

Clinical significance of anti-cyclic citrullinated peptide antibodies in Egyptian patients with chronic hepatitis C virus genotype IV infection

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Abstract

Background: Symmetric polyarthritis associated with hepatitis C virus (HCV) infection frequently displays a clinical picture like rheumatoid arthritis (RA). Antibodies to cyclic citrullinated peptide (CCP) have high specificity for the diagnosis of RA. This study examined the frequency and clinical significance of anti-CCP antibodies in patients with chronic HCV infection, with and without manifestations of joint involvement, compared to RA patients.

Methods: Serum anti-CCP antibodies and rheumatoid factor (RF) were evaluated in 30 patients with RA and 47 patients with chronic HCV infection. Of those with HCV infection, 20 patients had chronic HCV infection associated with articular involvement and 27 patients had chronic HCV infection without any articular involvement.

Results: Anti-CCP antibody level was positive in 70% of RA patients, 8.5% of HCV-infected patients, and in 20% of HCV patients with articular manifestations. RF was positive in 76% of RA patients and in 60% of HCV patients with articular involvement. Cryoglobulins were found in 29% of HCV-infected patients and in 16% of RA patients. Cryoglobulins were more frequent among HCV patients with articular affection (35%) compared to HCV patients without articular affection (26%).

Conclusions: Although anti-CCP antibodies remain a useful diagnostic tool for RA, their interpretation in HCV-infected patients with arthritis should be applied with caution. The possibility that those patients could be prone to develop RA cannot be ruled out. Those patients need careful clinical and radiological follow-up. Further large-scale studies are warranted.

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Keywords: anti-cyclic citrullinated peptide (anti-CCP); cryoglobulins; hepatitis C virus (HCV); rheumatoid arthritis (RA); rheumatoid factor (RF).

Introduction

Infection with hepatitis C virus (HCV) remains a severe life-threatening medical and public health problem worldwide (1). The highest prevalence has been reported from Egypt (2) where the main HCV genotype is type IV, representing 90% of the Egyptian HCV-infected population (3). It has emerged as a major cause not only of liver disease, but also of numerous extrahepatic conditions (4). Many rheumatologic manifestations were associated with HCV infection, some of which are related to mixed cryoglobulinemia, whose link to the HCV is now firmly established (5).

Among the different clinical disorders associated with HCV infection, articular involvement is a frequent complication. HCV arthropathy frequently displays a rheumatoid arthritis (RA)-like clinical picture, and most patients with RA-like HCV related polyarthritis fulfill the American College of Rheumatology (ACR) criteria for RA (6). However, compared to RA, HCV-associated arthritis is usually less severe and does not cause joint deformities or rheumatoid nodules (7). Because the classic clinical picture of RA is not entirely helpful in the differential diagnosis, other diagnostic tools such as the detection of serological abnormalities in sera of patients with RA, could be helpful in differentiating between these disorders (8).

The detection of classic immunoglobulin M (IgM) rheumatoid factor (RF) is of little utility as a diagnostic tool for RA because a high percentage of patients with chronic HCV infection display serum RF reactivity. Also, the frequency of RF increases in patients with articular involvement (9). During the last few years, a variety of circulating non-RF antibodies have been discovered and reported to have a potential diagnostic value (10, 11). Anti-cyclic citrullinated peptide (anti-CCP) antibodies are antibodies against synthetic citrullinated peptides, and are specific markers of RA (12). Anti-CCP antibodies are disease-specific and sensitive markers for RA. In addition to their diagnostic properties, anti-CCP antibodies have also been shown to be good prognostic markers, as they help predict the erosive or non-erosive progression of the disease and anti-CCP antibodies are usually present early in the course of disease (10). The present study aimed to examine the frequency and clinical significance of anti-CCP antibody in patients with chronic

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HCV infection, with and without manifestations of joint involvement, compared to patients with RA.

Materials and methods

Patients

All patients enrolled in this study were referred to the Rheumatology and Rehabilitation Department and Tropical Medicine Department of El-Kasr El-Aini Hospital, Cairo University, Egypt, from January 2006 to December 2007. All investigations were done in accordance with the Cairo University, health and human Ethical Clearance Committee guidelines for clinical researches. The local Ethics Committee approved the study protocol. All patients agreed to be enrolled in this study.

