Characterization of antiphospholipid antibodies in chronic hepatitis B infection

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Background

Many infections are associated with antiphospholipid antibodies (aPLs). The purpose of this study was to investigate the prevalence, persistence, clinical significance, and characteristics of aPLs in hepatitis B virus (HBV)-infected patients.

Methods

This study included 143 patients with HBV infection and 32 healthy individuals as controls. The presence of anticardiolipin antibodies (aCL Ab), anti-β2-glycoprotein I antibodies (β2GPI Ab), and lupus anticoagulant (LA) was assessed.

Results

The total prevalence of aPLs in HBV-infected patients was 12.6% (18 of 143). Of these 18 patients, 15 had low to medium titers of aCL Ab (10 with IgM, 4 with IgG, and 1 with both isotypes). β2GPI Ab and LA were detected in 3 (2.1%) and 2 (1.4%) patients with HBV infection, respectively. In follow-up specimens from 14 patients with elevated levels of aCL Ab or β2GPI Ab, 10 (71.4%) showed the persistent presence of aPLs. No clinical manifestations related to aPLs were identified.

Conclusion

In HBV-infected patients, the most frequently detected antiphospholipid antibodies were IgM aCL Ab, which have a weak association with the clinical manifestations of APS. Like other infections, aPLs were persistently detected over a 12-week period in patients with HBV infection.

Key Words

Anticardiolipin antibodies, Anti-β2-glycoprotein I antibodies, Lupus coagulation inhibitor, Hepatitis B virus
non-pathogenic [5, 8]; however, in patients with various infections, thrombotic manifestations such as portal vein thrombosis and pulmonary embolism have been reported [10-13].

Recently, several studies showed a higher prevalence of aCL in chronic viral hepatitis patients than in control individuals [14-17]. In the present study, we investigated the prevalence, persistence, clinical significance, and characteristics of aPLs in HBV-infected patients.

### MATERIALS AND METHODS

#### 1. Patient selection

The prevalence of aPLs was prospectively determined in HBV-infected patients and healthy controls who visited the Gastroenterology Department of the Bundang CHA hospital between 2008 and 2009. This study included 143 HBV-infected patients, irrespective of their treatment (59 women and 84 men; age range, 16-71 years; mean, 42.7 years), and 32 healthy individuals as controls (13 women and 19 men; age range, 27-65 years; mean, 40 years). All patients with HBV infection tested positive for HBV surface antigen (HBsAg) or HBV DNA and negative for anti-HCV antibody. Informed consent was obtained from all patients. The patients were divided into 2 groups on the basis of the hepatitis B e antigen and antibody (HBeAg, HBeAb) status and serum HBV DNA level: chronic hepatitis B patients (N=97) and patients with inactive HBsAg carrier state (N=46) [18]. Patients with positive HBeAg or high levels of HBV DNA (≥ 10^4 copies/mL) were considered chronic hepatitis B patients, and patients with negative HBeAg and low HBV DNA level (< 10^4 copies/mL) were considered inactive HBsAg carriers. All normal controls were negative for HBsAg, anti-HCV antibody, anti-HIV antibody, and antinuclear antibody. Alanine aminotransferase (ALT) levels were normal (5-40 IU/L) in all healthy controls. Thromboembolic complications associated with aPLs were identified through medical record review. The local ethics committee of CHA Bundang Medical Center approved this study.

#### 2. aCL Ab

aCL Ab (IgG and IgM isotype) were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Zeus Scientific Inc., Raritan, NJ). According to the manufacturer’s instructions, the cut-off values for positivity were 20 GPL or MPL (IgG or IgM phospholipid units). Specimens with less than 20 GPL or MPL in the initial ELISA were considered negative. Specimens with greater than 20 GPL or MPL in the initial ELISA were retested in duplicate. The specimens were considered positive, when the value was repeatedly greater than 20 GPL or MPL.

