

Anti-Cyclic Citrullinated Peptide Frequency in Patients with Chronic Hepatitis C Virus Infection and Effect of Presence of Systemic Disease

Kronik Hepatit C Virus Enfeksiyonlu Hastalarda Anti-Siklik Sitrülenmiş Peptid Sıklığı ve Sistemik Hastalık Varlığının Etkisi

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Abstract

Objective: Patients with chronic hepatitis C virus (HCV) infection may show a variety of rheumatic symptoms and signs. Anti-cyclic citrullinated peptide (anti-CCP) is widely used as a marker, particularly for rheumatoid arthritis (RA), and may be positive in some diseases that also cause arthritis, such as systemic lupus erythematosus, familial Mediterranean fever, Behçet's disease, and psoriatic arthritis.

Materials and Methods: Blood samples were obtained (in routine protocols) from 57 patients with chronic HCV infection from the Gastroenterology Clinic of Ataturk University and Infectious Disease Clinic of Erzurum Region Research and Education Hospital. Normal sera were obtained from volunteer blood donors at Ataturk University.

Results: Anti-CCP antibodies were found in 5 chronic HCV patients with RA. The patient with the highest anti-CCP antibody level had RA. No patient in the control group was positive for anti-CCP antibodies.

Conclusion: Anti-cyclic citrullinated peptide (anti-CCP) antibodies should be measured frequently in patients with HCV and an additional systemic disease, such as end-stage chronic renal failure, chronic obstructive airway disease, and decompensated liver cirrhosis, to differentiate RA from non-RA arthropathy.

Key Words: Anti-CCP, Chronic hepatitis C virus, Rheumatoid arthritis

Özet

Amaç: Kronik hepatit C virüs (HCV) enfeksiyonu çeşitli romatolojik belirti ve semptomlarla seyredebilir. Anti-siklik sitrülenmiş peptid (Anti-CCP) özellikle romatoid artrit (RA) tanısında yaygın şekilde kullanılan, ayrıca sistemik lupus eritematozus, ailevi akdeniz ateşi ve psöriatik artrit gibi artritle birlikte seyreden bazı hastalıklarda da pozitif olduğu gösterilmiş bir antikordur.

Gereç ve Yöntem: Çalışmada Atatürk Üniversitesi Gastroenteroloji Kliniği ve Erzurum Bölge Eğitim ve Araştırma Hastanesi Enfeksiyon Hastalıkları Kliniği'nden 57 kronik HCV enfeksiyonlu hastadan rutin tetkikleri sırasında alınmış olan kanlar kullanıldı. Kontrol kanları Atatürk Üniversitesi kan vericilerinden artan kanlardan toplandı.

Bulgular: Kronik HCV enfeksiyonuna ilaveten RA tanısı da almış olan 5 hastanın tamamında Anti-CCP pozitif bulundu. Anti-CCP seviyesi en yüksek olan hasta da RA'li olan bir hasta idi. Kontrol grubundaki hastalardan hiçbirinde Anti-CCP pozitif değildi.

Sonuç: Kronik HCV'li hastalarda kronik böbrek yetmezliği, kronik obstruktif akciğer hastalığı veya dekompanse karaciğer sirozu gibi sistemik hastalıkların varlığında, Anti-CCP daha sık olarak görülebilmektedir. Ayrıca Anti-CCP pozitifliğinin, bu hasta grubunda RA ile non-RA artropati ayırımında da kullanılabilmesi düşünülmektedir.

Anahtar Kelimeler: Anti-CCP, Kronik hepatit C virüs, Romatoid artrit

Introduction

Hepatitis C is a serious public health issue, with a global estimate of 170 million cases of hepatitis C virus (HCV) infection [1, 2]. Patients with chronic HCV infection may have a variety of rheumatic symptoms and signs, including immunological disorders with different clinical presentations, such as arthritis, arthralgia, and various forms of vasculitis [3-5]. The

prevalence of extrahepatic complications can vary between populations, possibly due to genetic and viral factors, such as viral genotype. Rheumatoid arthritis (RA)-like HCV-related arthropathy can be clinically indistinguishable from recent-onset RA, in which articular damage and deformities have not yet occurred. Two forms of articular involvement have been identified in association with HCV infection, but the more common form is an oligoarthritis of the large joints

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that is frequently associated with cryoglobulinemia [6, 7]. An association between HCV infection and cryoglobulinemia syndrome has been firmly established [8].