All participants underwent a general and locomotor clinical examination and gave a detailed history. Demographic, clinical, immunochemical, virological, and liver histology features were collected retrospectively for each patient. HCV antibodies were measured using a 3rd generation enzyme linked immunosorbent assay (ELISA) (Murex Biotech, Dartford, UK) and confirmed using reverse transcriptase polymerase chain reaction (RT-PCR) (Promega Co., Madison, WI, USA). Hepatitis B surface antigens (HBsAg) (Sorin Biomedica Co., Saluggia, Italy) were measured using commercially available kits. All patient groups were positive for HCV antibody and HCV-RNA.

Forty-seven HCV infected patients were enrolled. Two groups of HCV patients were identified on the basis of the presence of HCV-related arthropathy; Group I (20 patients with articular involvement) and Group II (27 patients without articular involvement). Patients with evidence of deformity, erosions on radiographs or subcutaneous nodules were excluded from the study. The control group for the diagnostic reliability of the anti-CCP antibody was comprised of 30 patients with a definitive diagnosis of RA as per ACR criteria (6). Controls were sero-negative for HCV.

Anti-CCP antibodies

Anti-CCP antibodies were measured using a commercial anti-CCP ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) according to the manufacturer's instructions. A cut-off value of five relative units was established, as recommended by the manufacturer's protocol. The cut-off value was established by evaluating 419 patients with RA, 744 patients without RA and 400 healthy controls. Values >5 units were considered positive for anti-CCP immunoglobulin G (IgG) antibodies. We considered the validation studies performed by the manufacturer to be sufficient and to preclude the need for use of a healthy control population in our study.

Rheumatoid factor (RF)

RF was determined using a latex fixation method. A suspension of uniform polystyrene particles sensitized in glycine buffer with heat-altered human IgG (BD Diagnostic Systems, Sparks, MD, USA) was incubated with progressive dilutions of human sera in microtitre wells. After incubation, the plates were visually inspected for agglutination.

Cryoglobulin detection

Venous blood was collected in a pre-warmed syringe and stored at 37°C until clotted. Serum was centrifuged at 37°C for 10 min at 2500 rpm (559 g), collected and stored at 4°C

for 7 days. After isolation and washing, the components of the cryoprecipitate were characterized by immunofixation (13).

Radiographic assessment

Plain X-rays for both hands and wrists were obtained on chronic HCV patients who had articular involvement at the time of anti-CCP antibody analysis. If erosions were present, the diagnosis of HCV related arthropathy was discarded, and the patients were now considered to have RA with concomitant HCV infection. The radiographs of all patients were reviewed by a senior rheumatologist.

Liver biopsy

Liver biopsy was performed whenever possible and assessed histopathologically (14).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 10 (LEAD Technology Inc., Charlotte, NC, USA) was used to analyze patient data. Descriptive statistics included the mean and standard deviation (SD) for quantitative variables, and number and percentage for qualitative variables. Unpaired t-test and Chi-square (χ^2), with Fisher exact test when appropriate, were used for statistical comparative analysis. A difference was considered to be statistically significant when the (p) value was ≤ 0.05 .

Results

Clinical and demographic data of studied population

We enrolled 47 HCV-infected patients, ranging from 20 to 57 years of age, and 28 being female. All had HCV genotype IV. Table 1 summarizes the demographic, clinical and serological characteristics of the HCV-infected patients, with and without articular involvement. Comparing HCV patients with articular involvement to those without articular involvement, no statistically significant differences were found between any of the variables mentioned in the table. Histological examination of hepatic biopsies showed grading of activity from minimum to severe, with no significant difference between patients with and without arthritis.

Table 2 shows the demographic, articular characteristics and erythrocyte sedimentation rate (ESR) levels of patients with RA, and of HCV patients with articular involvement. With respect to the pattern of articular involvement in HCV patients with articular involvement, all of them complained of polyarthralgias (20/20), three had mono-pauciarticular involvement of large joints with predominance of the knee, and nine had RA-like polyarthritides of the diarthroidal joints of the hands.

Mean anti-CCP antibodies level in patients studied

The mean for anti-CCP antibodies was 3.00 ± 5.64 U/mL (range 0.32–32.62) in the HCV group, while it was 19.84 ± 19.45 U/mL (range 1.3–75) in the RA

Table 1 Clinical, demographic and laboratory characteristics of HCV patients with and without articular involvement.

