# Anti-Cyclic Citrullinated Peptide Antibodies Distinguish Hepatitis B Virus (HBV)-associated Arthropathy from Concomitant Rheumatoid Arthritis in Patients with Chronic HBV Infection

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***ABSTRACT.*** ***Objective.*** To determine whether anti-cyclic citrullinated peptide (anti-CCP) antibodies, which are a highly specific test for rheumatoid arthritis (RA), could differentiate between hepatitis B virus (HBV)-associated arthropathy and concomitant RA in Korean patients with chronic HBV infection.
***Methods.*** We investigated 240 patients with HBV infection. Anti-CCP antibodies were measured by ELISA and rheumatoid factor (RF) by the latex fixation test. Patient records were reviewed, and a standard form was used to record all demographic, clinical, and laboratory characteristics. Patients were divided into 4 groups according to joint symptoms: asymptomatic, arthralgia, oligoarthritis, and RA. We categorized liver disease into 3 groups: carrier, chronic hepatitis, and cirrhosis.
***Results.*** Anti-CCP antibodies and RF were detected in 11 and 28 of 240 patients, respectively. Anti-CCP antibodies were detected in 9 of 10 RA (90%) and 2 of 230 non-RA patients (0.86%). The positive rate for RF was 90% in RA and 8.3% in non-RA. Eight of 10 RA patients were positive for both RF and anti-CCP antibodies. RF was detected in 11 patients without joint symptoms, 4 with arthralgia, and 4 with oligoarthritis, whereas anti-CCP antibodies were found in 1 patient without joint symptoms and 1 with oligoarthritis. Specificity of anti-CCP antibody for RA was 99.1%, whereas RF showed 91.7% specificity ($p < 0.0002$). We compared the titers and positive detection rates of anti-CCP antibodies and RF among liver disease subgroups. There was no significant between–subgroup difference.
***Conclusion.*** Measurement of anti-CCP antibodies is better than RF detection to discriminate HBV-associated arthropathy from concomitant RA in patients with chronic HBV infection.
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*Key Indexing Terms:*
HEPATITIS B VIRUS INFECTION ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES
RHEUMATOID FACTOR RHEUMATOID ARTHRITIS

Hepatitis B virus (HBV) infection is a major health problem, with approximately 400 million virus carriers worldwide[1]. HBV prevalence is highest in Asia and sub-Saharan Africa. In Korea, the prevalence is as high as 8.9%, much higher than that in the United States or Western Europe[2]. Rheumatoid factor (RF), the presence of which is one of the revised American College of Rheumatology (ACR) criteria for rheumatoid arthritis (RA)[3], is detected in patients with various infectious diseases including HBV infection, other rheumatic disorders, or even in healthy individuals. Studies have reported that the sera of patients with HBV infection might contain RF in up to 20% of cases[4]. This is of particular importance given that patients with HBV infection may present with arthralgia or arthritis, in which situation a differential diagnosis from RA is often challenging. A more specific marker is required to differentiate between RA and hepatitis B-associated arthropathy.

Recently, a novel diagnostic marker for RA, anti-cyclic citrullinated peptide (CCP) antibody measurement, has been developed, and it shows good diagnostic performance. Studies have shown that anti-CCP antibody assessment may be more useful than RF measurement to diagnose true RA in patients with chronic HBV infection[5,6]. Only one study has investigated anti-CCP antibody positivity in non-arthritic HBV patients[7]. No study has examined the value of anti-CCP antibody measurement in HBV-infected patients with arthritis. We evaluated the frequencies and levels of anti-CCP antibodies and RF in HBV-infected patients divided

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into 4 subgroups (asymptomatic, arthralgia, oligoarthritis, and polyarthritis) according to joint symptoms. In addition, we analyzed data on liver disease involvement.

The purpose of our study was to evaluate whether measurement of anti-CCP antibodies might be a useful marker in distinguishing between HBV-associated arthropathy and concomitant RA in patients with chronic HBV infection.

## MATERIALS AND METHODS

*Patients.* This was a retrospective, cross-sectional study performed on 240 Korean patients positive for HBV surface antigen (HBsAg). Patients attended the Division of Rheumatology and Gastroenterology at Eulji University Hospital, in which comprehensive medical examination was conducted between April 2006 and October 2007. All patients were subjected to careful historical interviews and rheumatologic examinations. Patient records were reviewed, and a standard form was used to record all information on demographic, clinical, and laboratory characteristics. Patients were divided into 4 subgroups according to joint symptoms: asymptomatic; arthralgia, with joint pain but no inflammatory sign; oligoarthritis, with joint pain and inflammatory signs in 2 to 4 joints; and polyarthritis, with joint pain and inflammation in ≥ 5 joints.