Anti-cyclic citrullinated peptide (anti-CCP) antibody has been reported as a new commercially available serological marker for RA. It is more specific than rheumatoid factor (RF) [9, 10] and is now widely used. Anti-CCP antibodies may also be present in some diseases that present arthritis as a symptom, such as systemic lupus erythematosus, familial Mediterranean fever, Behçet's disease, and psoriatic arthritis [10-13].

Differentiating patients with HCV-related arthritis from patients with RA represents both a diagnostic and therapeutic challenge. Anti-CCP antibody has been investigated as a possible factor that can be used to distinguish between these two conditions. Our aim in the present study was to investigate the levels of anti-CCP antibodies in HCV-infected Turkish patients with or without arthritis, RF, or cryoglobulinemia.

Materials and Methods

Sera were obtained from 39 normal (control group) volunteer blood donors chosen from patients who attended outpatient clinics but had no diseases. Blood samples from 57 patients with RA were obtained from the Gastroenterology Clinic of Ataturk University and the Infectious Disease Clinic of Erzurum Region Research and Education Hospital. The study was conducted at two hospitals (the Erzurum Region Education and Research Hospital and the Ataturk University Faculty of Medicine). Patients with HCV infection were diagnosed by the presence of HCV antibodies, and infection was confirmed by the detection of viral RNA in sera. After obtaining informed consent, serum samples were collected from all patients. Normal sera were obtained from volunteer blood donors at the Ataturk University. Sera previously stored at -80°C were evaluated for RF and anti-CCP antibodies.

A history was taken from all of the patients, and a clinical examination was performed, including a musculoskeletal examination, abdominal ultrasonography, and laboratory investigations in the form of routine laboratory tests and tests for RF and anti-CCP antibodies. HCV infection was diagnosed in patients with chronic HCV infection by the presence of HCV antibodies and the detection of HCV-RNA by real-time PCR. In total, 35.1% of the patients with chronic HCV had end-stage chronic renal failure (CRF), 5.3% had chronic obstructive airway disease (COBD), 5.3% had decompensated liver cirrhosis (DLC), 8.8% had RA, and 52.5% had no additional diseases. Patients were excluded if they were co-infected with human immunodeficiency virus or hepatitis B virus. Women who were pregnant or breast-feeding were also excluded. Patients were required to give written informed consent.

The serum concentration of anti-CCP antibodies was analyzed with an enzyme-linked immunosorbent assay (ELISA) using commercially available QUANTA Lite™ CCP IgG ELISA kits (INOVA Diagnostic, Inc., San Diego, USA) according to the manufacturer's protocol. All assays were performed in duplicate. The concentration of anti-CCP antibodies was estimated by interpolation from a dose-response curve based on standards included in the assay. Patients were considered to be anti-CCP positive when the absorbance was higher than the cutoff value of the kit (20 U/mL). Values <20 U were considered negative, while values between 20 and 39 U were considered "weakly" positive, 40-59 U "moderately" positive, and 60 U "strongly" positive. According to the manufacturer, the anti-CCP ELISA had a sensitivity of 76% for clinically confirmed RA patients and a specificity of 99% for healthy controls. Some results were expressed as the mean±standard deviation.

Results

The ages and genders of the patients in the two groups (patients with chronic HCV infection and the control group) are shown in Table 1. The ages of the patients ranged from 22-78 years for the HCV-infected patients and from 19-59 years for the control group. The concentration of anti-CCP antibodies was high in five chronic HCV patients with RA. The patient with the highest anti-CCP level was also one with RA (220.6 IU/mL). No patient in the control group was positive for anti-CCP antibodies. However, 2 of these patients (5.1%) were positive for RF (Table 1). Of the patients positive for anti-CCP antibodies (14.1%) in the chronic HCV group, 2 were "moderately" positive (20-39 IU/mL), and 6 were "strongly" positive (greater than 60 IU/mL). The patient with the highest anti-CCP titer was also one of the patients with RA in the study group, in which the RF titers were the highest. Only one of the patients with CRF was anti-CCP positive, whereas no anti-CCP positivity was noted in the patients with COBD or DLC.