Variable	HCV with articular involvement n=20	HCV without articular involvement n=27	p-Value
Age, years ^a	34.10 ± 10.10	38.27 ± 10.86	NS
Female/male	12/8	16/11	NS
Sicca syndrome ^b	6 (30)	4 (14)	NS
Parathesia ^b	5 (25)	6 (22)	NS
Myalgias ^b	7 (35)	8 (29)	NS
Vasculitis ^b	7 (35)	5 (18.5)	NS
Raynauds ^b	4 (20)	4 (14.8)	NS
ESR, mm/1st h ^a	35.4 ± 13.7	29.32 ± 10.12	NS
AST, U/L ^a	51.64 ± 10.20	56.6 ± 12.70	NS
ALT, U/L ^a	51.09 ± 13.60	45.8 ± 19.50	NS
Serum albumin ^a	3.82 ± 0.47	3.54 ± 1.92	NS
Creatinine, μmol/L ^a	65.416 ± 10.608	60.996 ± 15.912	NS
Hemoglobin, g/L ^a	127 ± 11.3	132 ± 15.2	NS
WBCs, × 10 ⁹ /L ^a	5.60 ± 1.81	4.8 ± 1.42	NS
PLT, × 10 ⁹ /L ^a	271.09 ± 88.08	233.4 ± 75.6	NS

^aValues are mean ± SD; ^bvalues are n (%); HCV, hepatitis C virus; ESR, erythrocyte sedimentation rate; AST, aspartate transaminase; ALT, alanine transaminase; WBCs, white blood cells; PLT, platelets; NS, not significant.

Table 2 Demographic, articular characteristics and ESR levels of patients with RA and HCV patients with articular involvement.

Variable	RA patients n=30	HCV with articular involvement n=20
Age, years ^a	39.2 ± 10.6	34.10 ± 10.10
Duration of articular manifestations, years ^a	6.5 ± 4.9	2.89 ± 1.73
Morning stiffness > 1 h ^b	22 (73)	9 (45)
Arthritis of 3 or more joint areas ^b	28 (93.3)	9 (45)
Symmetric arthritis ^b	28 (93.3)	7 (35)
Subcutaneous nodules ^b	3 (10)	0
Deformity ^b	14 (46)	0
Radiological erosions ^b	11 (36)	0
ESR, mm/1st h ^a	42 ± 24	35.4 ± 13.7

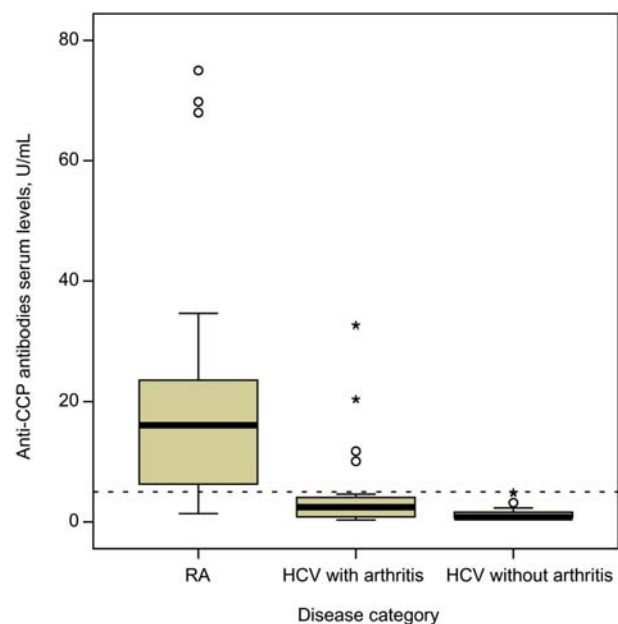
^aValues are mean ± SD; ^bvalues are n (%); ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; HCV, hepatitis C virus.

group ($p < 0.001$). Mean levels were highly significant ($p < 0.001$) when comparing the RA group to the HCV patients with arthritis (5.22 ± 8.13 U/mL). In addition, a significant difference ($p < 0.05$) was found when comparing mean anti-CCP antibodies in patients with HCV and articular involvement to those without articular involvement (1.36 ± 1.18 U/mL). Figure 1 shows the anti-CCP antibody levels for the various disease categories.

Prevalence of anti-CCP antibodies, RF, and cryoglobulins in studied patients

Anti-CCP antibodies were positive in only four patients, representing 8.5% of the HCV-infected group. Three patients tested positive for both anti-CCP antibodies and RF, and had symmetric polyarthritis (RA-like). Their ages ranged from 36 to 52 years and their duration of disease (with respect to arthritis) ranged from 4 to 6 years. Table 3 summarizes the characteristics of HCV patients with elevated anti-CCP antibody levels.

