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Individual and combined effects of hepatitis B surface antigen level and viral load on liver cancer risk

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Abstract

Background—Hepatitis B surface antigen (HBsAg) and viral load are both hallmarks of hepatitis B virus (HBV) infection and have potential to stratify liver cancer risk.

Methods—We carried out a nested case-control study including 211 liver cancer cases and 221 controls who were sero-positive for HBsAg within two population-based cohorts in Shanghai. Logistic regression was performed to estimate the odds ratios (ORs) and 95% confidence intervals (CIs).

Results—Risk of liver cancer was positively related to increasing levels of HBV DNA and HBsAg in dose-response manners. Compared to subjects with HBV DNA <2,000 IU/ml, the adjusted ORs increased from 2.11 (95%CI: 0.99–4.50) to 10.47 (95%CI: 5.06–21.68) for those with HBV DNA level at 2,000–19,999 IU/ml to ≥20,000 IU/ml. Compared to subjects at a low level of HBsAg (0.05–99 IU/ml), the adjusted ORs increased from 1.82 (95%CI: 0.90–3.68) to 2.21 (95%CI: 1.10–4.43) for those with HBsAg level at 100–999 IU/ml to ≥1,000 IU/ml. Compared to subjects with HBV DNA <2,000 IU/ml and HBsAg <100 IU/ml, the adjusted ORs were increased from 2.20 (95%CI: 1.07–4.49) for those with HBV DNA <2,000 IU/ml and HBsAg 100 IU/ml to 6.94 (95%CI: 3.39–14.23) for those with HBV DNA ≥2,000 IU/ml and HBsAg <1,000 IU/ml, and 16.15 (95%CI: 7.60–34.32) for those with HBV DNA ≥2,000 IU/ml and HBsAg ≥1,000 IU/ml.

Conclusion—Elevated levels of HBV DNA and HBsAg are associated with increased risks of liver cancer. Chronic HBsAg carriers may be suggested to simultaneously lower the viral load to <2,000 IU/ml and HBsAg level to <100 IU/ml to lower their liver cancer risk.

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Keywords

Hepatitis B virus; Hepatitis B surface antigen; Liver cancer; Prospective study; Viral load

Introduction

Hepatitis B virus (HBV) infection is a global health problem and roughly 30% of the world's population shows serological evidence of current or past HBV infection. In 2010, about 60%–80% of the total liver cancer incidence and half of the mortality was attributed to HBV infection¹. Thus, the control and treatment of chronic HBV infection is a major and effective approach to lower the morbidity and mortality of liver cancer.

In current clinical practice guidelines, effective suppression of serum HBV DNA is a marker of efficacy for antiviral therapy. Serum HBV DNA was indicated to be the major drive of disease progression in patients with chronic hepatitis B (CHB)^{2–5}, and was extensively used to monitor and predict hepatocellular carcinoma (HCC) occurrence^{6–9}. The quantification of serum hepatitis B surface antigen (HBsAg) was widely used in the management of CHB patients during recent years^{10, 11}. Increasing evidences have suggested that a lower HBsAg level is associated with better clinical outcomes, including a higher likelihood of HBsAg loss¹², lower risk of hepatitis B e antigen (HBeAg)-negative hepatitis, cirrhosis and HCC^{13, 14}.

The combined effect of quantitative HBsAg level and viral load on liver cancer has been investigated in a few hospital-based studies. Studies in Taiwan and Italy suggested that HBsAg quantification level might complement HBV DNA in the identification of low-risk inactive carriers^{13, 15}. Of note, hospital-based studies characterized by high proportion of patients with severe liver disease and worse health condition may expose to selection bias and limit the generalization of the result. To guide the future community-based surveillance and prediction of liver cancer occurrence, the large-scale population-based prospective studies are still needed to comprehensively evaluate the individual and combined effects of these serum markers of HBV infection on liver cancer risk. In particular, relevant data are still largely lacking in mainland of China, where chronic HBV infection accounts for half of the CHB in the world. Thus, we conducted a nested case-control study within two large population-based cohorts in Shanghai to prospectively assess the liver cancer risk by combining the quantitative HBsAg level and viral load.

