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Hepatocellular Carcinoma Among HBsAg Positive Blood Donors in Fukuoka, Japan

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Abstract—*In order to examine the association between hepatitis B virus carriage and hepatocellular carcinoma, 3765 HBsAg positive blood donors were followed from 1977 to 1983 in Fukuoka, Japan. The observed number of deaths was compared with the expected deaths calculated by applying cause-, sex- and age-specific death rates for Fukuoka in 1980 to sex- and age-specific population at risk of the subjects. Among 2595 male blood donors, mortality from liver cancer (or hepatocellular carcinoma) was specifically elevated compared with the general population, where the observed, expected deaths and O/E were 15, 2.07 and 7.25, respectively* ($P < 0.001$). *This relative risk was assumed to be underestimated partly because of a healthy donor effect. Neither the HBsAg titer nor the HBeAg-Ab system was related to the risk. Relative risk and population attributable risk % in Japan and various countries were estimated.*

INTRODUCTION

A STRONG association between the prevalence of hepatitis B surface antigen (HBsAg) and incidence of (or mortality from) hepatocellular carcinoma (HCC) has been suggested by studies on their geographical correlations in many parts of the world [1–4]. Such definite correlations by prefecture in Japan, and by city, town or village in Fukuoka Prefecture were also observed (Tokudome S, unpublished data).

Epidemiological studies (some prospetive [5–9] and some retrospective [10–22]) have demonstrated a significant relationship between hepatitis B virus (HBV) infection and risk of HCC in various countries as well as in Japan.

It is known that HBV DNA is integrated in the genomes of HCC and it seems probable that HBV plays a definitive role in the pathogenesis of HCC [23–28], but the precise carcinogenesis remains obscure.

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Abbreviations used: HBV = hepatitis B virus; HCC = hepatocellular carcinoma; O/E = observed deaths/expected deaths; HBsAg = hepatitis B surface antigen; HBeAg = hepatitis B e antigen; Anti-HBe = antibody to hepatitis B e antigen; GOT = glutamic oxaloacetic transaminase.

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This prospective study was done to examine whether HBsAg positive blood donors were at high risk of HCC in an area of moderate endemicity for HBV and whether such a risk was related to HBsAg titer or the hepatitis B e antigen–antibody (HBeAg-Ab) system. An etiologic fraction (or population attributable risk %) of HBV infection with respect to HCC was also estimated.

MATERIALS AND METHODS

Medical records of HBsAg positive blood donors from 1977 to 1979 were reviewed at Fukuoka Red Cross Blood Center. Demographic and clinical data as to name, sex, blood type, HBsAg. HBeAg, anti-HBe, glutamic oxaloacetic transaminase [GOT (Karmen Units)], dates of birth and donation, age at donation and occupation were obtained. HBsAg was screened by electrosyneresis and reverse passive hemagglutination. HBeAg and anti-HBe were detected by a micro-Ouchterlony technique at the center.

In all, 4458 medical records of HBsAg positive blood donors were reviewed at the center. Among them, there were 679 duplicate donations, 13 non-Japanese and one whose date of donation was out of the observation period, all of which were excluded. The remaining 3765 subjects were analyzed in the study.

The blood donors were followed-up from the date of donation to the date of death or the end of observation period (31 December 1983). The vital

Table 1. Number of subjects by sex and vital status as of 31 December 1983

Vital status	Male	Female
Alive	2325	1103
Deceased	30	3
Unknown	240	64
Total	2595	1170

status of the subject was investigated by referring to the resident's registration card from the city, town or village office where the subject's present address is registered. When the subject was known to have died, the death certificate was requested from the District Legal Affairs Bureau at his Honseki (where one's permanent address is registered). Those lost to follow-up were assumed to have survived up to the end of the observation period.

The number of subjects by sex and vital status as of 31 December 1983 is shown in Table 1. Among the females, only three deaths were observed but none of them were associated with liver disease. Therefore, further analysis was confined to the male subjects. Of the 2595 males, 2325 were known to be alive and 30 were known to have died. Two hundred and forty (9.2%) were lost to follow-up. The total person-years at risk was 15,214.44, and the average observation period was 5.86 years. The age distribution of subjects was apparently skewed to younger ones, and 81.8% were less than 40 years old.

The causes of the observed deaths were classified according to the ICD-8th revision. In comparison with the observed number of deaths, the expected deaths was calculated by applying cause-, sex- and age-specific mortality rates for Fukuoka Prefecture in 1980 [29] to sex- and age-specific person-years at risk of blood donors. For statistical analysis, observed vs. expected deaths (O/E) was calculated and the difference was tested under the assumption of a Poisson distribution [30].

