

HEPATITIS B e ANTIGEN AND THE RISK OF HEPATOCELLULAR CARCINOMA

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ABSTRACT

Background The presence of hepatitis B e antigen (HBeAg) in serum indicates active viral replication in hepatocytes. HBeAg is thus a surrogate marker for the presence of hepatitis B virus DNA. We conducted a prospective study to determine the relation between positivity for hepatitis B surface antigen (HBsAg) and HBeAg and the development of hepatocellular carcinoma.

Methods In 1991 and 1992, we enrolled 11,893 men without evidence of hepatocellular carcinoma (age range, 30 to 65 years) from seven townships in Taiwan. Serum samples obtained at the time of enrollment were tested for HBsAg and HBeAg by radioimmunoassay. The diagnosis of hepatocellular carcinoma was ascertained through data linkage with the computerized National Cancer Registry in Taiwan and with death certificates. We performed a multiple regression analysis to determine the relative risk of hepatocellular carcinoma among men who were positive for HBsAg alone or for HBsAg and HBeAg, as compared with those who were negative for both.

Results There were 111 cases of newly diagnosed hepatocellular carcinoma during 92,359 person-years of follow-up. The incidence rate of hepatocellular carcinoma was 1169 cases per 100,000 person-years among men who were positive for both HBsAg and HBeAg, 324 per 100,000 person-years for those who were positive for HBsAg only, and 39 per 100,000 person-years for those who were negative for both. After adjustment for age, sex, the presence or absence of antibodies against hepatitis C virus, cigarette-smoking status, and use or nonuse of alcohol, the relative risk of hepatocellular carcinoma was 9.6 (95 percent confidence interval, 6.0 to 15.2) among men who were positive for HBsAg alone and 60.2 (95 percent confidence interval, 35.5 to 102.1) among those who were positive for both HBsAg and HBeAg, as compared with men who were negative for both.

Conclusions Positivity for HBeAg is associated with an increased risk of hepatocellular carcinoma. (N Engl J Med 2002;347:168-74.)

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CHRONIC hepatitis B virus (HBV) infection is a serious clinical problem because of its worldwide distribution and potential for adverse sequelae, including hepatic cirrhosis and hepatocellular carcinoma. It is particularly prevalent in the Asian–Pacific region, where patients usually acquire the infection at the time of birth or in early childhood.

The natural course of chronic HBV infection acquired early in life can be divided into three phases.^{1,2} The first phase is characterized by active replication of HBV, positivity for hepatitis B e antigen (HBeAg), and normal-to-low levels of serum aspartate aminotransferase and alanine aminotransferase. The second phase, characterized by immune clearance, usually occurs from the age of 15 to 35 years, during which hepatitis flares may occur as the result of specific, T-lymphocyte–mediated cellular responses to viral antigens and apoptosis of hepatocytes. This phase subsides as viral replication declines, with the appearance of antibodies against HBeAg and clinical remission. In the third, or residual, phase, patients are positive for hepatitis B surface antigen (HBsAg) and negative for HBeAg, and they do not have active liver disease.^{1,2} Epidemiologic studies have shown that positivity for HBsAg is one of the most important risk factors for hepatocellular carcinoma.³⁻⁵

Positivity for HBeAg usually indicates active replication of HBV. Loss of detectable HBeAg, together with the emergence of antibodies against HBeAg, has been used as a key end point in studies of the efficacy of various agents, including lamivudine and interferon, for the treatment of chronic hepatitis B.^{6,7} However, the role of positivity for HBeAg in the prediction of hepatocellular carcinoma remains inconclusive. Various studies of case series have found that the prevalence of HBeAg was lowest among patients with hepatocel-

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lular carcinoma, highest among patients with chronic hepatitis B, and intermediate among those with liver cirrhosis.^{8,9} In several case-control studies, however, the prevalence of HBeAg was significantly higher among HBsAg-positive patients with hepatocellular carcinoma than among matched HBsAg-positive controls.¹⁰⁻¹⁴ All these studies were cross-sectional in design, with disease status and the prevalence of antigen positivity determined at the same time. The studies did not determine whether positivity for HBeAg preceded the onset of hepatocellular carcinoma. We conducted a prospective study of the relation between positivity for HBeAg and the risk of hepatocellular carcinoma among 11,893 men in Taiwan.

