VOLUME 46 · NUMBER 1 · March 2011

KJH

THE KOREAN JOURNAL OF HEMATOLOGY

ORIGINAL ARTICLE

Characterization of antiphospholipid antibodies in chronic hepatitis B infection

Ji Young Huh[1], Dae Young Yi[1], Seong Gyu Hwang[2], Jin Jung Choi[2], Myung Seo Kang[3]

Departments of [1]Laboratory Medicine, [2]Internal Medicine, CHA Bundang Medical Center, Seongnam, [3]Department of Laboratory Medicine, CHA Kangnam Medical Center, Seoul, Korea

p-ISSN 1738-7949 / e-ISSN 2092-9129
DOI: 10.5045/kjh.2011.46.1.36
Korean J Hematol 2011;46:36-40.

Received on November 5, 2010
Revised on February 13, 2011
Accepted on February 15, 2011

*This work was supported by a Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2008-331-E00313).

Correspondence to
Ji Young Huh, M.D., Ph.D.
Department of Laboratory Medicine, CHA Bundang Medical Center, CHA University, Yatap-dong, Bundang-gu, Sungnam 463-712, Korea
Tel: +82-31-780-5451
Fax: +82-31-780-5476
E-mail: jiyoungh@cha.ac.kr

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-β_2-glycoprotein I antibodies (β_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). β_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 β_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-β_2-glycoprotein I antibodies, Lupus coagulation inhibitor, Hepatitis B virus

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-β_2-glycoprotein I antibodies (β_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

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.

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 ($\geq 10^4$ 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. β_2GPI Ab

β_2GPI 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, β_2GPI 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, β_2GPI Ab, and lupus anticoagulant) in HBV-infected patients.

	aCL Ab			β_2GPI Ab		Lupus anticoagulant	Total (%)
	IgM type	IgG type	IgM & IgG	IgM type	IgG type		
HBV-infected patients (N=143)	10	4	1	1	2	2	18 (12.6%)
Normal controls (N=32)	1	0	0	0	0	0	1 (3.1%)

Abbreviations: HBV, hepatitis B virus; aCL Ab, anticardiolipin antibodies; β_2GPI Ab, anti-β_2-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 β_2GPI 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 β_2GPI Ab or LA; however, the difference between the groups was not significant (P= 0.40). The isotype distribution for β_2GPI 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 (β_2GPI Ab, IgG type), and the other had β_2GPI Ab (IgM type) and LA.

Follow-up specimens were obtained from 14 of the 17 patients with elevated levels of aCL Ab or β_2GPI Ab (11 with aCL Ab, 2 with anti-β_2GPI Ab, and 1 with both aCL Ab and β_2GPI 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 β_2GPI Ab persisted in all 3 patients with β_2GPI Ab (Table 3).

DISCUSSION

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

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

Patient ID	Gender	Age	Status of HBV infection[a)]	Type of antiphospholipid antibodies
021	M	43	Inactive HBsAg carrier	IgG and IgM aCL
058	F	39	Chronic hepatitis B	IgM β_2GPI and LA
062	M	34	Chronic hepatitis B	IgG β_2GPI
082	F	61	Chronic hepatitis B	IgM aCL
098	F	42	Chronic hepatitis B	IgG aCL
099	M	34	Chronic hepatitis B	IgM aCL
104	F	46	Chronic hepatitis B	IgM aCL
112	F	26	Chronic hepatitis B	IgG aCL
114	F	39	Chronic hepatitis B	IgG aCL
122	F	40	Chronic hepatitis B	IgM aCL
130	F	37	Chronic hepatitis B	IgM aCL and IgG β_2GPI
134	F	70	Inactive HBsAg carrier	IgM aCL
137	M	52	Chronic hepatitis B	LA
140	M	45	Chronic hepatitis B	IgG 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; β_2GPI, anti-β_2-glycoprotein I antibodies; LA, lupus anticoagulant.