RESULTS

The observed and expected number of deaths with O/E values by selected cause are shown in Table 2. For all causes, the observed, expected deaths and O/E were 30, 38.16 and 0.79, respectively. Accordingly, no increased mortality was observed for all causes. For all malignant neoplasms, on the other hand, the O/E of 2.08 was significantly increased ($P < 0.01$), where the observed and expected deaths were 21 and 10.09, in that order.

However, further observation revealed that the elevated risk was due only to liver cancer where the observed, expected deaths and O/E were 15, 2.07 and 7.25, respectively, with statistical significance at the 0.1% level. Although there were 16 liver cancers mentioned in the death certificates, one case was a pancreas cancer confirmed at autopsy. This case was eventually excluded from the observed deaths from liver cancer. The remaining 15 cases were considered unequivocally to be HCC. The risk of other cancers (all malignant neoplasms except liver cancer) was not elevated. The O/E of 0.33 for liver cirrhosis was not increased in this study.

Table 2. Observed and expected number of deaths with their ratios from selected causes among male HBsAg positive blood donors from 1977 to 1983

Cause of death	Obs.	Exp.*	O/E
All malignant neoplasms	21	10.09	2.08†
Liver cancer	15	2.07	7.25‡
All malignant neoplasms except liver cancer	6	8.02	0.75
Liver cirrhosis	1	3.07	0.33
All causes	30	38.16	0.79

*Based on cause-, sex- and age-specific mortality rates for Fukuoka Prefecture in 1980.
†Significant at the 1% level (two-tailed).
‡Significant at the 0.1% level (two-tailed).

Further analysis was made for HCC by GOT value, HBsAg titer, HBeAg-Ab system and time interval (years after donation) in order to specify the risk of HCC (Table 3). Although the number was small, the O/E for the subjects whose GOT values were more than 40 was larger than that for those whose values were within normal limits. The ratios were 25.00 for the former and 5.76 for the latter. The O/E for the cases with HBsAg titer of less than 10 was 9.45, which was larger than that for those with a titer of 10 or more. This meant that HBsAg titers of HCC were lower than those of the other donors. The O/E ratio for the subjects with HBeAg was 8.00 and 7.02 for those with anti-HBe. Twelve cases of HCC have already had seroconversion from HBeAg to Anti-HBe at the time of donation. The risk of developing HCC within a period of 3 years or less after donation was almost equal to that of those with a time interval of more than 3 years.

Although histological diagnosis was performed for two-thirds of the 15 liver cancers, eight cases were indisputably diagnosed as HCC. Others were diagnosed on the basis of angiography, scintiscan, echogram or computed tomography in combination with level of alpha-fetoprotein (AFP). Consequently, all the cases were considered to be unambiguous HCC or primary liver cancer. Neither cholangiocarcinoma nor secondary (metastatic) liver cancer seemed to be misclassified.

Table 3. Observed and expected number of deaths with their ratios from liver cancer among male HBsAg positive blood donors from 1977 to 1983

By GOT value*

	GOT ≤ 40	GOT > 40
Obs	11	4
Exp.	1.91	0.16
O/E	5.76‡	25.00‡

By HBeAg-Ab sytem†

	HBeAg	Anti-HBe
Obs.	2	12
Ex.	0.25	1.71
O/E	8.00	7.02‡

By HBsAg titer

	Titer < 2^{10}	Titer ≥ 2^{10}
Obs	12	3
Exp.	1.27	0.80
O/E	9.45‡	3.75

By time in years after donation

	≤ 3 years	> 3 years
Obs.	6	9
Exp.	0.89	1.17
O/E	6.74‡	7.69‡

*Karmen Units.
†HBeAg-Ab system was negative for one case of liver cancer.
‡Significant at the 0.1% level (two tailed).

DISCUSSION

Judging from the findings of correlation studies between HBsAg positivity and incidence of (or mortality from) HCC [1–4], and world-wide epidemiological studies [5–22], asymptomatic HBV carriers are at risk of developing HCC. The results of our prospective study are in agreement with these findings. However, the pathogenesis of HCC is still unsolved in spite of intensive molecular biological research on HBV DNA [4, 23–28]. The HBV DNA is integrated in the genomes of some HCC, but insertion sites are not consistent in different tumors. Oncogenes have not been identified in the HBV DNA. In the meantime, however, sequences of HBV DNA are being analyzed and the carcinogenesis of HCC may soon be untangled.

