Anti-cyclic citrullinated peptide and rheumatoid factor in patients with chronic hepatitis B and hepatitis B carriers

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Abstract

Objective: Rheumatoid factor (RF) positivity that may occur in a number of patients with hepatitis B (HBV) infection poses challenges in terms of differential diagnosis with rheumatoid arthritis (RA). On the other hand, antibodies to cyclic citrullinated peptide (anti-CCP) may prove to be an important marker for differential diagnosis of the two conditions. This study aimed to assess anti-CCP and RF positivity among patients with hepatitis B and rheumatoid arthritis.

Material and Methods: Anti-CCP and RF seropositivity was assessed in 61 patients with HBV infection (32 patients with chronic hepatitis, 29 patients with inactive HBV carrier status) and 40 patients with RA as the control group.

Results: RF positivity was found in 18.7% and 34.4% of the patients with chronic hepatitis B and inactive HBV carrier status, respectively. On the other hand, only one patient with chronic HBV had low positive anti-CCP. RF was positive in 24 (60%) and anti-CCP was positive in 26 (65%) patients among the 40 patients with RA.

Conclusion: Anti-CCP may be helpful in the differential diagnosis between RA and chronic HBV infection or inactive HBV carrier status.

Keywords: Hepatitis B, anti-cyclic citrullinated peptide, rheumatoid factor, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology associated with synovitis and joint destruction. Chronic Hepatitis B (HBV) infections may also present with articular findings, sometimes requiring a differential diagnostic work-up for rheumatic diseases diseases and mainly for RA. Similar to hepatitis C virus (HCV) infections, several autoantibodies including rheumatoid factor (RF) and anti-nuclear antibody (ANA) may be detected in the sera of patients with HBV infection (1, 2). RF positivity may occur in HCV and HBV infections and pose further challenges in the simultaneous presence of articular findings in terms of differential diagnosis with RA. In addition, there is some evidence suggesting a pathophysiological link between HBV infection and RA (3).

Antibodies to cyclic citrullinated peptide (Anti-CCP) show a high specificity (91%-97%) for RA, although its sensitivity is lower (64%-75%) and similar to that of RF. The RF-positive cases (80%-90%) also show CCP antibodies (4-6). CCP antibody positivity has been shown many years before the manifestation of the disease (7).

Although an increased occurrence of RF positivity is a well-established phenomenon in patients with HBV infections, there is a lack of information on the presence of CCP antibodies in these patients, which possess a higher specificity for RA. Therefore, we examined the occurrence RF and anti-CCP positivity in patients with chronic HBV infection or inactive HBV carrier status based on the assumption that anti-CCP may prove to be an important marker for the differential diagnosis of HBV infections with RF positivity. In addition, the incidence of RF and anti-CCP positivity was compared between patients with chronic HBV infection and HBV carrier status.

Material and Methods

Patients

A total of 32 consecutive patients with chronic HBV infection (mean age 47±13 years, 19 male and 13 female) and 29 patients with inactive HBV carrier status (mean age 47±12 years, 20 male and nine fe-

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Table 1. Distribution of RF and anti-CCP among patients with inactive carriers and chronic disease by articular findings

	RF (+)		Anti-CCP (+)	
	Articular findings present	Articular findings absent	Articular findings present	Articular findings absent
Inactive HBV carriers (n=29)	6	4	0	0
Chronic hepatitis B (n=32)	4	2	1	0
Rheumatoid arthritis (n=40)	24		26	

All articular findings were arthralgia.
RF: rheumatoid factor; HBV: hepatitis B; RA: rheumatoid arthritis; anti-CCP: antibodies to cyclic citrullinated peptide

male) attending to the outpatient facility of the Department of Gastroenterology, Medical Faculty of Uludag University were included in this study. The control group included 40 patients (mean age 50±12 years, five male and 35 female) who were diagnosed with RA based on the 1987 American College of Rheumatology (ACR) or 2010 ACR/European League Against Rheumatism (EULAR) RA diagnostic criteria for RA (8, 9). In all subjects, the presence of anti-CCP as well as RF positivity was determined. The study protocol was approved by the local ethics committee, and the study procedures were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient, and patient anonymity was preserved.