Materials and Methods

Study Population

The Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS) are two large population-based prospective cohort studies currently on-going in Shanghai, China. The study was approved by the institutional review boards of all collaborating institutions, and all participants provided written informed consent. Details on the cohorts have been described elsewhere^{16, 17}. Briefly, a total of 74,941 eligible women aged 40 to 70 years and 61,480 eligible men aged 40 to 74 years were enrolled in the SWHS

and SMHS in 1997 to 2000 and 2002 to 2006, respectively. We collected information on demographic characteristics, anthropometric measurements, lifestyle, dietary habits, physical activity, disease history, medication history and family history of cancer in baseline survey. Of the study participants in the SWHS and SMHS, 56,830 (75.8%) and 46,111 (75.0%) provided a blood sample. The samples were kept in a portable Styrofoam box with ice packs during transportation and processed within 6 hours of collection for long-term storage at -70°C . At the time of sample procurement, a bio-specimen collection form was completed for each participant, which included the information such as the date and time of sample collection and time of last meal.

All cohort members were followed for cancer occurrence through in-person follow-up surveys every 2 to 3 years and annual record linkage with databases of the population-based Shanghai Cancer Registry, Shanghai Vital Statistics Registry and Shanghai Resident Registry. For the SWHS, the response rates for the first (2000–2002), second (2002–2004), third (2004–2007) and fourth (2007–2011) in-person follow-up surveys were 99.7%, 98.7%, 94.9% and 92.3%, respectively. For the SMHS, the response rates for the first (2004–2008) and second (2008–2011) follow-up surveys were 97.6% and 93.7%, respectively. All possible cancer diagnoses were verified through home visits and review of medical charts by a panel of clinical and pathological experts.

In our previous main liver cancer study (i.e., the parent study of the present one)¹⁸, we included 363 incident liver cancer cases identified during follow-up of the two cohorts through December 2012. We randomly chose 10 control subjects per case among all cohort members, who were individually matched to each case by age (± 2 years), sex (male or female), and date (± 30 days) and time (morning or afternoon) at sample collection, interval since last meal (<2 hours) and menopausal status (premenopausal or postmenopausal, women only). We tested the baseline plasma samples of all 363 cases and the 3,511 matched controls for the presence of HBsAg, of which 432 subjects (211 cases and 221 controls) were sero-positive for HBsAg and were finally included in the current study.

Laboratory Test

Plasma samples at enrollment were quantified for HBsAg levels using Architect HBsAg QT (Abbott Diagnostic, Shanghai, China) according to the manufacturer's instructions. Sero-negative of HBsAg was defined as a titer of less than 0.05 IU/ml. For subjects with positive HBsAg status (i.e., HBsAg titer ≥ 0.05 IU/ml), the HBV DNA were further quantified using the Hepatitis B Virus Diagnostic Kit (Real-Time PCR, PerkinElmer, USA) according to the manufacturer's instructions, with a low detection limit of 20 IU/ml. The laboratory personnel were blinded as to the disease status of study subjects whose plasma samples they analyzed.

Statistical Analysis

According to the earlier reports^{13, 19}, we categorized the HBsAg levels into three groups (0.05–99, 100–999, and ≥ 1000 IU/ml) and categorized the HBV-DNA levels into three groups (< 2000 , 2000–19 999, and $\geq 20\ 000$ IU/ml). Ratio of HBsAg to HBV DNA was determined to reflect the proportion of subviral particles to virions. Continuous variables

were compared with t test or Mann-Whitney U test as appropriate, and categorical variables were compared with Chi-square test. Spearman's rank correlation coefficient was used to assess the correlation between HBV DNA and HBsAg levels.