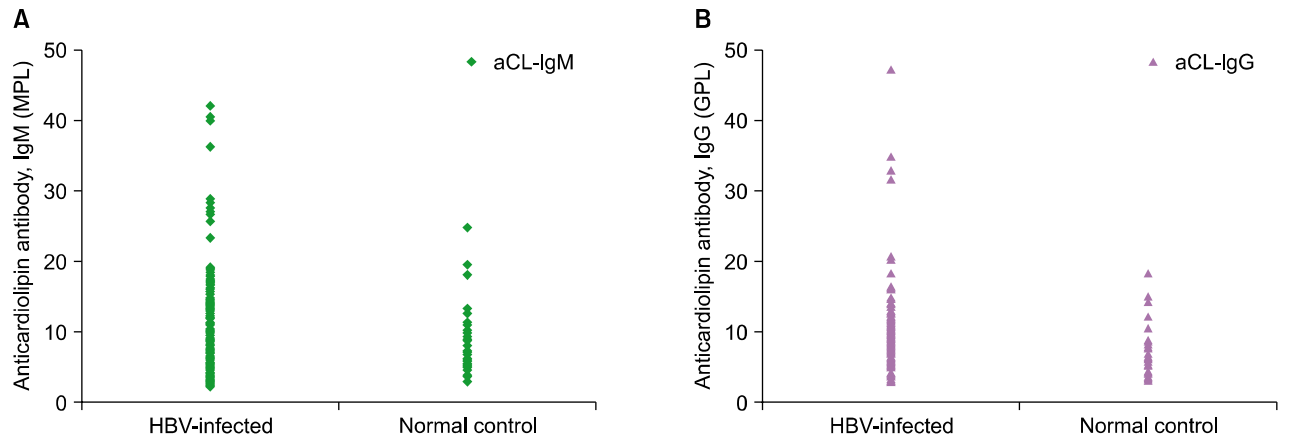

Fig. 1. Distribution of anticardiolipin antibody titers in hepatitis B virus (HBV)-infected patients and normal controls, IgM isotype **(A)** and IgG isotype **(B)**.

Table 3. Follow-up duration and results of aCL Ab and β_2GPI 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 (MPL or GPL)	021	26.6	47.2	–	66.0	100
	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
	134[a)]	39.8	–	NT	NT	
	140[a)]	–	31.5	NT	NT	
	153[a)]	25.8	–	NT	NT	
β_2GPI Ab (SMU or SGU)	058	50.1	–	30.0	–	63
	062	–	32.3	–	29.7	9
	130	–	38.8	–	24.6	28

[a)]Follow-up specimens of 3 patients (134, 140, and 153) could not be obtained.
Abbreviations: HBV, hepatitis B virus; aCL Ab, anticardiolipin antibodies; β_2GPI 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.

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 that lead 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 that of β_2GPI Ab was 2% [17] and 7.5% [14]. In the present study, the prevalence of aCL Ab and β_2GPI Ab was 10.5% and 2.1%, respectively, which was lower than that in previous reports. The lower prevalence of aCL Ab in the present study may be partly because of a higher cut-off value for positivity than that of previous reports. We used 20 GPL or MPL as the cut-off values, whereas 10 GPL or MPL were used as the cut-off values in the other reports [14, 23]. The prevalence of β_2GPI Ab in the present study was similar to those in previous reports.

In most cases, infection-associated aPLs appear temporarily and disappear within 2 or 3 months [24, 25], and except in rare cases, they are unrelated to thrombotic complications. Contrary to previous reports, in the present study, most aCL Ab and β_2GPI Ab showed persistence and were present over a 12-week period. We speculated that continuous antigenic stimulation might be associated with persistent aPL production, because in the present study, 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.

