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-\(\beta\)-2-glycoprotein I antibodies (\(\beta\)2GPI 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). \(\beta\)2GPI 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 \(\beta\)2GPI 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. Unlike the transient presence reported for other infection-associated aPLs, most aPLs were persistently detected over a 12-week period in patients with HBV infection.

**Key Words**

Anticardiolipin antibodies, Anti-\(\beta\)-2-glycoprotein I antibodies, Lupus coagulation inhibitor, Hepatitis B virus

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**INTRODUCTION**

Antiphospholipid antibodies (aPLs) are a heterogeneous group of autoantibodies or alloantibodies with an affinity for anionic phospholipids [1]. aPLs occur in patients with antiphospholipid syndrome (APS), systemic lupus erythematosus (SLE), and various other rheumatic diseases. Furthermore, an elevated level of aPLs is recognized as a risk factor for thrombosis [2]. Several studies have suggested that the pathogenic mechanisms of aPLs are related to platelet activation, endothelial cell activation, and activation of the complement cascade [3, 4]; however, their pathogenic functions in patients with autoimmune diseases like SLE and primary APS are not fully understood.

aPLs have also been detected in numerous infectious diseases, including those caused by parvovirus B19, cytomegalovirus, varicella-zoster virus, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), *Helicobacter pylori*, streptococci, and staphylococci [5-7]. The reported prevalence of aPLs in infected patients varies across studies [5, 8]. The variation in prevalence between studies may be partly due to differences in infection types, antibody types, and aPL measurement methods. For example, in HIV patients, a high prevalence of anticardiolipin antibodies (aCL Ab) (46.5%) [8] and lupus anticoagulant (LA) (43%) [9] was reported. In contrast, anti-\(\beta\)2-glycoprotein I antibodies (\(\beta\)2GPI Ab) were rarely detected in HIV patients [5].

The clinical significance of aPLs associated with various infections is controversial. In many studies, the presence of aPLs associated with infections has been regarded as
Antiphospholipid Ab in HBV infection

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 Ab 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.

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 (≥10⁴ copies/mL) were considered chronic hepatitis B patients, and patients with negative HBeAg and low HBV DNA level (<10⁴ 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...
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 β2GPI 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 β2GPI Ab or LA; however, the difference between the groups was not significant (P=0.40). The isotype distribution for β2GPI 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 (β2GPI Ab, IgG type), and the other had β2GPI Ab (IgM type) and LA.

Follow-up specimens were obtained from 14 of the 17 patients with elevated levels of aCL Ab or β2GPI Ab (11 with aCL Ab, 2 with anti-β2GPI Ab, and 1 with both aCL Ab and β2GPI 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 β2GPI Ab persisted in all 3 patients with β2GPI 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 infectiona) | Type of antiphospholipid antibodies |
|------------|--------|-----|---------------------------|-----------------------------------|
| 021        | M      | 43  | Inactive HBsAg carrier    | IgG and IgM aCL                    |
| 058        | F      | 39  | Chronic hepatitis B        | IgM β2GPI and LA                   |
| 062        | M      | 34  | Chronic hepatitis B        | IgG β2GPI                          |
| 082        | F      | 61  | Chronic hepatitis B        | IgM aCL                            |
| 098        | F      | 42  | Chronic hepatitis B        | IgM aCL                            |
| 099        | M      | 34  | Chronic hepatitis B        | IgM aCL                            |
| 104        | F      | 46  | Chronic hepatitis B        | IgM aCL                            |
| 112        | F      | 26  | Chronic hepatitis B        | IgM aCL                            |
| 114        | F      | 39  | Chronic hepatitis B        | IgM aCL                            |
| 122        | F      | 40  | Chronic hepatitis B        | IgM aCL                            |
| 130        | F      | 37  | Chronic hepatitis B        | IgM aCL and IgG β2GPI              |
| 134        | F      | 70  | Inactive HBsAg carrier     | IgM aCL                            |
| 137        | M      | 52  | Chronic hepatitis B        | LA                                 |
| 140        | M      | 45  | Chronic hepatitis B        | IgM aCL                            |
| 144        | M      | 47  | Chronic hepatitis B        | IgM aCL                            |
| 149        | F      | 64  | Inactive HBsAg carrier     | IgM aCL                            |
| 150        | M      | 47  | Chronic hepatitis B        | IgM aCL                            |
| 153        | F      | 28  | Chronic hepatitis B        | IgM aCL                            |

a)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, anticardiolipin antibodies; β2GPI, anti-β2-glycoprotein I antibodies; LA, lupus anticoagulant.
infections [5, 8]. Molecular mimicry is the proposed mechanism for the development of aPLs in infections. Previous reports showed that antigenic determinants shared between the antigens of infectious agents and host tissue might trigger the immune response [20, 21]. However, the mechanisms leading to the development of aPLs and their possible pathophysiological implications in patients with infections have not been well established.