METHODS

Study Cohort

From 1991 to 1992, we invited all 47,079 men between the ages of 30 and 65 years who were living in seven townships in Taiwan to participate in the study; the men were contacted by mail. A total of 11,893 men (25 percent) agreed to participate. Each subject provided written informed consent for us to conduct an interview, collect a blood specimen, and perform various serologic and biochemical assays. The study was conducted between February 1991 and September 2000.

Data Collection and Blood Tests

All the men were interviewed in person with the use of a structured questionnaire administered by well-trained public health nurses. The men were questioned about sociodemographic characteristics, diet, cigarette smoking, consumption of alcohol, betel-nut chewing, their medical and surgical history, and any family history of hepatocellular carcinoma or liver cirrhosis. A 10-ml specimen of blood was collected with the use of a disposable vacuum syringe. Samples of serum separated on the day of blood collection were kept in a freezer (at -70°C) until they were assayed. The specimens were tested at the time of enrollment for HBsAg and HBeAg by radioimmunoassay with the use of a commercial kit (Abbott Laboratories) and were tested for antibodies against hepatitis C virus by enzyme immunoassay with the use of a second-generation commercial kit (Abbott Laboratories).

For men in whom hepatocellular carcinoma was diagnosed during follow-up who were positive for HBsAg and negative for HBeAg at enrollment, tests for antibodies against HBeAg and HBV DNA in serum samples obtained at the time of enrollment were performed in 2002 as part of a nested case-control substudy. For each man with hepatocellular carcinoma, we identified two healthy controls matched for age, date of enrollment, and township, except that for each of two men with hepatocellular carcinoma, there was only one matched control for whom frozen serum samples were available. Thus, frozen serum samples collected at the time of enrollment were available for a total of 44 men with hepatocellular carcinoma and 86 matched controls who were positive for HBsAg and negative for HBeAg. These samples were tested for antibodies against HBeAg with the use of an enzyme-linked immunoassay (Abbott Laboratories) and were tested for HBV DNA with the use of a branched-chain DNA assay (Quantiplex, Chiron), according to the manufacturer's instructions. The threshold of detection for the quantitative assay of DNA was 2.5 pg per milliliter.

Follow-up for Hepatocellular Carcinoma

In Taiwan, interferon, lamivudine, or other antiviral or immune therapy was rarely used to treat chronic hepatitis B before 2001. The Bureau of National Health Insurance in Taiwan did not provide

reimbursement for the cost of treatment with lamivudine until March 2001. Since treatment with interferon alfa is expensive and has severe side effects that make it difficult to tolerate, the Bureau of National Health Insurance only recently began to provide reimbursement for the cost of this treatment. Other antiviral or immune therapies are also rarely used to treat chronic hepatitis B in Taiwan. For these reasons, we did not refer our subjects with chronic hepatitis B to the hospital for treatment after enrollment.

At the time of enrollment, none of the men had known hepatocellular carcinoma on the basis of screening tests (abdominal ultrasonography and serologic tests), interviews, and the absence of linkage with the National Cancer Registry. Cases of hepatocellular carcinoma were ascertained by computerized linkage of data with information from the National Cancer Registry in Taiwan for the period from January 1, 1984, through September 30, 2000. In addition, we linked data with information from death certificates to identify deaths from cases of hepatocellular carcinoma that were not included in the National Cancer Registry. The diagnosis was based on pathological examination in 56 men and on the results of abdominal ultrasonographic, angiographic, or computed tomographic studies combined with an elevated serum alpha-fetoprotein level (≥ 400 ng per milliliter) in 55 men.¹⁰⁻¹³

