis				
Yes	86,587	407	3.7	3.2–4.2
No	348,140	493		
Family history of HCC				
Yes	24,590	149	3.6	3.0–4.3
No	410,128	751		
Occupation				
Peasant	316,808	752	1.8	1.5–2.1
Nonpeasant	117,910	148		
Current cigarette smoking				
Yes	281,875	579	0.9	0.8–1.1
No	152,842	321		
Current alcohol drinking (≥4 drinks/week)				
Yes	261,740	490	0.8	0.7–0.9
No	172,978	410		
Current tea drinking (≥4×/week)				
Yes	68,380	118	0.8	0.7–1.0
No	366,338	782		
Drinking well water in 1980s				
Yes	211,383	397	0.9	0.7–1.0
No	223,335	503		
Corn as staple food in 1980s				
Yes	6999	16	1.1	0.6–1.7
No	427,718	884		

Table 3 Male cohort: Multivariate Cox proportional hazards model results, controlling for age

Status at study entry	RR	95% CI
HBsAg positive	18.8	16.0–22.1
History of acute hepatitis	2.3	2.0–2.7
Family history of HCC	2.3	1.9–2.7
Occupation (peasant)	1.5	1.3–1.8
Current alcohol drinker (≥4 drinks/week)	0.9	0.8–1.0
Current tea drinker (≥4×/week)	0.9	0.7–1.1
Drinking well water in 1980s	0.9	0.8–1.0

applied to the male cohort. Among female alcohol drinkers, 45.6% drank distilled spirits, 53.0% rice wine, 11.2% other grain wines, 2.9% grape wine, and 5.7% beer. (Total is >100% because individuals might drink more than one type of alcohol.) There was no significant association of either moderate or heavy alcohol drinking with HCC risk in the female cohort nor with consumption of any particular type of alcoholic beverage.

Cigarette Smoking Analyses. In the male cohort, 37,856 (64.8%) were current cigarette smokers. Smokers were more likely to be HBsAg-negative (65.3% *versus* 61.4%; $P < 0.001$), peasants (66.4% *versus* 60.7%; $P < 0.001$), alcohol drinkers (73.2% *versus* 52.0%; $P < 0.001$), or with a negative history of acute hepatitis (65.4% *versus* 62.4%; $P < 0.001$). Cigarette consumption categories were 1–5 cigarettes/day (13.9%), 6–10 cigarettes/day (27.3%), 11–20 cigarettes/day (47.1%), and >20 cigarettes/day (11.6%). In the multivariate analysis, smoking was not significantly associated with HCC risk for any of these categories or for a dose effect across categories of present consumption, years of smoking, or total pack-years of exposure (data not shown). Similarly, there was no association of HCC risk with smoking when the first 2 years of data were excluded,

nor when the analysis was restricted to the last 3 years of follow-up.

Smoking was uncommon in the female cohort, with only 891 individuals (3.5%) reporting current cigarette consumption. Female smokers were more likely to be peasants (4.0% *versus* 1.2%; $P < 0.001$) and alcohol drinkers (12.2% *versus* 2.2%; $P < 0.001$). Unlike with male smokers, however, there was no significant association between current smoking and negative HBsAg status (3.6% *versus* 3.1%; $P = 0.27$) or negative acute hepatitis history (3.6% *versus* 3.0%; $P = 0.07$). Females who smoked tended to smoke less than male smokers. The frequencies by category were 1–5 cigarettes/day (38.8%), 6–10 cigarettes/day (40.0%), 11–20 cigarettes/day (18.9%), and >20 cigarettes/day (2.4%). For the purposes of multivariate analysis, the two highest categories were combined in the female cohort.

Unlike the male cohort analysis, the RR associated with current smoking or dose-response over categories of current cigarette consumption in the female cohort was consistently >1.0, although not statistically significant in exploratory analyses. In the final model, controlling for age, HBsAg status, occupation, alcohol consumption, and acute hepatitis history, the RRs associated with each category of daily cigarette consumption were 1.5 (95% CI, 0.4–6.3), 2.0 (95% CI, 0.6–5.6.5), and 4.2 (95% CI, 1.3–13.8), respectively. The highest category of consumption (>10 cigarettes/day) included 3 HCC cases (all HBsAg positive) and 186 non-cases. To address the possibility that changes in smoking behavior may have occurred with impaired liver function in subjects who had undetected tumors at the time of cohort entry, we reanalyzed the survival data after removing the first 2 years of follow-up. In the same multivariate model, the RRs for cigarette smoking by category were increased to 1.2 (95% CI, 0.2–8.8), 2.1 (95% CI, 0.5–9.2), and 7.2 (95% CI, 2.1–24.5), while those for the other factors in the model remained essentially unchanged.