The reported prevalence of aPLs in infectious patients is variable, in part because of a methodological problem. Currently, the ideal approach for standardized measurement for aCL Ab is under debate [22]. A discrepancy is observed among the different assay kits and methods, particularly in the lower range of antibody levels. Furthermore, one of the critical problems in the standardization process is the absence of a defined cut-off value for positivity. In previous reports, the prevalence of aCL Ab in patients with HBV infection was between 14% and 42% [14, 17, 23], and the prevalence of aCL Ab in chronic hepatitis B patients was higher than that in inactive HBsAg carrier patients (15% vs. 6.5%). Furthermore, it was shown that in a patient with HCV infection and aPLs, elimination of the virus was accompanied by the disappearance of aPLs, and when HCV infection relapsed, aPLs also reappeared [26]. Therefore, aPLs associated with other chronic infections caused by HIV or HCV may also show persistent positivity.

According to a previous report [27], IgM aPLs are associated with the clinical manifestations of APS less often than the IgG isotype is, and the diagnostic criteria for APS include only a medium or high titer of aCL Ab (>40 GPL or MPL) [28]. In the present study, IgM aCL Ab were found more frequently (11 of 15) than the IgG isotype (5 of 15), and the titers of aCL Ab were mostly low. These findings support that infection-associated aPLs rarely manifest with the clinical features of APS. Because the rates of aCL Ab according to isotype were not provided in previous reports of aPLs in patients with HBV infection, comparison with our results was not possible.

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.

Table 3. Follow-up duration and results of aCL Ab and β2GPI Ab testing in antiphospholipid antibody-positive HBV-infected patients.

| Patient ID | Initial results | Follow-up results | Follow-up duration (weeks) |
|------------|----------------|------------------|----------------------------|
|            | IgM type | IgG type | IgM type | IgG type |                           |
| aCL Ab     | 021     | 26.6    | 47.2     | –         | 66.0                      | 100                        |
| (MPL or GPL) | 082     | 27.5    | –        | –         | –                         | 32                         |
|            | 098     | –       | 32.9     | –         | 24.6                      | 9                          |
|            | 099     | 42.2    | –        | 54.2      | –                         | 40                         |
|            | 104     | 36.3    | –        | –         | –                         | 71                         |
|            | 112     | –       | 20.5     | –         | 39.4                      | 63                         |
|            | 114     | –       | 34.7     | –         | 39.9                      | 36                         |
|            | 122     | 23.4    | –        | 27.5      | –                         | 25                         |
|            | 130     | 28.7    | –        | 46.3      | –                         | 28                         |
|            | 144     | 28.1    | –        | –         | –                         | 10                         |
|            | 149     | 40.4    | –        | 38.2      | –                         | 25                         |
|            | 150     | 26.9    | –        | 27.8      | –                         | 21                         |
| 134a)      | –       | 39.8    | –        | NT        | NT                        |                            |
| 140a)      | –       | 31.5    | NT       | NT        |                           |                            |
| 153a)      | –       | 25.8    | –        | NT        | NT                        |                            |
| β2GPI Ab   | 058     | 50.1    | –        | 30.0      | –                         | 63                         |
| (SMU or SGU) | 062     | –       | 32.3     | –         | 29.7                      | 9                          |
|            | 130     | –       | 38.8     | –         | 24.6                      | 28                         |

Follow-up specimens of 3 patients (134, 140, and 153) could not be obtained.

Abbreviations: HBV, hepatitis B virus; aCL Ab, anticardiolipin antibodies; β2GPI Ab, anti-2-glycoprotein I antibodies; MPL, IgM phospholipid unit; GPL, IgG phospholipid unit; SMU, standard IgM unit; SGU, standard IgG unit; -, negative; NT, not tested.
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