Statistical Analysis

The person-years of follow-up for each subject were calculated from the date of enrollment to the date of the diagnosis of newly developed hepatocellular carcinoma, the date of death, or the date of the last link to data from the National Cancer Registry (September 30, 2000), whichever came first. Incidence rates were calculated by dividing the number of incident cases of hepatocellular carcinoma by the number of person-years of follow-up. Data for men in whom hepatocellular carcinoma was not diagnosed were censored at the date of death or the last date of follow-up (September 30, 2000). A Cox's proportional-hazards model was used to estimate the relative risk of hepatocellular carcinoma associated with positivity for HBsAg and HBeAg and other risk factors, including advanced age, positivity for antibodies against hepatitis C virus, cigarette smoking, and consumption of alcohol. The 95 percent confidence intervals for the relative risks were also calculated. Significance levels were determined with the use of two-tailed tests. The cumulative incidence of hepatocellular carcinoma was calculated for men who were negative for both HBsAg and HBeAg, those who were positive for HBsAg alone, and those who were positive for both HBsAg and HBeAg, with the use of the Nelson-Aalen method (a nonparametric method for estimating the cumulative hazard).¹⁵⁻¹⁷ For the 130 men included in the nested case-control substudy described above, we used logistic-regression analysis to determine the association between the level of HBV DNA and hepatocellular carcinoma. Statistical analysis was performed with Stata software (Stata).

RESULTS

The prevalence of HBsAg among all men and the prevalence of HBeAg among those who were positive for HBsAg are shown in Table 1 according to age, cigarette-smoking status, and use or nonuse of alcohol. The older the age, the lower the prevalence of HBsAg and HBeAg. The prevalence was consistent with the rates reported previously in Taiwan.^{1,2} Thus, we considered the study cohort to be representative of the general population of Taiwanese men in terms of the prevalence of HBsAg and HBeAg.

Men who smoked and those who drank alcohol had a lower prevalence of HBsAg alone but a higher prevalence of HBeAg among those who were positive for HBsAg than did nonsmokers and nondrinkers. But

TABLE 1. PREVALENCE OF HEPATITIS B SURFACE ANTIGEN (HBsAg) AND HEPATITIS B e ANTIGEN (HBeAg) IN 11,893 MEN IN TAIWAN.

VARIABLE	NO. OF MEN	PREVALENCE OF	P VALUE	PREVALENCE OF	P VALUE
		HBsAg		HBeAg AMONG HBsAg- POSITIVE MEN	
		no. (%)		no. (%)	
Age at enrollment			<0.001*		<0.001*
30–39 yr	3242	771 (23.8)		180 (23.3)	
40–49 yr	2921	639 (21.9)		104 (16.3)	
50–59 yr	3684	695 (18.9)		72 (10.4)	
≥60 yr	2046	256 (12.5)		14 (5.5)	
Cigarette smoking†			0.004		0.80
No	5167	1088 (21.1)		168 (15.4)	
Yes	6713	1271 (18.9)		201 (15.8)	
Alcohol consumption‡			0.09		0.29
No	9434	1902 (20.2)		289 (15.2)	
Yes	2432	453 (18.6)		78 (17.2)	

*P values are based on a test for trend.

†Data were not available for 13 men.

‡Data were not available for 27 men.

the difference was statistically significant only for the prevalence of HBsAg alone among cigarette smokers and nonsmokers. Age and cigarette-smoking status differed significantly among the men who were negative for both HBsAg and HBeAg, those who were positive only for HBsAg, and those who were positive for both HBsAg and HBeAg ($P < 0.001$ and $P = 0.02$, respectively). The rates of use and nonuse of alcohol were similar in the three groups of men ($P = 0.14$).