Table 4 Female cohort: Age-adjusted association of HCC mortality rates with suspected risk factors

Status at study entry	Person-years	No. of HCC	RR	95% CI
HBsAg				
Positive	19,050	61	37.2	19.1–72.5
Negative	162,312	16		
History of acute hepatitis				
Yes	25,492	34	6.1	3.9–9.6
No	155,870	43		
Family history of HCC				
Yes	8459	7	2.3	1.0–4.9
No	172,903	70		
Occupation				
Peasant	153,600	73	2.5	0.9–6.9
Nonpeasant	27,762	4		
Smoking				
Yes	6278	8	2.0	0.9–4.2
No	175,084	69		
Drinking alcohol (≥4 drinks/week)				
Yes	24,471	9	0.6	0.3–1.3
No	156,891	68		
Drinking tea (≥4×/week)				
Yes	12,245	1	0.2	0.1–1.3
No	169,117	76		
Drinking well water in 1980s				
Yes	79,403	28	0.7	0.5–1.2
No	101,959	49		
Corn as staple food in 1980s				
Yes	4526	2	1.0	0.2–4.0
No	176,836	75		

Table 5 Female cohort: Multivariate Cox proportional hazards model results, controlling for age

Status at study entry	RR	95% CI
HBsAg	33.2	17.0–65.0
History of acute hepatitis	4.7	3.0–7.5
Family history of HCC	1.2	0.5–2.5
Cigarette smoking	1.0	
1–5/day	1.5	0.35–6.3
6–10/day	2.0	0.60–6.5
>10/day	4.2	1.3–13.8
Alcohol drinking	0.57	0.28–1.2

Family History. 3,052 of 4,552 subjects reporting a family history of HCC gave specific information on relatives that were affected. We analyzed first-degree (including mother) and second affected as separate independent variables in the multivariate analysis. For the male cohort, having an affected first-degree relative had a RR of 2.4 (95% CI, 1.9–3.0) and a second-degree relative an RR of 2.8 (95% CI, 1.6–4.8). The RR for having an affected mother was not significantly different from the RR for having an affected first-degree relative other than mother. For females, none of the affected relative variables was significantly associated with HCC risk, just as positive family history overall was not significant.

Interactions with Sex. Table 6 shows HCC mortality rates by sex for different combinations of risk factors. The male:female ratio was fairly consistent across strata of risk factors and ranged between 1.8 and 6.7 for the age-adjusted rates. To test formally for interactions with sex, we conducted additional analyses with the combined male and female cohorts. Interaction terms for sex*HBsAg status ($P = 0.11$), sex*occupation ($P = 0.34$), and sex*family history ($P = 0.0.14$) were not statistically significant and produced estimates of the sex-specific RRs that were also consistent with those from the separate analyses. The RR associated with acute hepatitis in the combined model was 2.5 (95% CI, 2.2–2.8), and the sex*acute hepatitis interaction term was statistically significant ($P = 0.009$). Calculation of the RR for acute hepatitis separately for males and females using the parameter estimates from the combined model produced, as expected, RR estimates that were similar to those from the separate analyses for males and females, *i.e.*, 2.3 and 4.4, respectively.

Interactions with HBsAg Status. In the combined dataset and for the male and female cohorts separately, we tested for interactions of HBsAg status with the major variables of interest-acute hepatitis history, family history, occupation, smoking, and alcohol use. None of these was statistically significant. Of particular interest was the effect of acute hepatitis history. In the combined dataset, controlling for age, sex, family history, and occupation, the RR for HBsAg-negative persons with a history of acute hepatitis was 2.0 (95% CI, 1.4–2.9). For HBsAg-positive persons without acute hepatitis history, the RR was 18.3 (95% CI, 14.9–22.4). For those with both risk factors, the RR was 46.4 (95% CI, 37.7–57.3). Although this is suggestive of somewhat higher than multiplicative risk in the highest category, the interaction term was not statistically significant ($P = 0.23$). Similar effects were seen from analyzing the male and female cohorts separately.