#### 3. β2GPI Ab

β2GPI Ab (IgG and IgM isotype) were measured using a commercial ELISA (INOVA Diagnostics, Inc., San Diego, CA, USA). According to the manufacturer’s instructions, the cut-off values for positivity are 20 SGU or SMU (standard IgG or standard IgM units). Specimens with less than 20 SGU or SMU in the initial ELISA were considered negative. Specimens with greater than 20 SGU or SMU in the initial ELISA result were retested in duplicate. The specimens were considered positive when the value was repeatedly greater than 20 SGU or SMU.

#### 4. LA

All plasma samples from patients and normal individuals were evaluated for the presence of LA according to the criteria defined by the Subcommittee on LA/antiphospholipid antibody of the International Society of Thrombosis and Haemostasis [19]. LA was measured by performing the dilute Russell’s viper venom test using the HemosIL Kit (Instrumentation Laboratory, Milano, Italy).

#### 5. Statistical analysis

Statistical analyses were performed using SAS Statistical Analysis Software Version 9.1 (SAS Institute Inc., Cary, NC, USA). A chi-square test was used to determine the difference in the presence of aPLs between the chronic viral hepatitis patient group and the control group.

### RESULTS

The total prevalence of aPLs (aCL Ab, β2GPI Ab, or LA) in HBV-infected patients was 12.6% (18 of 143) (Table 1). Among these 18 patients, aPLs were detected in 15 of 97 chronic hepatitis B patients and 3 of 46 inactive HBsAg carrier patients. No clinical manifestations related to aPLs were identified. Among the 143 HBV-infected patients, 15 patients (10.5%) had a low to moderate level of aCL Ab (10 with IgM, 4 with IgG, and 1 with both isotypes) compared

### Table 1. Prevalence of antiphospholipid antibodies (aCL Ab, β2GPI Ab, and lupus anticoagulant) in HBV-infected patients.

<table>
<thead>
<tr>
<th></th>
<th>aCL Ab</th>
<th>β2GPI Ab</th>
<th>Lupus anticoagulant</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM type</td>
<td>IgG type</td>
<td>IgM &amp; IgG</td>
<td>IgM type</td>
</tr>
<tr>
<td>HBV-infected patients (N=143)</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normal controls (N=32)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: HBV, hepatitis B virus; aCL Ab, anticardiolipin antibodies; β2GPI Ab, anti-β2-glycoprotein I antibodies.
to only 1 of the 32 control subjects (3.1%). The difference between the groups was not significant \((P=0.19)\). The median values for aCL Ab IgM and IgG isotypes were 28.1 MPL (range, 23.4-42.2) and 32.9 GPL (range, 20.5-47.2), respectively (Table 2 and Fig. 1).

The prevalence of \(\beta_2\)GPI Ab and the LA activity in the HBV-infected patient group was 2.1% (3 of 143) and 1.4% (2 of 143), respectively. In contrast, none of the healthy controls had elevated levels of \(\beta_2\)GPI Ab or LA; however, the difference between the groups was not significant \((P=0.40)\). The isotype distribution for \(\beta_2\)GPI Ab in HBV-infected patients was 1 with IgM and 2 with IgG. Two HBV-infected patients simultaneously had 2 types of aPLs; 1 patient had IgM aCL Ab with cofactor dependency (\(\beta_2\)GPI Ab, IgG type), and the other had \(\beta_2\)GPI Ab (IgM type) and LA.

Follow-up specimens were obtained from 14 of the 17 patients with elevated levels of aCL Ab or \(\beta_2\)GPI Ab (11 with aCL Ab, 2 with anti-\(\beta_2\)GPI Ab, and 1 with both aCL Ab and \(\beta_2\)GPI Ab). The median follow-up duration was 30 weeks (range, 9-100 weeks). The aPLs persisted in 10 of the 14 patients (71.4%), and \(\beta_2\)GPI Ab persisted in all 3 patients with \(\beta_2\)GPI Ab (Table 3).