Our Institutional Review Board approved the study. All patients gave written informed consent.

*HBV infection*. Hepatitis B surface antigen was determined by the CLIA-approved method (Aovia Centaur; Bayer Healthcare Co., Morristown, NJ, USA).

*Anti-CCP antibodies*. Anti-CCP antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) employing a Diastat anti-CCP kit (MBL Co., Nagoya, Japan) and read on an automated EIA analyzer (CODA, Bio-Rad Co., Japan). According to the manufacturer's guidelines, a sample was considered positive for anti-CCP antibodies when the absorbance was higher than the cutoff value of 5 U/ml.

*RF assay.* RF was measured by the latex fixation test using the Hitachi 7170S kit (Hitachi Co., Tokyo, Japan). The positive cutoff value was 18 IU/ml RF.

*Statistical analysis.* Data were analyzed using SPSS (v 14.0) and SAS (release 8.2). The titers of both RF and anti-CCP antibodies were compared to joint involvement by the Kruskal-Wallis test, followed by Dunn's multiple-comparison post-hoc analysis when the overall significance level was $p < 0.05$. Similarly, we compared RF and anti-CCP antibody titers between cases of different liver disease. Comparisons of sensitivity and specificity of assays for anti-CCP antibody and RF were made using McNemar's test.

## RESULTS

The characteristics of 240 patients (162 men, 78 women) enrolled in the study are summarized in Table 1. The mean age of the subjects was 46.6 years (range 24–83 yrs). When joint involvement was assessed, 140 patients were without joint symptoms, 72 had arthralgia, 18 oligoarthritis, and 10 polyarthritis. All patients with polyarthritis were classified as RA cases by ACR criteria[3]. Bone erosions were observed in 8 of the 10 patients with RA. This study showed that the prevalence of concomitant RA in patients with chronic HBV infection was 4.1%. Among patients without joint symptoms, 11 patients (7.9%) were RF-positive, whereas only 1 patient (0.7%) was positive for anti-CCP antibodies. Among patients with arthralgia, RF was present in 5.6%, whereas anti-CCP antibodies were not detected. RF positivity was 22.2% among patients with oligoarthritis, whereas anti-CCP antibodies were found in only 1 patient (5.6%), who had arthritis in both knee and elbow joints; the titer was 13.4 U/ml. Whereas 8 of 10 RA patients were positive for both anti-CCP antibodies and RF, the other 2 patients were positive for either anti-CCP antibodies or RF (Figure 1). When we divided patients into RA or non-RA cases, both positive rates and titers of RF and anti-CCP antibodies were significantly higher in the RA group than in non-RA patients ($p < 0.001$; Figure 2). Specificity and sensitivity of anti-CCP antibody presence in diagnosis of RA were 99.1% and 90%, respectively, whereas the corresponding values for RF measurement were 91.7% and 90%. There was a significant difference between measurement of anti-CCP antibodies and RF in specificity ($p < 0.0002$; Table 2). As the data were non-normally distributed (p values in the Kolmogorov-Smirnov test < 0.05), we used the nonparametric Kruskal-Wallis test and Dunn's multiple comparison post-hoc test for further data evaluation.

In addition, we compared titers among liver disease subgroups. Twenty-four patients had fatty liver and 1 patient had a hepatoma. These 25 patients were excluded to allow only the effect of HBV infection on autoantibody levels to be analyzed. There was no significant difference between

*Table 1.* Demographic and clinical characteristics of patients with HBV infection.

| Characteristic | Patients (n = 240) |
|---|---|
| Male/female | 162/78 |
| Age, yrs (range) | 46.6 (27–83) |
| Liver involvement (%) | 215 |
| Carrier | 170 (79.1) |
| Chronic hepatitis | 27 (12.6) |
| Cirrhosis | 18 (8.3) |
| Joint involvement (%) | |
| No symptom | 140 (58.3) |
| Arthralgia | 72 (30.0) |
| Oligoarthritis | 18 (7.5) |
| Polyarthritis | 10 (4.2) |