The prevalence of anti-CCP antibody positive patients increased to 20% (4/20) in patients having chronic HCV and articular involvement, while the prevalence was zero in chronic HCV patients without articular involvement. This finding was statistically significant ($p < 0.05$). Anti-CCP antibody level was positive in 70% of the RA patients. A significant difference ($p < 0.001$) was found between the frequency of anti-CCP antibodies between the RA group and chronic HCV patients with arthritis. RF was positive in 76% of RA patients, whereas it was found in 60% of patients affected by HCV with articular involvement (12/20); this difference was statistically insignificant. However, a statistically significant difference ($p < 0.05$) was

**Figure 1** Serum anti-CCP antibody levels in the various disease categories.

Data are presented as box plots with lines inside the box representing medians, boxes representing the 25th and 75th percentiles and the lines outside the boxes indicating the 10th and 90th percentiles.

Table 3 Characteristic of HCV patients with elevated anti-CCP antibody levels.

Patient no.	2	7	8	14
Age, years	36	43	52	43
Sex	M	F	F	F
Arthralgia	Y	Y	Y	Y
Duration of arthritis, years	4	6	4	4
RA like arthritis	Y	N	Y	Y
Pauciarticular arthritis	N	Y	N	N
Sicca syndrome	N	Y	Y	Y
Parathesia	N	N	Y	N
Myalgia	Y	N	N	Y
Vasculitis	Y	N	N	Y
Raynauds phenomenon	Y	Y	N	N
ALT, U/L	52	34	79	46
AST, U/L	45	61	54	59
ESR, mm/1st h	20	48	35	52
RF	Y	N	Y	Y
Cryoglobulin	N	Y	Y	N
Anti-CCP antibodies	10.09	11.77	32.62	20.30

Y, yes; N, no; M, male; F, female; ALT, alanine transaminase; AST, aspartate transaminase; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptides.

found when comparing the frequency of RF in HCV patients with arthritis to those without arthritis. Cryoglobulins were found in 14 of the chronic HCV-infected patients (29%), and in five patients with RA (16%). Although cryoglobulins were more frequent among chronic HCV patients with articular affection (35%) compared to those without affection (26%), the difference was not statistically significant. Figure 2 shows the prevalence of anti-CCP antibodies, RF and cryoglobulins in RA and HCV-infected patients.

Discussion

The articular manifestations of chronic HCV and RA may be very similar, and the prevalence of RF is the same in the two conditions (15). Numerous studies

have corroborated the high specificity (>95%) of the anti-CCP assay for the diagnosis of RA, and its association with erosive inflammatory joint disease has added to the prognostic value of anti-CCP antibodies (10). However, the control sera used to assess the specificity of this assay were derived primarily from normal subjects and patients with a wide variety of rheumatic diseases; patients with HCV were not well-represented (10). Thus, the objective of our study was to assess the presence of anti-CCP antibodies in patients with HCV infection, with and without manifestations of joint involvement, in comparison to patients with RA.

Van Boekel et al. (16) concluded that anti-CCP antibodies had the most value for diagnosis of RA. Despite the high specificity described for the anti-CCP antibody test, four (i.e., 8.5%) patients from the HCV-

