REPLICATION OF HEPATITIS B VIRUS IN FIRST-DEGREE RELATIVES OF PATIENTS WITH HEPATOCELLULAR CARCINOMA

DAR-IN TAI, CHI-SIN CHANGCHIEN, CHING-SHU HUNG, AND CHIU-JUNG CHEN
Liver Unit, Chang Gung Memorial Hospital, Kaohsiung Medical Center, and Chang Gung University, Taipei, Taiwan, Republic of China

Abstract. Hepatocellular carcinoma (HCC) may occur in family clusters. No genetic mechanism has been identified as responsible for this familial tendency. We suspected that a longer hepatitis B virus (HBV) replication phase might be the reason for a higher risk of HCC in families with this disease. We performed liver biochemical tests, test for viral hepatitis markers and hepatitis B e antigen (HBeAg), and liver ultrasonography in relatives of patients with HCC. A total of 1,885 first-degree relatives from 688 families participated in this study. Seven hundred fifty-two relatives were found to be carriers of hepatitis B surface antigen (HBsAg) and 675 of them were tested for HBeAg. The prevalence of HBeAg was 27.4% in relatives of those with HCC and 20% in asymptomatic HBsAg carriers. The HBeAg prevalence rate was higher in relatives of those with HCC ≥ 40 years old than in asymptomatic HBsAg carriers. Moreover, HBeAg was more likely to persist in men than in women ≥ 40 years old. We conclude that families with HCC showed a prolonged HBV replication phase that may be one of the cofactors for a familial tendency for HCC.

A familial tendency is one of the risk factors for hepatocellular carcinoma (HCC). Between 6% and 20% of patients with HCC have more than 1 case of HCC in their family. Among the known factors involved in hepatocarcinogenesis, genetic mechanisms may be responsible for familial HCC. However, this has not been confirmed. Families with HCC are usually clustered with carriers of hepatitis B surface antigen (HBsAg), and their infections with hepatitis B virus (HBV) have usually been acquired through vertical transmission. Since 50% of HBV carriers are infected by vertical transmission in Taiwan, vertical transmission alone does not explain the familial tendency of HCC. It had been postulated that damage to genomic DNA by prolonged hepatic inflammation and regeneration may be the main reason for replication of HBV. A familial tendency of prolonged HBV replication could be a possible mechanism for familial HCC.

Both serum HBV DNA and hepatitis B e antigen (HBeAg) are good indicators of HBV replication. Testing for HBeAg in serum is the less expensive than testing for HBV DNA and has a clear end point that makes interpretation of results and comparisons easier. Therefore, we studied HBeAg in the HBsAg-positive relatives of families with HCC.

RESULTS

We identified the relationship between the first-degree relatives and the index case of HCC in each family, and then acquired demographic data on their relatives. Twenty milliliters of blood was drawn from the relatives and used for liver biochemistry and tests for HBsAg (Abbott Ausria-II; Abbott Laboratories, Abbott Park, IL), antibody to hepatitis C virus (HCV) (HCV enzyme immunoassay II; Abbott Laboratories), and α-fetoprotein (α-Feto-radioimmunoassay-II; Dainabot, Tokyo, Japan). Liver ultrasonography was performed at the same time. Sera were stored at −80°C. Patients with abnormal findings in any of the assays were retested at 6–12-month intervals.

Those relatives who were HBsAg-positive were also tested for HBeAg, antibody to HBe, and antibody to hepatitis D virus (HBeAg-radioimmunoassay and anti-delta; Abbott Laboratories). The first data available were used for analysis. The study relatives were stratified into 4 groups: parents, siblings, children, and grandchildren, according to their relation to index cases of HCC. Statistical analysis was conducted using Student’s t-test, the chi-square test with Yates’ correction, and multiple logistic regression (BMDP Statistical Software, Los Angeles, CA) as appropriate.

PATIENTS AND METHODS

Over a 5-year period, we performed a survey in families with HCC. First-degree relatives > 15 years old were included in this survey. The study was reviewed and approved by the Department of Health, Republic of China and Chang Gung Memorial Hospital. The families with an index case of HCC that was diagnosed in our hospital were encouraged to participate in this study. The relatives of those families with an index case of HCC diagnosed at other hospitals were also accepted into this study if they presented certification from the original hospital. They entered this study as a result of our announcements at an outpatient clinic, in newspapers, and on television. Informed consent was obtained from all participants in the study. For comparison, asymptomatic HBsAg carriers reported in our earlier study were included as a control group.

From 1992 to 1997, 1,885 first-degree relatives from 688 families underwent 3,523 examinations in this study. Five hundred ninety-four index patients with HCC were diagnosed at our hospital and 94 index patients with HCC were diagnosed at other hospitals. Thirty-five patients were found in this study to have HCC.

Among the 1,885 first-degree relatives of patients with HCC, 752 patients were carriers of HBsAg. Six hundred seventy-five relatives were enrolled in this study. Demographic data for the study relatives in relation to HBeAg are shown in Table 1.

Relatives who were HBeAg positive were significantly younger than those who were HBeAg negative. When the relatives were stratified into different generations, the generation of children who were HBeAg positive were significantly younger than those who were HBeAg negative. No
age difference in HBeAg clearance was found between the parental and sibling generations. There were no different in gender and maternal HBsAg status between HBeAg-positive and HBeAg-negative groups. Levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were higher in HBeAg-positive relatives than in HBeAg-negative relatives. Superinfection with hepatitis C virus (6%) or hepatitis D virus (3%) was uncommon.