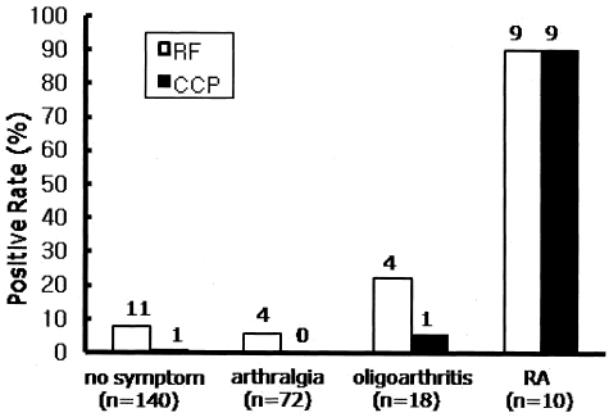


*Figure 1.* Percentages of cases positive for rheumatoid factor (RF) and anti-CCP antibodies according to joint involvement (n: number of patients).

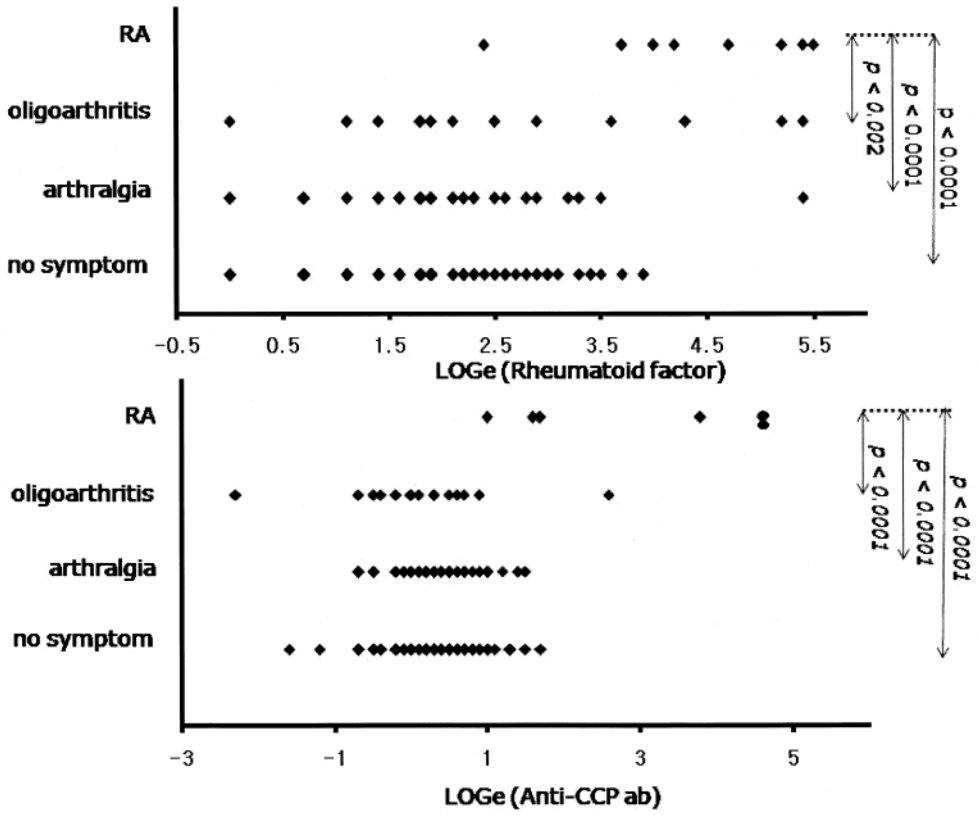


*Figure 2.* Titers of rheumatoid factor (RF) and anti-CCP antibodies according to joint involvement. To expand information distribution, the titer scale is in natural logarithms. The cutoff values for RF and anti-CCP antibodies on the log scale were 2.89 and 1.61, respectively. Titers were compared by Kruskal-Wallis test for both RF and anti-CCP antibodies, followed by Dunn's multiple-comparison post-hoc analysis.

*Table 2.* Diagnostic sensitivity and specificity of anti-CCP antibodies and RF. Values are numbers (%) of patients.

| | RF | | Anti-CCP | | |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | p* |
| RA | 9 (90) | 1 (10) | 9 (90) | 1 (10) | |
| Non-RA | 19 (8.3) | 211 (91.7) | 2 (0.8) | 228 (99.2) | |
| Sensitivity, % | 90 | | 90 | | 1 |
| Specificity, % | 91.7 | | 99 | | 0.0002 |

* Statistically significant difference; 2-sided McNemar test.

the subgroups (data not shown). We also assessed the effect of liver disease on autoantibody levels in non-RA patients alone. There was no significant difference in autoantibody levels among non-RA patients with different degrees of liver disease (Figure 3). The 10 RA patients included 1 case of chronic hepatitis, 1 of cirrhosis, and 8 carriers. The HBV DNA viral load in the chronic hepatitis and cirrhosis patients was 2098 pg/ml and 4.68 pg/ml, respectively.