Chronic HBV infection was defined as follows: presence of HBV in addition to >6 months of necroinflammatory activity in the liver [HBsAg(+) and HBc IgM(-) >6 months; HBV DNA >10^4 copies/mL; persistent or intermittent elevation of serum transaminases; and necroinflammatory activity±fibrosis on liver biopsy]. Inactive HBV carrier status was defined as follows: negligible virus replication despite ongoing HBV infection [HBsAg(+) and HBc IgM(-) >6 months, HBeAg(-), anti-HBe(+), and Serum HBV DNA <2000 IU/mL] and persistent normal serum transaminases.

Anti-CCP and RF assays
RF was assayed with a quantitative immunonephelometry test (Siemens N Latex RF Kit, Germany). RF was considered positive when the concentration was higher than the cut-off value of the kit (15 IU/mL).

Anti-CCP was determined using the enzyme-linked immunosorbent assay (ELISA) method. For the semi-quantitative determination of CCP-specific IgG autoantibodies, a flexible test protocol referred to as the Chemiflex System (Architec System i2000 SR, USA) was used in conjunction with CMIA technology and automatic sample preparation. The sam-

ples were pre-diluted with washing buffer. The pre-diluted samples were mixed with CCP-covered paramagnetic microparticles and sample diluents. Anti-CCP antibodies present in the sample were attached to the CCP-covered microparticles, and a conjugate labeled with anti-human IgG acridinium was added following a washing step. Following another washing step, pre-tigger and tigger solutions were added into the reaction mixture. The resultant chemiluminescence reaction was measured using relative light units. Anti-CCP antibodies were considered positive when the absorbance was higher than the cut-off value of the kit (5 U/mL).

Statistical analysis
SPSS statistical package version 13.0 (SPSS, Inc, Chicago, IL, USA) was used for data analysis. The results are expressed in frequencies and percentages. Patients with chronic HBV infection and inactive HBV carrier status were compared using Fisher's chi-square test for the frequency of RF and anti-CCP positivity. A p value <0.05 was considered statistically significant.

Results
Among 61 patients with HBV infection, RF positivity occurred in 16 patients (26.2%), with only one patient (1.63%) showing weak positivity for anti-CCP. Of the 32 patients with chronic HBV infection, six (18.7%) were positive for RF, and the only anti-CCP positive patient was in this group. The latter patient had an anti-CCP value of 7 U/mL (normal range: 0-5 U/mL), tested negative for RF, was not a smoker, and did not meet the 1987 ACR or 2010 ACR/EULAR RA diagnostic criteria for RA despite having articular signs. Ten out of the 29 patients (34.4%) with inactive HBV infection were positive for RF, with no cases testing positive for anti-CCP. Although RF positivity was more frequent among inactive HBV infection patients compared with that in the patients with chronic HBV, the difference did not reveal a statistical significance (p=0.244). On the other hand, RF was positive in 24 patients (60%) and anti-CCP was positive in 26 patients (65%) among the 40

patients with RA. Table 1 shows the distribution of RF and anti-CCP positivity among patients with inactive carriers and chronic disease by articular findings. Among RF-positive patients, the frequency of articular findings were similar in inactive carriers and in patients with chronic hepatitis (p=1.0).

Discussion
The common occurrence of positivity for certain serologic tests in patients with HBV or HBC infection may result in some diagnostic difficulties. This is particularly true for patients with HBV infection who have been shown to have an increased incidence of RF positivity compared to that of the general population (2). Furthermore, articular signs that may occur in some patients with HBV infection may also mimic RA closely. If such a patient is tested positive for RF, differential diagnosis may be even more complicated.

Anti-CCP, a relatively novel antibody, is among an antibody family against several citrullinated peptides, and of these, CCP is the most frequently used antigen for laboratory procedures (10, 11). After a period of 5-6 years, with a number of studies conducted on its role for the diagnosis of RA, it has been included in the latest ACR/EULAR 2010 diagnostic criteria for RA. Despite having a similar sensitivity with RF, it demonstrates significant superiority in terms of specificity for the disease. In addition, anti-CCP positivity has been associated with a more erosive course in early RA (12, 13).