Table 1. The age, gender properties and the percentage of the two tests for the of the two groups

Mean±SD (minimum-maximum)	Number of samples	Age	Gender (M/F)	RF positivity	Anti-CCP positivity
HCV-infected patients	57	52.4±13.3 (22-78)	54.4%/45.6%	7%	14.1%
Control	39	34.4±11.4 (19-59)	56.4%/43.6%	2.5%	0%

SD: standard deviation, HCV: hepatitis C virus, M/F: male/female, RF: rheumatoid factor, Anti-CCP: anti-cyclic citrullinated peptide.

Discussion

Rheumatological manifestations are common during HCV infection, and in some cases, symptoms may mimic the onset of RA. Arthralgia is the most common extrahepatic manifestation of HCV infection. The distinction between HCV-associated arthropathy and RA has great relevance for clinicians [2]. Many autoantibodies are common in HCV-infected patients, including rheumatoid factors and antinuclear antibodies, but anti-CCP antibodies are also found, which may help to differentiate early RA from HCV-associated joint manifestations. Therefore, many studies have explored the relationship between HCV and anti-CCP antibodies. The frequency of anti-CCP positivity in patients with HCV is inconsistent between studies (Table 2) [1, 5, 6, 8, 14-19]. However, almost all of the patients researched previously had no additional systemic diseases and had isolated HCV and/or HCV+ arthropathy. In the present study, unlike these previous studies, we included patients with additional systemic diseases in the study group.

In our study, the frequency of anti-CCP positivity for patients with HCV appears to be the highest ever reported (Table 2). However, our study evaluated a limited number of patients (n=57). In addition, differences may have existed in the methods and kits used to measure anti-CCP antibodies. However, we believe that the high frequency of anti-CCP antibodies observed in our study was primarily due to the inclusion of HCV patients with additional systemic diseases.

The presence of RF is not a reliable diagnostic tool for RA, as many cases of recent-onset RA are seronegative. Additionally, many non-rheumatoid conditions, such as connective tissue

Table 2. Summary of published reports of the prevalence of anti-CCP antibodies in HCV-infected patients

First author, year (reference no.)	HCV		Control group	
	Patients (n)	Anti-CCP (%)	Patients (n)	Anti-CCP (%)
Bombardieri, 2004 (1)	39	0		
Wener, 2004 (5)	50	0	20	0
Lienesch, 2005 (14)	50	2		
Sene, 2006 (15)	147	1.4		
Koga, 2008 (16)	45	0	23	0
Liu, 2008 (8)	34	8.8	42	0
Orge, 2010 (17)	41	4.9	87	5.7
Ezzat, 2011 (19)	22	4.5		
Zehairy, 2011 (6)	55	1.8		
Our results, this article	57	14.1	39	0

HCV: hepatitis C virus, n: number of patients, Anti-CCP: anti-cyclic citrullinated peptide.

diseases and HCV infection, may be RF seropositive. Indeed, 30-68% of HCV-infected patients are RF positive [15, 20-22]. Some previous studies have stated that the presence of anti-CCP antibodies in patients with HCV can differentiate RA from non-RA conditions [1, 5, 6, 8, 14, 15, 17, 18]. However, the patients with HCV examined in these studies did not have additional systemic diseases. While anti-CCP antibodies were found in 14% of patients in these studies, RF was found in 7% of HCV-positive patients in our study. Our study group included 5 RA patients, and these patients were positive for both RF and anti-CCP antibodies. The patient with the highest concentration of anti-CCP antibodies was one of the RA patients positive for RF. However, 3 patients with a high anti-CCP antibody concentration did not have RA. Therefore, in the presence of an additional systemic disease, such as CRF, DLC, and COBD, anti-CCP positivity is important for diagnosing RA in HCV-positive patients.

Our study demonstrates that anti-CCP antibodies should be measured in the presence of additional systemic diseases, such as CRF, DLC, and COBD, in patients with HCV to differentiate RA from non-RA arthropathy.

Conflict of interest statement: The authors declare that they have no conflict of interest to the publication of this article.

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