Regarding the strength of the relationship between HBV infection and HCC, Beasley *et al.* [5] reported a relative risk as high as 223 in Taiwan. Relevant figures (relative risk in prospective studies [6–9] or odds ratios from retrospective studies [10–22]) were estimated to be 5–20, 10–20 and 40–135 in the countries of high (over 10%), medium (1–10%) and low (less than 1%) prevalence of HBsAg, respectively. Relative risk seems to be inversely related to the prevalence of HBsAg. By means of a retrospective approach, Chien *et al.* [15] reported that the prevalence of HBsAg was 12.0% in healthy individuals in Taiwan and the odds ratio of HBV infection with regard to HCC was 17.6. The disrepancy between the values is so large that explanations are needed to account for it. As the relative risk of Beasley *et al.* [5] was based on a small number of deaths among a reference group, they may need a longer observation period to resolve this question.

On the contrary, the relative risk in the present study seems to be somewhat underestimated as in other prospective studies [6–9]. There are a number of plausible explanations. Firstly, our study was based on blood donors who were asymptomatic healthy individuals and the O/E was far less than one for all causes of death. This may be called 'healthy donor effect' similar to the 'healthy worker effect' in occupational studies. Secondly, the data are limited both in terms of the observation period and study subjects' age distribution. Therefore, in order to estimate the degree of relative risk more precisely, it would be preferable to have a longer observation period as well as a large number of older blood donors. Thirdly, the expected number of deaths was calculated by using the death rates for a general population which includes HBV carriers. Theoretically such estimations should be computed on the basis of a HBsAg negative population. Furthermore, in addition to primary liver cancer [155.0 (ICD-8th)], liver cancer (primary or secondary unspecified) (197.8), intrahepatic biliary duct carcinoma (155.1) and some secondary forms of liver cancer (197.7) are included in the mortality rates.

Case studies of primary liver cancer have been extensively carried out in Japan [31, 32], reporting that the HBsAg positive rate in HCC was 30–40%. The rate for the general male population is known to be 2%. From these values, the odds ratios of HBV infection with respect to HCC were estimated to be 21–33, which was almost equivalent to 20 reported by Kubo *et al.* [21].

There is another index for the strength of association: attributable risk (etiologic fraction), which is helpful to show what percentage of HCC is

accounted for by HBV infection. Here we estimated population attributable risk % (PAR%) [33] using published data [10–22]. The PAR% were 50–80, 45–55 and 15–30 in the countries having a high (over 10%), medium (1–10%) and low (less than 1%) prevalence of HBsAg, respectively. Contrary to relative risk, PAR% seemed to correlate with positive rates of HBsAg with few exceptions. Here again, utilizing the data reported by the Liver Cancer Study Group of Japan [31, 32]l, we estimated PAR% to be 29–39, which was somewhat smaller than the 44 reported by Kubo *et al.* [21].

Although the number was small, the HBeAg-Ab system was not related to the risk of HCC in the present study. As is well known, HBeAg is considered to be an index of prognosis of hepatitis B [34–37] as well as infectivity of HBV [38–40]. Some investigators have reported that seroconversion from HBeAg to anti-HBe is favorable for prognosis [34–36], but others disagree [15, 18, 20, 22, 37]. As the timing of seroconversion may have something to do with the prognosis, we need well designed and long-term follow-up studies to answer this question. Furthermore, HBsAg titer was not associated with the risk of HCC in this study; however, the precise reasons are unknown to us. If persistent infection of HBV and integration of HBV DNA in the liver cell are essential in the carcinogenesis, irrespective of virulence or immune response, HBsAg titer itself may be unrelated to the risk of HCC.

A similar increased risk of HCC was not detected among female HBsAg positive blood donors in the present study. This is partly because of the limited number of study subjects. Male preponderance in regard to the risk of HCC is well known in many countries, and the male vs. female ratio of mortality from cancer of the liver in Japan is more than 2 [3, 41]. Exposure to smoking and alcohol drinking, which are considered to be cofactors or promoters in hepatocarcinogenesis, are far less common among Japanese females than males. Since, to our knowledge, every study seems to have failed to detect the elevated risk of HCC specifically among females, a similar prospective study is now in progress on the basis of about 6000 medical records of female HBsAg positive blood donors in order to determine whether the strength of the association between HBV infection and HCC among females is equal to that of males.

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