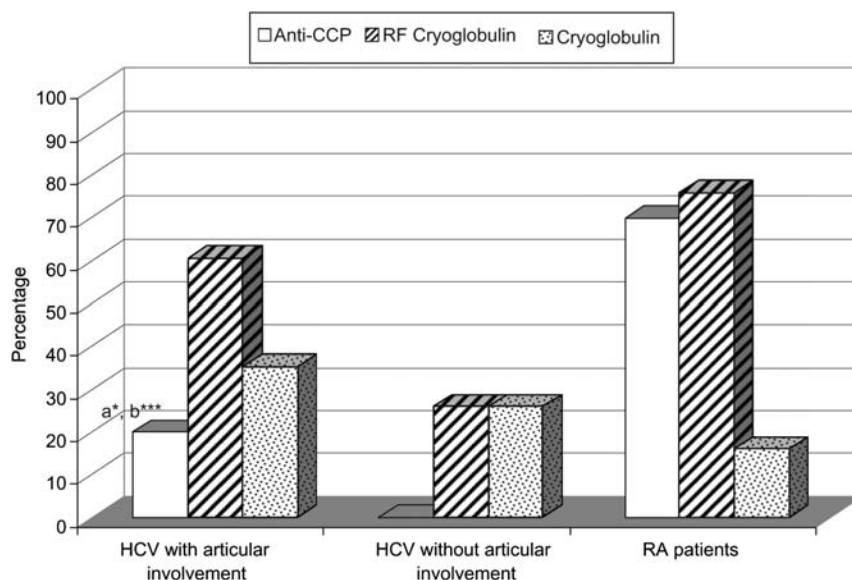


Figure 2 Prevalence of anti-CCP antibody, RF and cryoglobulins in RA and HCV-infected patients.

(a) Statistically significant from HCV patients without articular involvement. (b) Statistically significant from RA patients. * $p < 0.05$; *** $p < 0.001$.

infected group were positive for anti-CCP antibodies. The prevalence increased to 20% (4/20) when considering only HCV with articular manifestations, while anti-CCP antibodies were absent in patients without articular involvement. On the other hand, the frequency of cryoglobulins and RF was comparable between HCV patients and RA patients in our patient cohort. The previously reported prevalence of anti-CCP antibodies among patients with HCV ranged from none to 9%, depending on the population studied and whether it was associated with cryoglobulinemia (17–20). For example, Sène et al. (17) found anti-CCP antibodies positive in 5.7% of HCV-infected patients with arthralgia, whereas they were present in 78% of patients with RA. Another study detected anti-CCP antibodies in 6.9% of patients with mixed cryoglobulins (18). Similarly, Liu et al. (19) detected a significant number of HCV-infected patients with RF and cryoglobulinemia who were also positive for anti-CCP antibodies. On the other hand, Girelli and colleagues (20) reported that RF was present in 91% of patients with RA and 86% of the patients who had HCV-related arthritis, whereas anti-CCP antibodies were seen in 71% and 0% of patients, respectively.

Although our study reconfirms the association of anti-CCP antibodies with RA (present in 70%), the number of our patients with HCV arthritis who have anti-CCP antibodies is greater than would be expected with respect to the high specificity of the test (16). The high prevalence of anti-CCP antibodies might reflect ethnic differences or different genotypes of the HCV virus (i.e., IV) found in our patients, which differ compared to patients evaluated in other studies. It has been suggested that chronic HCV with genotypes IV through IX may exhibit epidemiologic, clinical, and treatment outcomes different from those for genotypes I, II, or III (21).

The synthesis of citrullinated proteins has been shown to increase as a result of both apoptosis and inflammation-induced cellular death (necroinflammation). This suggests that this process is inflammation-dependent rather than disease-dependent (22, 23). Moreover, the presence of anti-CCP antibody has been reported, in low prevalence, in association with systemic lupus erythematosus, systemic sclerosis, polymyositis (24), Sjögren syndrome (25) and psoriasis (26, 27). Recently, a relatively high percentage (9%) of patients with anti-CCP antibody was detected in a large group of individuals with autoimmune hepatitis, but without apparent overlap with RA (28).

The basis for the association of anti-CCP antibodies with HCV arthritis in our study of patients with chronic HCV is speculative. It might be a part of the heightened immune reactive state characteristic of HCV infection, in which epitope spread and molecular mimicry result in multiple serological reactivities, or concurrent immune disorders with the production of a wide range of autoantibodies produced against liver- and non-liver-specific autoantigens (29–31).

Extravascular citrullinated fibrin is one of the citrullinated proteins that trigger production of anti-CCP antibodies in patients with RA. HCV infection can

cause chronic liver injury with the release of large amounts of fibrin and fibrin fragments that can be citrullinated. These citrullinated antigens may then activate CD4+ T helper cells and stimulate production of anti-CCP (30). The presence of mRNA of peptidyl-arginine deiminase (PAD) enzyme in fetal liver, with the occurrence of citrullination processes within this organ, and subsequent release of liver-derived substrates could lead to the generation anti-CCP antibodies (32).

To summarize, our results suggest that anti-CCP antibodies may be present in HCV patients presenting with articular manifestations, but must be interpreted with caution. However, it is possible that patients with anti-CCP antibody could be prone to develop RA. It is well known that anti-CCP antibodies can be present years before the first sign of RA. Careful clinical and radiological follow-up is required to confirm the absence of evolution to RA in anti-CCP antibody positive patients. Further large-scale studies are warranted.

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