We broke the matched case-control sets of the initial study to maximize the sample size. Unconditional logistic regression was performed to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) for liver cancer risk associated with HBV DNA and HBsAg levels. Covariates were selected as they were known to be associated with liver cancer development. Covariates included in the final model were as follows: education level, family income, family history of liver cancer, HBeAg status, history of chronic hepatitis/other chronic liver diseases, and vegetable intake. Besides the aforementioned covariates, the original matching factors (age, sex, date and time at sample collection) were also included in the model. Further analyses by additionally adjusting for body mass index, total physical activity, tea consumption, smoking status, alcohol drinking, fruit intake, total energy intake, history of diabetes, menopausal status (for women) did not change the results materially, thus we did not enter them into the final model. Besides, no significant interaction effect between HBsAg and HBV DNA was detected based on the first-degree multiplicative model.

Statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC). A two-sided *P* value of <0.05 was considered statistically significant.

Results

Baseline characteristics of liver cancer cases and controls were shown in Table 1. The cases had a median follow-up time of 4.42 years, which was less than the controls (9.53 years). Compared with control subjects, cases had lower family income and education level, less vegetable intake, and were more likely to have reported a family history of liver cancer, a history of chronic liver disease and sero-positive HBeAg. There were no significant differences between cases and controls in BMI, history of diabetes, total energy intake, fruit intake, physical activity, smoking status, alcohol consumption, and tea drinking.

As shown in Table S1, HBV DNA and HBsAg levels were correlated moderately in control subjects ($r=0.63$, $P<0.01$). The correlation was higher in subjects sero-positive for HBeAg ($r=0.93$, $P<0.01$), lower in those sero-negative for HBeAg ($r=0.58$, $P<0.01$) and those concurrent with a low HBV DNA of <2,000 IU/ml ($r=0.56$, $P<0.01$), and lowest in those sero-negative for HBeAg concurrent with a high HBV DNA of ≥2,000 IU/ml ($r=0.18$, $P=0.27$). In addition, the median of HBsAg/HBV DNA ratio was 0.69 (Quartile Range=1.27) in the HBeAg-negative subjects concurrent with HBV DNA<2,000 IU/ml, which was higher than that in the HBeAg-positive subjects (Median=0.51, Quartile Range=0.45) and the HBeAg-negative subjects concurrent with HBV DNA of ≥2,000 IU/ml (Median=0.57, Quartile Range=0.24) (Table S2).

Risk of liver cancer was positively related to increasing levels of HBV DNA ($P_{trend}<0.01$) and HBsAg ($P_{trend}=0.03$) in dose-response manners (Table 2). Compared to subjects at a low level of HBV DNA (<2,000 IU/ml), the adjusted ORs increased from 2.11 (95%CI: 0.99–

4.50) for those at an intermediate level of HBV DNA (2,000–19,999 IU/ml) to 10.47 (95% CI: 5.06–21.68) for those at a high level of HBV DNA ($\geq 20,000$ IU/ml). The adjusted OR was 3.12 (95% CI: 2.19–4.44) for each level of HBV DNA increase. Compared to subjects at a low level of HBsAg (0.05–99 IU/ml), the adjusted ORs increased from 1.82 (95% CI: 0.90–3.68) for those at an intermediate level of HBsAg (100–999 IU/ml) to 2.21 (95% CI: 1.10–4.43) for those at a high level of HBsAg ($\geq 1,000$ IU/ml). The adjusted OR was 1.48 (95% CI: 1.04–2.09) for each level of HBsAg increasing. Furthermore, such dose-response associations were not materially altered in the HBeAg-negative subjects.

Subgroup results for different HBsAg cutoff levels on liver cancer risk stratified by HBV DNA were shown in Table 3 and 4. In lowly viremic subjects (HBV DNA $< 2,000$ IU/ml), subjects with HBsAg level ≥ 100 IU/ml may be at an increased risk of liver cancer (OR=1.98, 95% CI: 0.96–4.11) compared to those with HBsAg level < 100 IU/ml. The risk was comparable for subjects with HBsAg level $< 1,000$ IU/ml and $\geq 1,000$ IU/ml (OR=1.05, 95% CI: 0.42–2.59). In highly viremic subjects (HBV DNA $\geq 2,000$ IU/ml), the risk was higher for subjects with HBsAg level ≥ 100 IU/ml than those with HBsAg level < 100 IU/ml (OR=3.72, 95% CI: 1.17–11.82). Moreover, a higher risk was also observed for subjects with HBsAg level $\geq 1,000$ IU/ml than those with HBsAg level $< 1,000$ IU/ml (OR=2.60, 95% CI: 1.11–6.05).