A total of 111 cases of hepatocellular carcinoma were diagnosed during a follow-up period of 92,359 person-years; the overall incidence rate was 120.2 cases per 100,000 person-years. The incidence rate was 39.1 among men who were negative for both HBsAg and HBeAg, 324.3 among those who were positive only for HBsAg, and 1169.4 among those who were positive for both HBsAg and HBeAg (Table 2). The prevalence of HBeAg was 39 percent among the men who were positive for HBsAg. The cumulative incidence of hepatocellular carcinoma is shown in Figure 1 for the three groups. The men who were positive for both HBsAg and HBeAg had a much higher cumulative incidence of hepatocellular carcinoma than those who were positive only for HBsAg and an even higher incidence than those who were negative for both ($P < 0.001$ for both comparisons). The longer the follow-up, the greater the differences among the three groups.

Table 3 shows the results of the multiple-regression analysis with the use of the Cox proportional-hazards model. After adjustment for other risk factors, the relative risk of hepatocellular carcinoma was 9.6 (95 percent confidence interval, 6.0 to 15.2) for men who were positive for HBsAg alone and 60.2 (95 percent

confidence interval, 35.5 to 102.1) for those who were positive for both HBsAg and HBeAg, as compared with men who were negative for both. An older age at the time of enrollment, the presence of antibodies against hepatitis C virus, cigarette smoking, and consumption of alcohol were also associated with an increased risk of hepatocellular carcinoma. The findings were similar when the data were stratified according to age, cigarette-smoking status, and use or nonuse of alcohol, in an analysis adjusted for other risk factors (Table 4).

In the nested case-control analysis that involved 130 men who were positive for HBsAg and negative for HBeAg (44 men with newly diagnosed hepatocellular carcinoma and 86 matched controls), 120 men (92 percent) were positive for antibodies against HBeAg, and 29 (22 percent) were positive for HBV DNA. The proportion of men who were positive for antibodies against HBeAg did not differ significantly between men with hepatocellular carcinoma and controls ($P = 0.16$). Seventeen men with hepatocellular carcinoma (39 percent) and 12 controls (14 percent) had detectable serum levels of HBV DNA (> 2.5 pg per milliliter). The odds ratio for the development of hepatocellular carcinoma was 3.9 (95 percent confidence interval, 1.6 to 9.2) for men who had detectable HBV DNA, as compared with those who had undetectable levels. The odds ratio increased with increases in the level of HBV DNA (Table 5).

DISCUSSION

It has been reported that in most patients with hepatocellular carcinoma, the disease develops after the

TABLE 2. INCIDENCE OF HEPATOCELLULAR CARCINOMA DURING FOLLOW-UP.*

RESULTS OF HBV ANTIGEN TESTS	PERSON-YR OF FOLLOW-UP	NO. OF MEN	NO. OF CASES OF HEPATOCELLULAR CARCINOMA	INCIDENCE RATE (95% CI)
Negative for HBsAg and HBeAg	74,205	9532	29	39.1 (26.2–56.1)
Positive for HBsAg, negative for HBeAg	15,418	1991	50	324.3 (240.7–427.5)
Positive for HBsAg and HBeAg	2,736	370	32	1169.4 (799.9–1650.9)

*HBV denotes hepatitis B virus, CI confidence interval, HBsAg hepatitis B surface antigen, and HBeAg hepatitis B e antigen.