Chronic infections such as streptococcus, hepatitis B, tuberculosis, and autoimmune thyroid diseases can produce elevated levels of RF and anti-CCP (14). Singh U et al. (14) examined 33 cases of undifferentiated arthritis (UA) in which features of RA were not present; however, anti-CCP antibody was positive. Out of the 33 cases of UA, 19 had well-known diseases such as hyperthyroidism, hypothyroid-

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ism, tubercular arthritis, traumatic arthritis, pneumonia with arthritis, varicose vein with pain in legs, and cervical spondylitis . All 33 cases were positive for anti-CCP and approximately 62.5% cases showed RF positivity. Lee et al. (15) examined 176 patients with chronic HBV infection who had no articular signs and found a high percentage of RF positivity (42.7%, 75 patients) while detecting only one patient (0.4%) with anti-CCP positivity. In addition, Lim et al. (16) examined 240 patients with HBV infection to determine whether anti-CCP antibodies could differentiate between HBV-associated arthropathy and concomitant RA in Korean patients with chronic HBV infection. Anti-CCP antibodies and RF were detected in 11 and 28 of 240 patients, respectively. Anti-CCP antibodies were detected in nine of 10 RA patients (90%) and two 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, four with arthralgia, and four with oligoarthritis, whereas anti-CCP antibodies were found in one patient without joint symptoms and one with oligoarthritis .Measurement of anti-CCP antibodies seems to be better than RF detection to discriminate HBV-associated arthropathy from concomitant RA in patients with chronic HBV infection. Until now, studies have focused on anti-CCP among patients with chronic HCV infection rather than HBV infection. For example, Lienesch et al. (17) and Orge et al. (18) found no increase in anti-CCP in non-arthritic patients with chronic HCV infection. Similarly Bombardieri et al. (19) examined RF and anti-CCP in patients with chronic HCV infection and found RF positivity in 37% of the patients vs. no patients with anti-CCP positivity.

In our study, RF positivity occurred in 26.2% of the 61 patients with chronic HBV infection, and anti-CCP was weak positive in only one case, which tested negative for RF, did not exhibit clinical features supporting a diagnosis of RA, and was not a smoker. Although RF is commonly used for the diagnosis of RA, its diagnostic value is diminished in HBV carriers and patients with chronic HBV infection. As shown in our study, absence or low incidence of anti-CCP positivity in such patients may prove to be a more reliable indicator for the differential diagnosis between HBV infection and RA. Although the number of the patients in the group seems to be low,

the finding that only 1.63% of these patients show weak positivity for anti-CCP suggests that this test can be used for the differential diagnosis in RF-positive HBV infections. The control group did not get to participate in our study because of the low presence of anti-CCP in our country (20). Higher incidence of RF compared to that of anti-CCP suggests that B cell activity due to HBV is responsible for RF positivity while pointing out to a possible non-B cell dependent mechanism for the emergence of anti-CCP.

Most agents used for the treatment of RA, including methotrexate, have hepatotoxic effects that require close monitoring of the liver function tests. An increased presence of RF positivity in HBV infections may result in misdiagnoses, leading to unnecessary use of hepatotoxic agents, particularly in patients with articular signs. This further reinforces the importance of a reliable indicator such as anti-CCP in HBV infections.

In conclusion, anti-CCP appears to be a reliable and important marker that can be used for differential diagnosis in RF-positive HBV infections.

Ethics committee approval: The study protocol was approved by the local ethics committee and the study procedures were conducted in accordance with Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.D., M.F.O.; Design - E.D., Y.P.; Supervision - E.D., YP.; Materials - B.N.C., A.N.T.; Data Collection and/or Processing - A.Ö., A.N., M.E.D.; Analysis and/or Interpretation - M.F.O.; Literature Review M.F.O.; Writer - E.D., M.F.O.; Critical Review - E.D.,YP.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study has received no financial support.

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