The combined effects of HBV DNA and HBsAg levels on liver cancer risk were shown in Table 5. Compared to subjects with the minimal risk of liver cancer (HBV DNA $< 2,000$ IU/ml and HBsAg level < 100 IU/ml), the adjusted ORs were increased from 2.20 (95% CI: 1.07–4.49) for those with HBV DNA $< 2,000$ IU/ml and HBsAg level ≥ 100 IU/ml to 6.94 (95% CI: 3.39–14.23) for those HBV DNA $\geq 2,000$ IU/ml and HBsAg level $< 1,000$ IU/ml, and 16.15 (95% CI: 7.60–34.32) for those with HBV DNA $\geq 2,000$ IU/ml and HBsAg level $\geq 1,000$ IU/ml.

Discussion

It was the first prospective study to comprehensively evaluate the individual and combined effects of HBV DNA and quantitative HBsAg levels on liver cancer risk in urban Chinese residents. Using data from two large population-based prospective cohorts in urban Shanghai, we demonstrated that the elevated levels of HBV DNA and HBsAg were associated with higher risk of developing liver cancer in dose-response manners. The risk started to increase significantly at a HBV DNA level of 2,000 IU/ml and a HBsAg level of 1,000 IU/ml. But in lowly viremic subjects (HBV DNA $< 2,000$ IU/ml), HBsAg > 100 IU/ml was observed to be associated with liver cancer risk. Compared to individuals with HBV DNA $< 2,000$ IU/ml and HBsAg levels < 100 IU/ml, those with HBV DNA $\geq 2,000$ IU/ml and HBsAg level $\geq 1,000$ IU/ml were at the high risk of developing liver cancer.

We observed a positive correlation between HBV DNA and HBsAg levels. Previous studies indicated that the correlation changed during the natural history of HBV infection, higher at HBeAg-positive phase and lower at HBeAg-negative phase and the lowly replicative phase, which was consistent with our findings^{20, 21}. Furthermore, we observed the highest HBsAg/HBV DNA ratio in HBeAg-negative subjects with HBV DNA $< 2,000$ IU/ml.

Previous studies indicated that the ratio was significantly higher in the low-replicative phase which was characterized by HBeAg negativity, HBV DNA<2,000 IU/ml and normal serum alanine aminotransferase, compared to immune-tolerant, immune-clearance and HBeAg negative phases, respectively^{20, 21}. This data indicated that the HBV DNA may be decreased more significantly than HBsAg from the phases of immune-tolerant to immune-clearance. Thus, it was hypothesized that immune control over viral replication may be the first step of immune clearance. Moreover, the discrepancy between HBV DNA and HBsAg level at the HBeAg-negative phase and lowly replicative phase might be caused by accumulation of integrated viral envelope sequences in infected hepatocytes. During the lowly replicative phase, serum HBsAg levels may mainly derive from the integrated form of HBV DNA rather than the episomal form, and therefore decrease discordantly with serum HBV DNA¹³. Of note, lowly viremic patients who have high HBsAg level might harbor more hepatocytes with HBV integration than those who have low HBsAg level¹³. The integrated viral sequences may lead to an increased genomic instability which may play an important role in hepatocarcinogenesis¹³.