REFERENCES

1. Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. N Engl J Med 2002;346:752-63.
2. Tarr T, Lakos G, Bhattoa HP, et al. Clinical thrombotic manifestations in SLE patients with and without antiphospholipid antibodies: a 5-year follow-up. Clin Rev Allergy Immunol 2007;32:131-7.
3. Giannakopoulos B, Passam F, Rahgozar S, Krilis SA. Current concepts on the pathogenesis of the antiphospholipid syndrome. Blood 2007;109:422-30.
4. Vega-Ostertag ME, Pierangeli SS. Mechanisms of aPL-mediated thrombosis: effects of aPL on endothelium and platelets. Curr Rheumatol Rep 2007;9:190-7.
5. Cervera R, Asherson RA. Antiphospholipid syndrome associated with infections: clinical and microbiological characteristics. Immunobiology 2005;210:735-41.
6. Avcin T, Toplak N. Antiphospholipid antibodies in response to infection. Curr Rheumatol Rep 2007;9:212-8.
7. Sorice M, Pittoni V, Griggi T, et al. Specificity of anti-phospholipid antibodies in infectious mononucleosis: a role for anti-cofactor protein antibodies. Clin Exp Immunol 2000;120:301-6.
8. Sène D, Piette JC, Cacoub P. Antiphospholipid antibodies, antiphospholipid syndrome and infections. Autoimmun Rev 2008;7:272-7.
9. Bloom EJ, Abrams DI, Rodgers G. Lupus anticoagulant in the acquired immunodeficiency syndrome. JAMA 1986;256:491-3.
10. Blank M, Shoenfeld Y. Beta-2-glycoprotein-I, infections, antiphospholipid syndrome and therapeutic considerations. Clin Immunol 2004;112:190-9.
11. Gómez RM, García ES, Lacomba DL, Marchante I, Grande L, Fernandez MC. Antiphospholipid antibodies are related to portal vein thrombosis in patients with liver cirrhosis. J Clin Gastroenterol 2000;31:237-40.
12. Kida Y, Maeshima E, Yamada Y. Portal vein thrombosis in a patient with hepatitis C virus-related cirrhosis complicated with antiphospholipid syndrome. Rheumatol Int 2009;29:1495-8.
13. Ramos-Casals M, Cervera R, Lagrutta M, et al. Clinical features related to antiphospholipid syndrome in patients with chronic viral infections (hepatitis C virus/HIV infection): description of 82 cases. Clin Infect Dis 2004;38:1009-16.
14. Guglielmone H, Vitozzi S, Elbarcha O, Fernandez E. Cofactor dependence and isotype distribution of anticardiolipin antibodies in viral infections. Ann Rheum Dis 2001;60:500-4.
15. Ordi-Ros J, Villarreal J, Monegal F, Sauleda S, Esteban I, Vilardell M. Anticardiolipin antibodies in patients with chronic hepatitis C virus infection: characterization in relation to antiphospholipid syndrome. Clin Diagn Lab Immunol 2000;7:241-4.
16. Harada M, Fujisawa Y, Sakisaka S, et al. High prevalence of anticardiolipin antibodies in hepatitis C virus infection: lack of effects on thrombocytopenia and thrombotic complications. J Gastroenterol 2000;35:272-7.
17. Zachou K, Liaskos C, Christodoulou DK, et al. Anti-cardiolipin antibodies in patients with chronic viral hepatitis are independent of beta2-glycoprotein I cofactor or features of antiphospholipid syndrome. Eur J Clin Invest 2003;33:161-8.
18. Lee KS, Kim DJ. Management of Chronic Hepatitis B. Korean J Hepatol 2007;13:447-88.
19. Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. Thromb Haemost 1995;74:1185-90.
20. Harel M, Aron-Maor A, Sherer Y, Blank M, Shoenfeld Y. The infectious etiology of the antiphospholipid syndrome: links between infection and autoimmunity. Immunobiology 2005;210:743-7.
21. Amin NM. Antiphospholipid syndromes in infectious diseases. Hematol Oncol Clin North Am 2008;22:131-43.
22. Devreese K, Hoylaerts MF. Laboratory diagnosis of the antiphospholipid syndrome: a plethora of obstacles to overcome. Eur J Haematol 2009;83:1-16.
23. Elefsiniotis IS, Diamantis ID, Dourakis SP, Kafiri G, Pantazis K, Mavrogiannis C. Anticardiolipin antibodies in chronic hepatitis B and chronic hepatitis D infection, and hepatitis B-related hepatocellular carcinoma. Relationship with portal vein thrombosis. Eur J Gastroenterol Hepatol 2003;15:721-6.
24. Uthman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. Semin Arthritis Rheum 2002;31:256-63.
25. Dalekos GN, Zachou K, Liaskos C. The antiphospholipid syndrome and infection. Curr Rheumatol Rep 2001;3:277-85.
26. Alric L, Oskman F, Sanmarco M, et al. Association of antiphospholipid syndrome and chronic hepatitis C. Br J Rheumatol 1998;37:589-90.
27. Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. Blood 2003;101:1827-32.
28. Devreese K, Hoylaerts MF. Challenges in the diagnosis of the antiphospholipid syndrome. Clin Chem 2010;56:930-40.