In those less than 40 years old, the prevalence of HBeAg decreased with age in both the relatives of HCC patients and in asymptomatic HBsAg carriers (Table 2 and Figure 1). A difference between HCC relatives and asymptomatic HBV carriers was observed in those ≥ 40 years old. The prevalence of HBeAg was higher in HCC relatives than in asymptomatic HBsAg carriers.

Among the 4 stratified generations, the HBeAg prevalence rate was highest in children. For relatives ≥ 40 years old, women were more likely to clear HBeAg than men, especially in the children (Table 3).

Multivariate analysis showed that age was the only independent factor related to HBeAg clearance in all relatives. Gender was an important factor in HBeAg clearance in those ≥ 40 years old. Women were more likely to clear HBeAg than men.

### DISCUSSION

The relatives of patients with HCC showed a higher prevalence of HBeAg in those ≥40 year old. This age range is also the higher risk period for development of HCC. Long-term, active replication of HBV with liver injury is thought to be a critical factor in hepatocarcinogenesis.11-17

The mean age of the children was younger for HBeAg-positive patients than for HBeAg-negative patients. This is a normal phenomenon in the natural course of a chronic hepatitis B carrier because the HBeAg prevalence decreases with age. However, there was no age difference in the parental and sibling generations. An age-independent mechanism that delayed HBeAg clearance may have acted in these relatives. Multivariate analysis showed that age was the only independent factor related to clearance of HBeAg for all relatives. In those ≥ 40 years old, the gender factor became an independent factor in clearance of HBeAg, and women were more likely to clear HBeAg than men. This difference probably explains the higher number of men with HCC. It is notable that a lower prevalence of HBeAg in men was found in asymptomatic HBsAg carriers.18 The significance of this discrepancy awaits further study.

Children showed the highest HBeAg prevalence rate in

### TABLE 1

Demographic data in relation to HBeAg in the HBsAg carrying first-degree relatives of HCC families*

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample Size</th>
<th>Mean ± SEM age (years)</th>
<th>HBeAg (−)</th>
<th>HBeAg (+)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>185/675</td>
<td>27.4 ± 2.6 (490)</td>
<td>34.0 ± 0.8 (185)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Parents</td>
<td>609/4,428</td>
<td>27.4 ± 2.6 (490)</td>
<td>34.0 ± 0.8 (185)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Siblings</td>
<td>609/4,428</td>
<td>27.4 ± 2.6 (490)</td>
<td>34.0 ± 0.8 (185)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>609/4,428</td>
<td>27.4 ± 2.6 (490)</td>
<td>34.0 ± 0.8 (185)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Grandchildren</td>
<td>609/4,428</td>
<td>27.4 ± 2.6 (490)</td>
<td>34.0 ± 0.8 (185)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Values in parentheses are numbers of cases. HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; HDV = hepatitis D virus; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase.

† NS = not significant.

### TABLE 2

Prevalence of HBeAg in the HBsAg carrying first-degree relatives of patients with HCC and asymptomatic HBsAg carriers*

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>First-degree relatives of HCC</th>
<th>Asymptomatic HBV carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBeAg (+)/total no.</td>
<td>% 95% confidence interval</td>
</tr>
<tr>
<td>&lt;20</td>
<td>22/40</td>
<td>55.5</td>
</tr>
<tr>
<td>20–29</td>
<td>52/117</td>
<td>44.4</td>
</tr>
<tr>
<td>30–39</td>
<td>67/282</td>
<td>24.0</td>
</tr>
<tr>
<td>40–49</td>
<td>31/152</td>
<td>20.4</td>
</tr>
<tr>
<td>50–59</td>
<td>9/58</td>
<td>15.5</td>
</tr>
<tr>
<td>≥60</td>
<td>4/26</td>
<td>15.4</td>
</tr>
<tr>
<td>Overall</td>
<td>185/675</td>
<td>27.4</td>
</tr>
</tbody>
</table>

* HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HCC = hepatocellular carcinoma; HBV = hepatitis B virus.

† NS = not significant.
FIGURE 1. Prevalence of hepatitis B e antigen (HBeAg) in different age groups in hepatitis B surface antigen (HBsAg)–positive first-degree relatives of patients with hepatocellular carcinoma (HCC) and in asymptomatic HBsAg carriers. The relatives ≥ 40 years old of those with HCC showed a higher HBeAg prevalence rate than the asymptomatic HBsAg carriers. The relatives of those with HCC were divided into 2 generations: children and family other than children. The HBeAg prevalence rate was higher in the children, especially in the relatives ≥ 40 years old.

Table 3
Prevalence of HBeAg as a function of gender and generation for HBsAg carrying HCC families ≥40 years old

<table>
<thead>
<tr>
<th>Generation</th>
<th>Sex</th>
<th>HBeAg (−)</th>
<th>HBeAg (+)</th>
<th>Total</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>M</td>
<td>52</td>
<td>22</td>
<td>74</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>34</td>
<td>4</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Siblings</td>
<td>M</td>
<td>48</td>
<td>14</td>
<td>62</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>46</td>
<td>9</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>M</td>
<td>105</td>
<td>37</td>
<td>142</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>103</td>
<td>15</td>
<td>118</td>
<td></td>
</tr>
</tbody>
</table>

*HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HCC = hepatocellular carcinoma.

In conclusion, the clearance of HBeAg was delayed in families with HCC. Prolonged replication of HBV may be one of the cofactors involved in the familial tendency for HCC.

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Authors’ address: Dar-in Tai, Chi-Sin Changchien, Ching-Shu Hung, and Chiu-Jung Chen, Liver Research Unit, Chang Gung Memorial Hospital and Chang Gung University, 199 Tun-Hwa North Road, Taipei, Taiwan 105, Republic of China.

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