It has been extensively reported that HBV DNA was the main drive to HCC development. One of the largest community-based cohort study in Taiwan (the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus cohort) found that increasing levels of HBV DNA at study entry was associated with a stepwise increase in HCC risk². High HBV DNA that started at 10000 copies/ml was significantly associated with an increased risk of HCC², which was consistent with our observations of the start point at 2000 IU/ml of HBV DNA. Recently, HBV DNA has been incorporated into several risk prediction models to predict HBV-related HCC occurrence, with promising results^{6, 8, 9, 22}. In the parent study of the present one, we observed a strong positive dose-response relationship between HBsAg levels and liver cancer risk in a general healthy population¹⁸. Compared to HBsAg-negative subjects, the corresponding ORs increased from 7.27 to 7.16, 34.30 and 47.33 in men and 1.37 to 3.81, 7.36 and 16.86 in women, with HBsAg level increased from 0.05–9 IU/ml to 10–99 IU/ml, 100–999 IU/ml, and 1000 IU/ml¹⁸. Here, after further adjusting for HBV DNA, such dose-response risk remained significant in HBsAg carriers. HBV DNA and HBsAg were independent risk factors of HBV-related HCC, which suggested that infectious virions and noninfectious HBsAg particles may have their own unique mechanism of inducing hepatocarcinogenesis. Integration of the new biomarker, quantitative serum HBsAg levels into the risk prediction models may increase the predictability for HCC.

For highly viremic subjects (HBV DNA ≥2,000IU/ml), HBsAg ≥100 or ≥1,000IU/ml were both identified to be associated with increased risk of liver cancer. Of note, for lowly viremic subjects (HBV DNA<2,000IU/ml) who has reported to be at similar risk of liver cancer in earlier reports^{2, 4}, HBsAg ≥100IU/ml may be associated with increased risk of liver cancer. These data suggested that HBsAg level might complement HBV DNA in predicting HCC risk. Another cohort study in Taiwan(the ERADICATE-B study) also reported that high levels of HBsAg increased risk of HCC in patients with low HBV load¹³. However, a HBsAg level ≥1,000 rather than ≥100 IU/ml was identified as an independent risk factor for HCC development in the ERADICATE-B cohort members with HBV DNA<2,000 IU/ml. The discrepancy might be partly due to the hospital-based design for the ERADICATE-B

[Elucidation of Risk Factors for Disease Control or Advancement in Taiwanese Hepatitis B Carriers] study, which may have higher possibility to enroll subjects with severe liver disease condition (more subjects with HBsAg >1,000 IU/ml than <1,000 IU/ml) compared to a population-based study¹³. Because of limited liver cancer cases (<5 cases) in subjects with HBsAg of 100–999 IU/ml in the ERADICATE-B study, it may be under-powered to detect the risk associated with lower level of HBsAg level. In present study, a relative conservative value of 100 IU/ml of HBsAg may be a more suitable cut-point to further stratify the lowly viremic HBV carriers (HBV DNA <2,000 IU/ml). The findings indicated the importance of lowering serum HBsAg levels in those who already have low serum HBV DNA levels. When this cutoff value is validated in future studies, physicians may be suggested to adopt it as the intermediate treatment goal to stop nucleos(t)ide analogue therapy.

A strength of this study is based on its prospective design. The HBV DNA and quantitative HBsAg levels were determined in plasma collected before the development of liver cancer, minimizing the possibility that the HBV DNA and HBsAg levels were affected by the malignant transformation of hepatocytes or clinical treatment for liver cancer. Second, it is the first population-based study among urban residents in mainland China, where HBV infection is highly endemic. This study could provide valuable data for future community-based prediction and surveillance of liver cancer among urban Chinese residents. The present study also has some limitations. Our quantifications of HBV DNA and HBsAg level used a single plasma sample obtained at study entry; thus, the changes in HBV DNA and HBsAg levels over time during follow-up could not be assessed. Second, antiviral therapy may decrease the viral load and be associated with reduced risk of liver cancer. Thus, the effect estimate of viral load may be overestimated without adjusting it in the analysis. However, the study population were recruited from communities, not patients in hospital. They were supposed to have better health condition or asymptomatic infection, minimizing the possibility of medical treatments. Third, other potential confounders such as intake of aflatoxin B1, hepatitis C virus, HIV infection and serum metabolic markers were not considered in present study, which may bias the observed associations.