**DISCUSSION**

aPLs have been found not only in patients with autoimmune diseases like SLE, but also in patients with various

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**Table 2. Characteristics of HBV-infected patients with antiphospholipid antibodies and the types of antiphospholipid antibodies.**

| Patient ID | Gender | Age | Status of HBV infection
d | Type of antiphospholipid antibodies |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>021</td>
<td>M</td>
<td>43</td>
<td>Inactive HBsAg carrier</td>
<td>IgG and IgM aCL</td>
</tr>
<tr>
<td>058</td>
<td>F</td>
<td>39</td>
<td>Chronic hepatitis B</td>
<td>IgG (\beta_2)GPI and LA</td>
</tr>
<tr>
<td>062</td>
<td>M</td>
<td>34</td>
<td>Chronic hepatitis B</td>
<td>IgG (\beta_2)GPI</td>
</tr>
<tr>
<td>082</td>
<td>F</td>
<td>61</td>
<td>Chronic hepatitis B</td>
<td>IgM aCL</td>
</tr>
<tr>
<td>098</td>
<td>F</td>
<td>42</td>
<td>Chronic hepatitis B</td>
<td>IgG aCL</td>
</tr>
<tr>
<td>099</td>
<td>M</td>
<td>34</td>
<td>Chronic hepatitis B</td>
<td>IgM aCL</td>
</tr>
<tr>
<td>104</td>
<td>F</td>
<td>46</td>
<td>Chronic hepatitis B</td>
<td>IgM aCL</td>
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<tr>
<td>112</td>
<td>F</td>
<td>26</td>
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<td>IgG aCL</td>
</tr>
<tr>
<td>114</td>
<td>F</td>
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<td>IgG aCL</td>
</tr>
<tr>
<td>122</td>
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<td>Chronic hepatitis B</td>
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<tr>
<td>130</td>
<td>F</td>
<td>37</td>
<td>Chronic hepatitis B</td>
<td>IgM aCL and IgG (\beta_2)GPI</td>
</tr>
<tr>
<td>134</td>
<td>F</td>
<td>70</td>
<td>Inactive HBsAg carrier</td>
<td>IgM aCL</td>
</tr>
<tr>
<td>137</td>
<td>M</td>
<td>52</td>
<td>Chronic hepatitis B</td>
<td>LA</td>
</tr>
<tr>
<td>140</td>
<td>M</td>
<td>45</td>
<td>Chronic hepatitis B</td>
<td>IgG aCL</td>
</tr>
<tr>
<td>144</td>
<td>M</td>
<td>47</td>
<td>Chronic hepatitis B</td>
<td>IgM aCL</td>
</tr>
<tr>
<td>149</td>
<td>F</td>
<td>64</td>
<td>Inactive HBsAg carrier</td>
<td>IgM aCL</td>
</tr>
<tr>
<td>150</td>
<td>M</td>
<td>47</td>
<td>Chronic hepatitis B</td>
<td>IgM aCL</td>
</tr>
<tr>
<td>153</td>
<td>F</td>
<td>28</td>
<td>Chronic hepatitis B</td>
<td>IgM aCL</td>
</tr>
</tbody>
</table>

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**Fig. 1. Distribution of antcardiolipin antibody titers in hepatitis B virus (HBV)-infected patients and normal controls, IgM isotype (A) and IgG isotype (B).**

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\[\text{Patients were divided into 2 groups on the basis of the hepatitis B e antigen and antibody status and the HBV DNA level: chronic hepatitis B patients and patients with inactive HBsAg carrier state.}\]

Abbreviations: HBV, hepatitis B virus; M, male; F, female; aCL, antcardiolipin antibodies; \(\beta_2\)GPI, anti-\(\beta_2\)-glycoprotein I antibodies; LA, lupus anticoagulant.
follow-up results

IgG type

Follow-up duration

IgG type

In conclusion, the most frequently detected aPLs in HBV-infected patients were IgM aCL Ab, which has a weak association with the clinical manifestations of APS. Unlike the transient presence of other infection-associated aPLs, most aPLs were persistently detected over a 12-week period in patients with HBV infection.
REFERENCES