## DISCUSSION

Chronic HBV infection is associated with extrahepatic manifestations including arthropathy, glomerulonephritis, polyarteritis nodosa, polymyalgia rheumatica, dermatomyositis, uveitis, myocarditis, and neurogenic disease. Among these, arthropathy, which mimics RA, is a frequent symptom (10%–35%) and sign associated with HBV infection (10%–35%)[8]. RA is a common chronic autoimmune disorder, affecting roughly 1% of the population worldwide. RF is an antibody directed against the Fc region of IgG; RF has been used as a diagnostic marker for RA, but is also known to be very frequently present in HBV carriers[9]. When RF was assayed in 140 asymptomatic carriers, 20% of patients were positive, compared to 2.7% of controls[9]. In our study, the prevalence of concomitant RA in patients with chronic HBV infection was 4.1%, 4-fold higher than in the general population. However the level of RF-positive cases was 11.6%, which was almost 3-fold higher than the prevalence of RA; this indicates that RF cannot serve as a specific marker of RA in patients with HBV infection.

The recent development of serological tests for antibodies to CCP in patients with RA has provided a new diagnostic tool. Most patients with RA produce antibodies that recognize peptides containing the unusual amino acid citrulline. In general, assay of anti-CCP antibodies is better than RF measurement for the diagnosis of RA. The

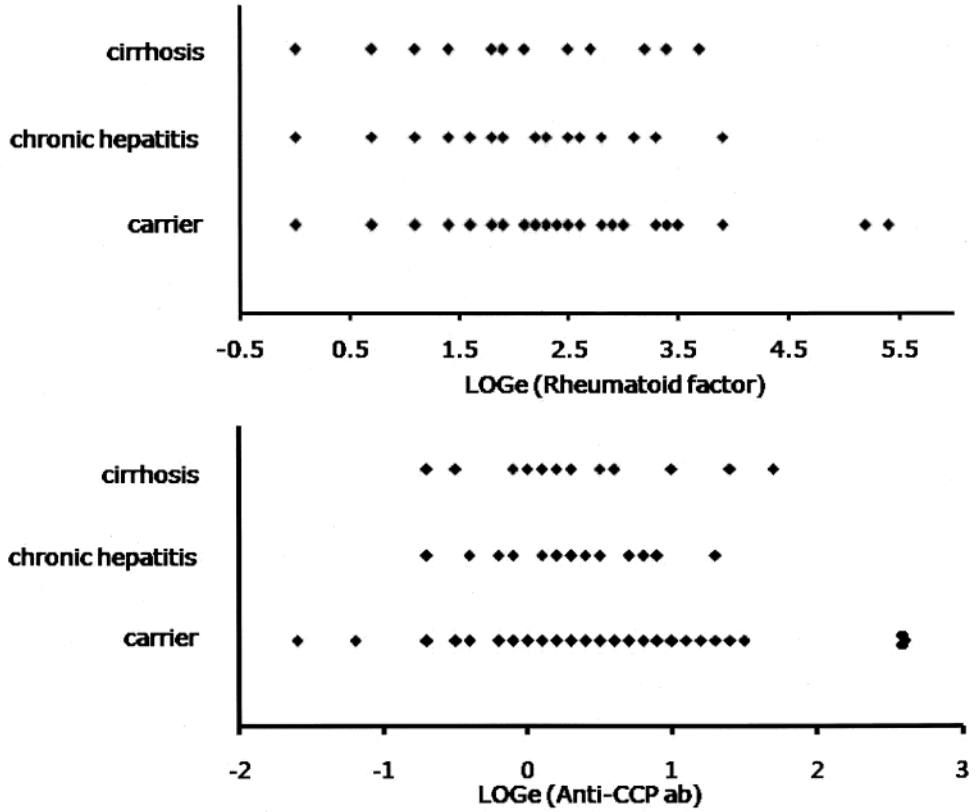


*Figure 3.* Titers of rheumatoid factor and anti-CCP antibodies, according to extent of liver involvement in non-RA patients (patients with hepatoma and fatty liver were excluded). There was no significant difference in either antibody with varying extent of liver involvement.

anti-CCP assay has a reported sensitivity of 41%–68%, with a specificity of 96%–98% in patients with established RA[10-16]. Several studies have also suggested that anti-CCP antibodies identify RA patients at risk for more severe joint damage and functional disability[11,15].