We fit a multivariate model with the same covariates for the subset of cases and controls whose HBsAg status was determined by testing in our laboratories in Philadelphia (5,940 individuals, 677 cases in males; 2,503 individuals, 61 cases in females) to determine whether misclassification of HBsAg status by the less sensitive test used in Haimen accounted for some of the observed association of acute hepatitis history to HCC risk. For both males and females, the main effect of acute hepatitis history was somewhat lower than in the full cohort

Table 6   HCC mortality rates and rate ratios (male:female) by gender and combinations of other risk factors

Risk factors				Male			Female			RR	Adjusted RR[a]
HBsAg	Peasant	Acute hepatitis	Family history	HCC cases	Total person-years	Rate/10^5 py	HCC cases	Total person-years	Rate/10^5 person-years		
−	−	−	−	50	103,940	48	0	24,798	0	∞	∞
−	−	+/−	+/−	207	268,326	77	16	137,509	12	6.6	5.3
+	−	−	−	34	7,792	436	2	2,056	97	4.5	4.8
+	−	+/−	+/−	64	6,173	1037	2	903	221	4.7	4.8
+	+	−	−	230	28,272	814	24	11,324	212	3.8	4.1
+	+	+	−	207	15,074	1373	27	2,993	902	1.5	1.8
+	+	−	+	50	2,736	1827	4	1,304	307	6.0	6.6
+	+	+	+	58	2,396	2421	2	465	430	5.6	6.7

[a] Adjusted for age.

analysis (RR in males, 1.8; 95% CI, 1.5–2.1; RR in females, 4.0; 95% CI, 2.4–6.7). The test for HBsAg*acute hepatitis history interaction was not significant for males or females ($P = 0.86$ and 0.27, respectively).

Discussion

In this extremely high-risk population, HCC is the major cause of adult mortality. Because Haimen City is also an endemic area for HBV infection, all adults are likely to have been exposed to HBV in their lifetimes, regardless of their current HBsAg status. Our study confirms the findings of many previous studies that current HBsAg positivity is still the major risk factor for development of HCC. Unfortunately, it is also apparent that even noncarriers are at high risk for HCC development in endemic populations. In a recent study in Taiwan, Huo *et al.* (9) showed that adult subjects who clear HBsAg remain at high risk for adverse outcomes of HBV infection, including HCC.

The major aim of our study was to discover other risk factors that may lead to higher HCC risk in this population, particularly those risk factors amenable to intervention. To our surprise, alcohol consumption did not appear to contribute to HCC risk. If the risk associated with alcohol consumption is confined only to very heavy drinkers, we may not have detected it because of misclassification inherent in the constraints on our data collection. At cohort entry, we were restricted to a one-page questionnaire because the number of subjects to be screened was large with a high illiteracy rate.

Our finding of a lack of association with cigarette smoking in males is consistent with many, but not all, prior studies of this exposure (5, 10, 11). Numbers of cigarettes consumed per day by smokers, even among males, is low in this area compared with most Western populations. Female smoking was rare in Haimen City, and females who smoke tend to smoke even less than male smokers at the inception of this cohort. Nevertheless, we found that females in the highest category of current smoking consumption (>10 cigarettes/day) did have a significantly increased risk for HCC in a multivariate model controlling for other major risk factors and potential confounders. There was no suggestion of a similar effect in male smokers, despite their higher levels of exposure. Interpretation of this finding requires some caution because it is based on only 8 HCC cases in smoking women, and further investigation is required to determine whether there are other factors that may be significant in these unusual women. The possibility of unmeasured confounding by unknown lifestyle, reproductive, or occupational factors must be considered and will be addressed in future work in this population. Because anecdotal evidence

suggests that rates of female smoking have increased in Haimen City since the early 1990s, the potential public health impact of this finding is considerable.

Other suspected lifestyle and environmental exposures in our study also yielded negative results, including drinking water source which has long been considered a potentially important exposure in this region (12) and corn consumption because of aflatoxin contamination (13). The corn consumption variable was used as a proxy for past exposure to aflatoxin because public health measures have greatly reduced both corn consumption and current aflatoxin exposure in this region of China, making measurement of current consumption a poor proxy of lifetime exposure. It is possible, however, that food sources other than corn were substantial sources of aflatoxin exposure in the past. No data are available for aflatoxin levels in foodstuffs in Haimen City in past decades, but studies in other parts of China [*e.g.*, Wang *et al.* (13)] have implicated a wider range of foods including soy products, rice, and cooking oils.

We did find, however, a strong association of occupation with risk in male adults. Peasants were at 50% higher risk of HCC than nonpeasants, independent of other risk factors including HBsAg status. Occupation was not a significant risk factor in the female cohort, but there were few nonpeasant females overall. This suggests a number of possible carcinogenic exposures, including pesticides. Our brief questionnaire asked subjects about past pesticide exposure, and this variable was not significantly associated with HCC risk in our analysis. It is likely, however, that self-reported pesticide exposure is unreliable, and that biological measurements of exposure and dose would yield more relevant results.