Conclusion

There is a strong positive dose-response relationship between HBV DNA and HBsAg levels and risk of liver cancer in these population-based cohorts of residents in urban Shanghai. Individuals with HBV DNA <2,000 IU/ml and HBsAg <100 IU/ml should be considered as the minimal-risk HBV carriers, and HBV DNA 2,000 IU/ml and HBsAg 1,000 IU/ml as the high-risk persons of developing liver cancer. Clinical therapy of CHB to simultaneously lower serum levels of both HBV DNA and HBsAg may be suggested to lower the risk of liver cancer, especially for those high-risk persons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosure statement

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Abbreviations

BMI	body mass index
CHB	chronic hepatitis B
CI	confidence interval
HBV	hepatitis B virus
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HCC	hepatocellular carcinoma
ICD-9	the International Classification of Disease, Ninth Revision
OR	odds ratio
SWHS	the Shanghai Women's Health Study
SMHS	the Shanghai Men's Health Study

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Table 1

Baseline characteristics of participants in the nested case-control study

Characteristic	All subjects				Women		Men		
	Cases (n=211)	Controls (n=221)	P value*	Cases (n=53)	Controls (n=62)	P value*	Cases (n=158)	Controls (n=159)	P value*
Age at interview (years)	56.37 ± 9.24	58.12 ± 9.18	0.05	54.29 ± 8.87	58.42 ± 8.51	0.01	57.07 ± 9.28	57.99 ± 9.45	0.38
Gender									
Female	25.12	28.05	0.49						
Male	74.88	71.75							
Body mass index (kg/m ² ,Continuous)	23.70 ± 3.20	23.62 ± 2.77	0.77	24.28 ± 3.54	23.35 ± 3.18	0.14	23.51 ± 3.07	23.72 ± 2.60	0.51
Body mass index (kg/m ² , Categorized)			0.18			0.35			0.16
<18.50	5.21	3.17		5.66	8.06		5.06	1.26	
18.50–23.99	51.18	50.23		43.40	43.55		53.80	52.83	
24.00–27.99	34.12	41.18		32.08	40.32		34.81	41.51	
28.00	9.48	5.43		18.87	8.06		6.33	4.40	
Family income (%)			0.05			0.34			0.05
Low	13.33	12.67		22.64	14.52		10.19	11.95	
Low to middle	52.86	42.99		43.40	46.77		56.05	41.51	
Middle to high	26.19	29.41		24.53	19.35		26.75	33.33	
High	7.62	14.93		9.43	19.35		7.01	13.21	
Education level (%)			<0.01			0.39			<0.01
Elementary school or less	12.32	18.10		26.42	38.71		7.59	10.06	
Middle school	39.34	37.56		43.40	29.03		37.97	40.88	
High school	35.07	22.62		24.53	25.81		38.61	21.38	
College or above	13.27	21.72		5.66	6.45		15.82	27.67	
Ever had chronic hepatitis/other chronic liver disease (%)	48.34	15.84	<0.01	39.62	12.90	<0.01	51.27	16.98	<0.01
Family history of liver cancer (%)	15.17	6.33	<0.01	18.87	4.84	0.02	13.92	6.92	0.04
Ever had diabetes (%)	8.53	8.14	0.89	7.55	6.45	0.82	8.86	8.81	0.99
Ever had cholelithiasis or cholecystectomy (%)	12.80	11.31	0.64	11.32	17.74	0.33	13.29	8.81	0.20
Total energy intake (kcal/day)	1900.20 ± 546.60	1898.70 ± 529.80	0.98	1742.00 ± 612.00	1715.70 ± 399.10	0.79	1953.20 ± 514.20	1970.10 ± 557.70	0.78