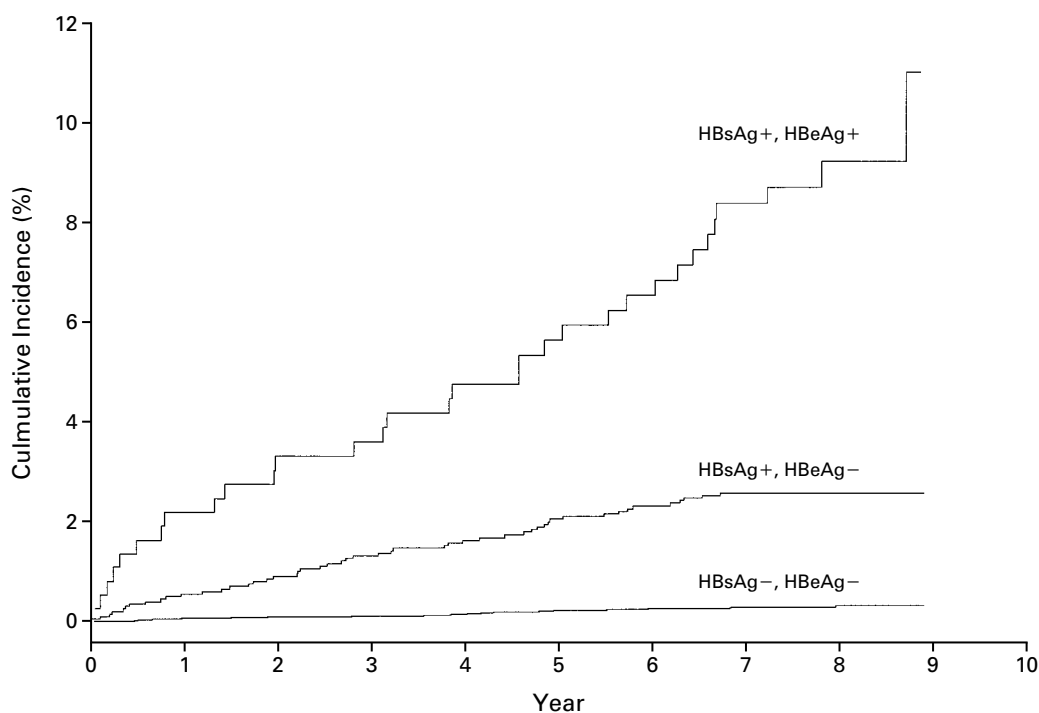


Figure 1. Cumulative Incidence of Hepatocellular Carcinoma during Follow-up among 11,893 Men in Taiwan, According to the Presence or Absence of Hepatitis B Surface Antigen (HBsAg) and Hepatitis B e Antigen (HBeAg) at Enrollment.

The cumulative incidence was estimated with the use of the Nelson–Aalen method.

development of antibodies against HBeAg.¹⁸ In our follow-up study, with all blood samples collected and assayed before the diagnosis of hepatocellular carcinoma, the prevalence of HBeAg was 39 percent among men who were positive for HBsAg at the time of enrollment and who subsequently received a diagnosis of hepatocellular carcinoma — a much higher prevalence than that reported in a previous case-series study

(18 percent)⁹ and in previous case-control studies (22 and 25 percent).^{12,14} Similarly, in our study, the relative risks of hepatocellular carcinoma among men who were positive for HBsAg alone (9.6) and among those who were positive for both HBsAg and HBeAg (60.2), as compared with men who were negative for both, were much higher than in previous case-control studies.¹⁰⁻¹⁴

TABLE 3. ADJUSTED RELATIVE RISK OF HEPATOCELLULAR CARCINOMA ACCORDING TO VARIOUS RISK FACTORS.*

VARIABLE	NO. OF CASES OF HEPATOCELLULAR CARCINOMA	ADJUSTED RELATIVE RISK (95% CI)
Age at enrollment		
30–39 yr†	6	1.0
40–49 yr	23	5.4 (2.2–13.2)‡
50–59 yr	55	13.5 (5.8–31.7)‡
≥60 yr	27	17.7 (7.1–43.9)‡
Results of HBV antigen tests		
Negative for HBsAg and HBeAg†	29	1.0
Positive for HBsAg, negative for HBeAg	50	9.6 (6.0–15.2)‡
Positive for HBsAg and HBeAg	32	60.2 (35.5–102.1)‡
Antibodies against hepatitis C virus		
No†	98	1.0
Yes	13	2.7 (1.5–4.9)‡
Cigarette smoking		
No†	41	1.0
Yes	70	1.5 (1.0–2.2)§
Alcohol consumption		
No†	80	1.0
Yes	31	1.5 (1.0–2.3)§

*Data were not available on cigarette smoking for 13 men, on alcohol consumption for 27, and on antibodies against hepatitis C virus for 77. CI denotes confidence interval, HBV hepatitis B virus, HBsAg hepatitis B surface antigen, and HBeAg hepatitis B e antigen.