The strongest risk factor other than HBsAg positivity that we found in both males and females was a history of acute hepatitis. Self-reported acute hepatitis was considered reliable in this population because until quite recently hospitalization as an infection control measure was required for this disease. Unfortunately, determination of the etiology of an acute hepatitic episode has not always been possible, and subject's reports of a specific diagnosis would in any case be unreliable. It is unlikely that these hepatitis episodes represent initial infection with HBV, which usually occurs in infancy or early childhood in this population. Flares of chronic hepatitis in HBV carriers, however, can produce symptoms similar to acute hepatitis and may occur with higher frequency during seroconversion from hepatitis B e antigen positive to negative or HBsAg positive to negative (14–16). It is likely that a substantial portion of the hepatitis episodes reported by cohort members were exacerbations of chronic HBV infection, some in patients who subsequently cleared HBsAg. Without prospective data, however, we

cannot exclude the possibility of other etiological factors, both viral and nonviral. In pilot studies of viral causes of current adult cases of acute hepatitis in Haimen City, we have found evidence of acute hepatitis A virus and hepatitis E virus infections (17).

Whatever the cause of these acute hepatitis episodes, the finding strongly suggests that liver damage is an important risk factor for HCC. HBV is not directly cytopathic, and liver damage that occurs during infection is immune mediated. Some measure of immune tolerance to the virus is necessary for chronic infection to be maintained, and many carriers remained infected for decades with no serological evidence of liver inflammation (18), presumably because of their tolerant state. Increases in serum transferases and other indices of inflammation typically occur during spontaneous clearance of viral replication in chronic carriers (15, 16). Liver damage followed by regeneration may promote the carcinogenic process in those already infected with HBV.

Although family clusters of HCC have been described in several populations (19, 20), it has been difficult to distinguish the effect of intrafamilial transmission of HBV from potential genetic factors predisposing to HCC itself. Shen *et al.* (21) analyzed pedigrees from Haimen City cases and found that familial aggregation could be explained by the interaction of HBV infection and a major gene. Yu *et al.* (22) found that male HBV carriers with a first-degree relative with HCC were at 2.4-fold elevated risk of HCC themselves. This finding is consistent with our estimation of 2.3-fold elevated risk in males. Our study also confirms that subjects who are not currently HBsAg positive have a similarly increased risk of HCC if they have an affected first- or second-degree relative.

Previous studies of familial aggregation of HCC have not included women at all or have not analyzed them separately. Our study did not find an elevated risk of HCC for female cohort members with a first- or second-degree affected relative. Overall, 17% of male cases reported an affected relative compared with only 9% of female cases. There are several possible explanations for this. Because women in Haimen City generally leave their family homes when they marry, our finding may point to important lifestyle or shared environment in adulthood as being more important than genetic factors. It may also suggest a gene-environment interaction involving a sex-linked gene and/or an environmental exposure that is more common in adult males than females. As with the smoking finding, this result should be interpreted with caution because of the comparatively small number of female HCC cases.

Although women in Haimen City were at overall lower risk of HCC than men, their relative risk associated with HBsAg positivity was higher, and the number of other risk factors identified for them in our analysis was fewer. Although our cohort size was large, we had only 77 HCC deaths in women, which may have limited our ability to detect weak associations. Other epidemiological studies of HCC have often omitted women or included them in numbers too small for separate analysis. We believe that the higher relative risk among female HBV carriers in Haimen City is attributable to lower exposure of women to non-HBV risk factors. The usual suspect for hepatocarcinogenic exposures more common in men than women, alcohol consumption, does not, however, appear to explain the difference in this case. Other investigators have hypothesized a role for sex hormones in liver cancer etiology, both a protective effect of estrogens (23) and an increased risk from testosterone (24). Further studies that include sufficient numbers of women with adequate measurements of hormone levels and other reproductive factors may help to elucidate this mystery.

In summary, HCC remains a public health problem of enormous significance in much of the developing world. Universal HBV vaccination remains the primary means of cancer prevention available, but for the millions of adults in these populations who have already been infected, new approaches are needed. We have strong evidence that there are environmental and genetic factors that may be acting independently of chronic HBV infection to increase HCC risk in endemic populations. These factors may also help to explain differences in HCC mortality rates between HBV carriers in different populations. It is hoped that further investigation of these findings will lead to appropriate interventions to reduce HCC mortality.

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