Characteristic	All subjects			Women			Men		
	Cases (n=211)	Controls (n=221)	P value [*]	Cases (n=53)	Controls (n=62)	P value [*]	Cases (n=158)	Controls (n=159)	P value [*]
Vegetable intake (g/day)	318.20 ± 173.50	361.20 ± 202.80	0.02	326.70 ± 204.50	352.90 ± 189.20	0.48	315.30 ± 162.40	364.50 ± 208.30	0.02
Fruit intake (g/day)	183.70 ± 146.90	186.00 ± 156.60	0.88	243.60 ± 182.40	250.80 ± 161.80	0.82	163.70 ± 127.30	160.80 ± 147.40	0.85
Physical activity (MET-h/week)	77.28 ± 43.86	77.23 ± 42.45	0.10	102.30 ± 46.98	107.9 ± 44.06	0.51	59.55 ± 37.16	65.27 ± 35.32	0.16
Ever smoker (%)	55.45	47.51	0.10	1.89	4.84	0.62	73.42	64.15	0.08
Ever alcohol drinker (%)	21.33	26.70	0.19	0	4.84	0.25	28.48	35.22	0.20
Ever tea drinker (%)	52.61	49.77	0.56	18.87	24.19	0.49	63.92	59.75	0.44
Post-menopausal status (%)	–	–	–	58.49	74.19	0.07	–	–	–
Positive HBsAg status (%)	37.91	5.88	<0.01	43.40	4.84	<0.01	36.08	6.29	<0.01
HBsAg level (log ₁₀ IU/ml) [‡]	3.07 ± 1.20	1.78 ± 2.75	<0.01	3.41 ± 0.79	2.06 ± 2.44	<0.01	2.95 ± 1.24	1.72 ± 3.03	<0.01
HBV DNA (log ₁₀ IU/ml) [‡]	5.29 ± 2.80	2.29 ± 1.75	<0.01	4.97 ± 3.14	2.23 ± 1.53	<0.01	5.30 ± 2.51	2.31 ± 2.07	<0.01
HBsAg/HBV DNA ratio	0.55 ± 0.26	0.59 ± 0.86	0.88	0.58 ± 0.31	0.73 ± 0.87	0.84	0.54 ± 0.25	0.56 ± 1.00	0.97

HBsAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

^{*}Continuous variables were compared with t test or Mann-Whitney U test as appropriate, and categorical variables were compared with chi-square test.

[‡]HBV DNA (IU/ml) and HBsAg levels (IU/ml) were logarithmically transformed.

Table 2
Odds ratios and 95% confidence intervals for liver cancer risk associated with HBV DNA and HBsAg levels

	Cases/Controls	Model 1 OR (95% CI) [†]	Model 2 OR (95% CI) [‡]	Model 3 OR (95% CI) [§]	Model 4 OR (95% CI)
<i>All subjects</i>					
HBV DNA (IU/ml)					
<2,000	50/170	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	3.12 (2.19–4.44)
2,000–19,999	21/27	2.65 (1.38–5.09)	2.59 (1.24–5.41)	2.11 (0.99–4.50)	
20,000	140/24	20.46 (11.81–35.45)	15.11 (7.79–29.34)	10.47 (5.06–21.68)	
<i>P</i> for trend		<0.01	<0.01	<0.01	
HBsAg levels (IU/ml)					
0.05–99	42/120	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.48 (1.04–2.09)
100–999	56/46	2.65 (1.38–5.09)	4.05 (2.17–7.54)	1.82 (0.90–3.68)	
1,000	113/55	20.46 (11.81–35.45)	5.68 (3.11–10.35)	2.21 (1.10–4.43)	
<i>P</i> for trend		<0.01	<0.01	0.03	
<i>HBsAg-negative subjects</i>					
HBV DNA (IU/ml)					
<2,000	47/166	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	3.02 (2.08–4.38)
2,000–19,999	21/27	2.79 (1.45–5.39)	2.50 (1.19–5.22)	1.97 (0.92–4.24)	
20,000	63/15	15.27 (7.90–29.54)	14.57 (7.17–29.59)	9.99 (4.62–21.59)	
<i>P</i> for trend		<0.01	<0.01	<0.01	
HBsAg levels (IU/ml)					
0.05–99	32/116	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.55 (1.07–2.25)
100–999	41/45	3.51 (1.95–6.32)	3.87 (2.01–7.45)	1.78 (0.85–3.73)	
1,000	58/47	5.30 (2.96–9.48)	5.91 (3.07–11.38)	2.41 (1.14–5.09)	
<i>P</i> for trend		<0.01	<0.01	0.02	

OR, odds ratio; CI, confidence interval, HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

[†] Adjusted for age and sex.