†This was the reference group.

‡P<0.001.

§P=0.06.

Both indirect and direct carcinogenic mechanisms are involved in the pathogenesis of hepatocellular carcinoma induced by chronic HBV infection.¹⁹ HBV may induce hepatocellular carcinoma indirectly by causing chronic necroinflammatory hepatic disease.²⁰ When HBV replication is sustained, as indicated by positivity for HBeAg, malignant transformation may occur as a result of continuous or recurrent cycles of hepatocyte necrosis and regeneration. The accelerated rate of cell turnover may act as a tumor promoter through the accumulation of spontaneous mutations or DNA damage caused by exogenous factors, resulting in an increased selective growth advantage for transformed cells. The accelerated turnover rate may also result in cleavage of viral DNA at specific motifs, resulting in linear DNA that is inserted into chromosomal DNA through increased intracellular topoisomerase I activity.²¹ Chronic necroinflammation may induce malignant transformation by generating mutagenic reactive oxygen species during the inflammatory process.²²

Active replication of HBV may also initiate malignant transformation through a direct carcinogenic mechanism by increasing the probability of insertion of viral DNA in or near proto-oncogenes, tumor-suppressor genes, or their regulatory elements of cellular DNA.^{23,24} The integration of viral DNA may increase the production of transactivator protein hepatitis B X antigen, which may induce the malignant transfor-

TABLE 4. ADJUSTED RELATIVE RISK OF HEPATOCELLULAR CARCINOMA, WITH STRATIFICATION ACCORDING TO AGE, CIGARETTE-SMOKING STATUS, AND USE OR NONUSE OF ALCOHOL.

VARIABLE	ADJUSTED RELATIVE RISK (95% CI)*		
	NEGATIVE FOR HBsAg AND HBeAg	POSITIVE FOR HBsAg, NEGATIVE FOR HBeAg	POSITIVE FOR HBsAg AND HBeAg
Age at enrollment†			
≤55 yr	1.0	6.1 (3.3–11.4)	25.4 (13.3–48.6)
>55 yr	1.0	14.8 (7.5–29.5)	95.5 (42.5–214.5)
Cigarette smoking‡			
No	1.0	14.3 (6.1–33.8)	67.0 (26.1–171.7)
Yes	1.0	8.9 (5.1–15.5)	76.9 (39.4–150.3)
Alcohol consumption§			
No	1.0	9.7 (5.6–16.9)	69.0 (37.1–128.2)
Yes	1.0	11.4 (5.0–26.3)	52.1 (18.0–150.8)

*The reference group was the group of men who were negative for both hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). CI denotes confidence interval. P<0.001 for all comparisons.

†The analysis was adjusted for the presence or absence of antibodies against hepatitis C virus, cigarette-smoking status, and use or nonuse of alcohol.

‡The analysis was adjusted for age (as a continuous variable), the presence or absence of antibodies against hepatitis C virus, and use or nonuse of alcohol.

§The analysis was adjusted for age (as a continuous variable), the presence or absence of antibodies against hepatitis C virus, and cigarette-smoking status.

TABLE 5. LEVEL OF HEPATITIS B VIRUS (HBV) DNA IN MEN WITH HEPATOCELLULAR CARCINOMA AND MATCHED CONTROLS WHO WERE POSITIVE FOR HEPATITIS B SURFACE ANTIGEN AND NEGATIVE FOR HEPATITIS B e ANTIGEN AT ENROLLMENT.

HBV DNA	MEN WITH HEPATOCELLULAR CARCINOMA (N=44)	MATCHED CONTROLS (N=86)	ADJUSTED ODDS RATIO (95% CI)*
	no. of men		
Undetectable (<2.5 pg/ml)†	27	74	1.0
2.5–13.0 pg/ml	7	7	2.3 (0.7–7.3)
>13.0 pg/ml	10	5	6.0 (1.7–21.4)‡

*The analysis was adjusted for age (as a continuous variable), the presence or absence of antibodies against hepatitis C virus, cigarette-smoking status, and use or nonuse of alcohol. P for trend=0.003.