Recent studies have reported that anti-CCP antibodies are not detected in the sera of patients with chronic hepatitis C virus (HCV) infection. Bombardieri and colleagues[6] measured anti-CCP antibodies and RF in 8 chronic HCV patients with articular involvement and in 31 chronic HCV patients with no joint involvement. Anti-CCP antibodies were not detected in patients with chronic HCV infection irrespective of the presence of articular involvement. Conversely, RF was detected in 37.5% of patients with HCV-related arthropathy and 9.7% of cases of HCV infection without joint involvement[6]. Wener and coworkers reported that elevated anti-CCP was not found in patients with HCV infection, whereas 44% of cases were positive on RF testing[5]. Lienesch and colleagues studied 50 nonarthritic patients with chronic hepatitis C infection. RF was detectable in 26 cases, but elevated CCP antibody was detected in only a single patient (2%)[17].

Only one study has measured anti-CCP antibodies and RF in the patients with HBV infection. Lee and coworkers studied 176 nonarthritic patients with HBV infection and reported that IgM RF-positive patients were found at a frequency of 18.8%; only 0.6% of cases had anti-CCP antibodies[7].

We analyzed the frequencies of anti-CCP antibodies and RF in patients with chronic HBV infection, according to joint and liver involvement. Anti-CCP antibodies were detected in 9 of 10 RA (90%) and 2 of 230 non-RA patients (0.86%). The frequency of patients positive for RF was 90% in RA cases (9 of 10 patients) and 8.3% in non-RA patients (19 of 230 cases). Eight of 10 RA patients were positive for both RF and anti-CCP antibodies. Of patients without joint symptoms, 1 was positive for anti-CCP antibodies and 11 were positive for RF. Among patients with arthralgia, RF was present in 5.6% of cases whereas anti-CCP antibodies were not detected in any patient. In cases of oligoarthritis, anti-CCP antibodies were present in 1 patient who complained of pain in the knee and elbow joints, and the titer was 13.4 U/ml. Therefore, anti-CCP antibody measurement showed a low rate of false-positive RA results. Also, it is possible that very early-stage RA was present in this patient, who is receiving careful followup to ensure that potential evolution to RA is noted and treated. But how can we be sure that the 10 patients with polyarthritis are true RA cases

rather than individuals with virus-induced arthritis? Virus-induced arthritis is characteristically an asymmetrical polyarthritis and significant joint destruction is lacking. In our study, all RA patients showed chronic persistent symmetric polyarthritis, and bone erosion was observed in 8 of 10 cases. Of the 2 patients without bone erosion, 1 had chronic hepatitis and 1 was a carrier. Csepregi and colleagues reported that 2 patients (1 with chronic active hepatitis; 1 liver cirrhosis) who showed poor responses to disease modifying antirheumatic drugs (DMARD) responded excellently to antiviral treatment[18]. In our study, the 10 RA patients comprised 1 case of chronic hepatitis, 1 of cirrhosis, and 8 carriers. The carriers were not treated with antiviral agents. The HBV DNA loads of the chronic hepatitis and cirrhosis patients were 2098 pg/ml and 4.68 pg/ml, respectively. The chronic hepatitis patient did not receive antiviral treatment, but showed marked joint symptom improvement with cyclosporine. We have no data on possible response to antiviral treatment. However, the fact that most RA patients had bone erosions and showed good responses to DMARD suggests that virus-induced arthritis was probably absent.

In addition, we investigated whether the extent of liver involvement might affect the development of anti-CCP antibodies or RF. There was no difference between liver sub-disease groups in expression of anti-CCP antibodies or RF. Therefore, the severity of liver involvement did not correlate with anti-CCP antibody or RF levels.

We provide evidence that anti-CCP antibodies are a very useful marker for distinguishing HBV-associated arthropathy from concomitant RA in patients with chronic HBV infection. Our study has some limitations; the work was retrospective, and no data on response to antiviral treatment are given. To confirm and extend our results, a well executed randomized prospective study in a large population, including antiviral treatment measures in statistical assessment, is required.

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