[‡] Adjusted for age, sex, HBeAg (for all subjects only), family history of liver cancer, education, income, vegetable intake, history of chronic liver disease and time at sample collection.

[§] Adjusted for covariates in Model 2. Additionally, HBV DNA and HBsAg level were mutually adjusted in the models.

ORs and 95% CIs was estimated by assigning an ordinal value to each range of HBV DNA and HBsAg, and treating them as continuous variables in the unconditional logistic regression models, with covariates adjusted as Model 3.

Table 3

Risk of liver cancer for HBsAg level 100IU/ml in comparison to <100IU/ml, stratified by HBV DNA level.

HBsAg Levels (IU/ml)	Cases/Controls	Model 1 OR (95% CI) [†]	Model 2 OR (95% CI) [‡]
HBV DNA<2,000IU/ml			
<100	24/110	1.00 (Reference)	1.00 (Reference)
100	26/60	2.03 (1.05–3.93)	1.98 (0.96–4.11)
HBV DNA 2,000IU/ml			
<100	18/10	1.00 (Reference)	1.00 (Reference)
100	143/41	2.06 (0.86–4.94)	3.72 (1.17–11.82)

OR, odds ratio; CI, confidence interval, HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

[†]Adjusted for age and sex.

[‡]Adjusted for age, sex, hepatitis B e antigen, family history of liver cancer, education, income, and history of chronic liver disease, and time at sample collection.

Table 4

Risk of liver cancer for HBsAg level 1,000IU/ml in comparison to <1,000IU/ml, stratified by HBV DNA level.

HBsAg Levels (IU/ml)	Cases/Controls	Model 1 OR (95% CI) [†]	Model 2 OR (95% CI) [‡]
HBV DNA<2,000IU/ml			
<1,000	40/138	1.00 (Reference)	1.00 (Reference)
1,000	10/32	1.07 (0.48–2.42)	1.05 (0.42–2.59)
HBV DNA 2,000IU/ml			
<1,000	58/28	1.00 (Reference)	1.00 (Reference)
1,000	103/23	2.43 (1.23–4.82)	2.60 (1.11–6.05)

OR, odds ratio; CI, confidence interval, HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

[†] Adjusted for age and sex.

[‡] Adjusted for age, sex, hepatitis B e antigen, family history of liver cancer, education, income, and history of chronic liver disease, and time at sample collection.

Table 5

Combined effects of HBV DNA and HBsAg levels on liver cancer risk

HBV DNA (IU/ml) & HBsAg Level (IU/ml)	Cases/Controls	OR (95% CI) [†]	OR (95% CI) [‡]
HBV DNA<2,000 & HBsAg level<100	24/110	1.00 (Reference)	1.00 (Reference)
HBV DNA<2,000 & HBsAg level 100	26/60	2.09 (1.09–4.00)	2.20 (1.07–4.49)
HBV DNA 2,000 & HBsAg level<1,000	58/28	9.47 (5.02–17.88)	6.94 (3.39–14.23)
HBV DNA 2,000 & HBsAg level 1,000	103/23	22.08 (11.37–42.87)	16.15 (7.60–34.32)
<i>P</i> for trend		<0.01	<0.01

OR, odds ratio; CI, confidence interval, HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

[†] Adjusted for age and sex.[‡] Adjusted for age, sex, hepatitis B e antigen, family history of liver cancer, education, income, and history of chronic liver disease, and time at sample collection.