†This was the reference group.

‡P=0.005.

mation of hepatocytes, as well as bind to the p53 tumor-suppressor gene and disrupt its functions.^{25–30}

The presence of antibodies against HBeAg has been used as an important marker for the resolution of active HBV infection and for a sustained response to treatment with lamivudine alone or in combination with interferon alfa.⁷ In the natural history of chronic hepatitis B, the spontaneous or interferon alfa-induced development of antibodies against HBeAg leads to improvement in the clinical outcome.^{6,31} One study showed that patients who had a response to treatment with interferon alfa (i.e., clearance of HBeAg) were much less likely to have major liver complications during a five-year period of follow-up than were treated or untreated patients with persistent HBeAg.³¹ A long-term controlled trial of treatment with interferon alfa showed that hepatocellular carcinoma developed more frequently in untreated patients than in treated patients and that it developed only in those with persistent HBeAg.⁶ Our study showed that a negative test for HBeAg was associated with a low risk of hepatocellular carcinoma, suggesting that the earlier the development of antibodies against HBeAg occurs, the lower the risk of hepatocellular carcinoma.

We performed tests for HBsAg and HBeAg only at the time of enrollment. The effect of the appearance of antibodies against HBsAg and HBeAg on the development of hepatocellular carcinoma was not assessed. If the appearance of antibodies against HBeAg occurred after enrollment, the risk of hepatocellular carcinoma among men who were initially positive for HBeAg was underestimated. Tests for antibodies against HBeAg were not performed at the time of enrollment for men who were negative for HBeAg. However, we did perform tests for antibodies against

HBeAg and for HBV DNA in frozen serum samples collected at the time of enrollment from the 44 men in whom hepatocellular carcinoma was subsequently diagnosed and the 86 matched controls, all of whom were initially positive for HBsAg and negative for HBeAg. In this nested case-control analysis, more than 90 percent of the men were positive for antibodies against HBeAg, and there was no significant difference in this respect between the men with hepatocellular carcinoma and the controls. However, positivity for HBV DNA was associated with a significantly increased risk of hepatocellular carcinoma.

HBV DNA is found in most patients who are positive for HBeAg, in a substantial proportion of those with antibodies against HBeAg (20 to 25 percent), and in some who are negative for HBeAg. Therefore, HBV DNA is the most important predictor of the development of hepatocellular carcinoma in HBsAg-positive Taiwanese men. However, the assay for HBV DNA is expensive and is performed only at specialized laboratories, whereas the assays for HBeAg, a surrogate marker of HBV DNA, and antibodies against HBeAg are inexpensive and are performed routinely. At present, a test for HBeAg may therefore be a more practical method for predicting the risk of hepatocellular carcinoma.

In conclusion, HBeAg, in addition to HBsAg, may be a useful marker of the risk of hepatocellular carcinoma. Persons considered to be at high risk because of positivity for HBeAg would be candidates for antiviral-drug treatment and close monitoring for the development of liver diseases associated with chronic HBV infection.

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APPENDIX

The following institutions and investigators, in addition to the authors, participated in the Taiwan Community-Based Cancer Screening Project Group: *National Taiwan University Hospital* — G.-T. Huang, H.-S. Lee, P.-M. Yang, C.-H. Chen; *Huhsi Health Center, Penghu County* — S.-C. Ho, T.-G. Lu; *Provincial Penghu Hospital* — W.-P. Wu, C.-T. Chen; *Sanchi Health Center, Taipei County* — C.-G. Lin; *Provincial Chutung Hospital* — C.-H. Lo; *Provincial Potzu Hospital* — Y.-Y. Kuo; *Kaohsiu Health Center, Pingtung County* — C.-T. Lee, C.-C. Chen; and *Paihsa Health Center, Penghu County* — Y.-C. Ou, W.-C. How.

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