Anti-Cyclic Citrullinated Peptide Frequency in Egyptian Patients with Chronic Hepatitis C Virus Infection with and without Articular Manifestation

M.Elnadry[1], M. Abdel-Rashed[2], M.A.Abdel-Monem[2], S.El-Eter[3] and W. Fathy[4]

Tropical M.[1], Internal M [2], Rheumatology M. (Girls) [3] Al-Azhar University, Clinical Pathology Elmenofia University[4]
prof_nadry@yahoo.com

Abstract: Background. The presence of extra hepatic manifestations is a relatively common feature in patients with chronic hepatitis C. Besides the role in the etiology of liver diseases, this virus is known to produce autoimmune phenomena. Among them, rheumatic diseases. **Aim of the work.** Our aim in the present study was to investigate the presence of anti-CCP antibodies in Egyptian patients with chronic hepatitis C virus infection, with and without articular manifestation. **Patients and Methods.** Blood samples were obtained from 86 patients with HCV from the Rheumatology, Internaland Tropical Medicine outpatient clinicsof Elzhraa, Elhussen and Bab Elshaaria University hospitals Alazhar University Cairo Egypt.. A history was taken from all patients, and a clinical examination was performed, including a musculoskeletal examination, abdominal ultrasonography, and laboratory investigations in the form of routine laboratory tests (CBC, ESR, LFT, RFT, viral marker, and tests for RF and anti-CCP antibodies). **Results** The studied group included 86 patients classifiedinto three groups:, Group I (HCV and RA) included 29 (33.7%) patients 15 females and 14 males, with the mean age of 44.6 ± 8.79 years. The disease duration among this group was less than one year in 12 patients and more than one year in 17 patients Group II (HCV with articular manifestation) included 31 (36 %) patients 12 females and 19 males, with the mean age of 42.5 ± 8.07years, The disease duration among this group was less than one year in 11 patients and more than one year in 20 patients. Group II (HCV without articular manifestation) included 26 (30.2%) patients 8 females and 18 males, with the mean age of 45.0 ± 9.10years, The disease duration among this group was less than one year in 20 patients and more than one year in 6 patients. Regarding to clinical exam 22(75.9%) and 10 (32.3%) of our patients in group I & II respectively presented with morning stiffness while no patients experienced morning stiffness in group III (p 0.001). 23 (79.3%) and 5 (16.1%) experienced symmetric arthritis in group I & II respectively while one patients (2.7%) experienced symmetric arthritis in group III ($p< 0.001$). Regarding to the X ray finding 19 (65.5%) and 8 (25.8%) patients in group I & II respectively showed erosions on X ray finding while no patients with erosions were found in group III ($p<0.001$).The mean ESR 1st hour were 40.55 ± 12.6 mm/h, 13.90 ± 3.5 mm/h and 13.19 ± 5.6 mm/h in group I,II, and III respectively. There was a statistically significant increases in the level of ESR 1ST hour in group I compared to group II and III.Regarding to the rheumatoid factor (RF) level the mean level were 32.2±19.6 U/L, 28.9±16.7 U/L and 26.1±15.5 U/L in group I, II and III respectively with no statistical significant differences between the groups ($p< 0.4$).The mean level of anti-CCP antibodies was significant higher (55.8±6.3 U/mL) in group I, compared to (21.28±3.4 U/mL) in group II and (15.5±2.7 U/mL) in group IIIp (0.001). **Conclusions** The distinction between HCV associated arthropathy and rheumatoid arthritis has great relevance for clinicians. Our results showed that antiCCP antibodies were a significant marker for diagnosis of HCV associated with RA and rarely present in HCV infected patients with rheumatoid like manifestations and were a reliable serological marker to discriminate between patients with HCV associated rheumatological manifestations and patients with rheumatoid arthritis.

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1. Introduction

Arthralgia is one of the most common extra-hepatic manifestations in patients with hepatitis C virus (HCV) infection or HCV-related cryoglobulinemia[1, 2]. In addition, associations of rheumatoid arthritis (RA) in autoimmune liver diseases, such as primary biliary cirrhosis (PBC), have also been observed [3]. The serological test routinely used is the determination of the IgM rheumatoid factor (RF) which constitutes one of the classification criteria proposed by the American College of Rheumatology (ACR) [4]. Many autoantibodies, including RF, are common in HCV-infected patients. In the light of these features, the distinction between liver disease-associated arthropathy and the occurrence of rheumatoid arthritis may be difficult.Therefore, the detection of classical RF is of little utility as a diagnostic tool because a high percentage of patients with HCV infection or autoimmune liver diseases have been shown to display serum RF reactivity [5].

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) [6] 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, Behcet's disease, and psoriatic arthritis [7]. 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. [8]

Our aim in the present study was to investigate the presence of anti-CCP antibodies in chronic hepatitis C Egyptian patients with and without articular manifestation

2.Patients and Methods

Blood samples were obtained from 86 patients with HCV from the Reumatology and Tropical Medicine outpatient clinic of Elhussien and Bab Elshaaria University hospital Al-azhar University Cairo Egypt.Patients were consecutively included in the study in the period from March 2011 to November 2012. 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.. 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 (CBC, ESR, LFT,RFT). Viral marker, tests for Rheumatoid factor (RF), and anti-CCP antibodies (Anti CCP). 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. 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. The serum concentration of RF were considered negative when the value were less than 20 U/mL, Values more than20 U/mL were considered positive. 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. 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/mL were considered "weakly" positive, 40-59 U/mL "moderately" positive, and 60 U/mL "strongly" positive. The patients will planned to divided into three groups:GroupI: included 29 patients who were suffered of RheumatoidArthritis with HCV infection, Group II: included 31patients with HCV infection and RA like manifestation andGroup III: included 26 patients with HCVinfection without articular manifestation.Data were analyzed using SPSS software (version 16, SPSS Inc., USA) and chi square and one way ANOVA test were used, when appropriate.

3.Results

The studied group included 86 patients classified into three groups, Group I (HCV and RA) included 29 (33.7%) patients 15 females and 14 males, with the mean age of 44.6 ± 8.79 years. While the disease duration among this group was less than one year in 12 patients and more than one year in 17 patients. Group II (HCV with articular manifestation) included 31 (36 %) patients 12 females and 19 males, with the mean age of 42.5 ± 8.07years, While the disease duration among this group was less than one year in 11 patients and more than one year in 20 patients. Group III (HCV without articular manifestation) included 26 (30.3%) patients, 8 females and 18 males, with the mean age of 45.0 ± 9.10 years. While the disease duration among this group was less than one year in 20 patients and more than one year in 6 patients. There was no statistically significant differences between the three groups regarding age, sex and disease duration (Table 1).

Table (1) Patients Characterization In Studied Groups

Studied Groups	NO (%)	Age Mean ± Sd	Gender		Disease Duration	
			Male	Female	Less than one year	More than one year
GROUP I	29 (33.7%)	44.6 ± 8.79	14 (48.3%)	15 (51.7%)	12 (41.4%)	17 (58.6%)
GROUP II	31 (36 %)	42.5 ± 8.07	19 (61.3%)	12 (38.7%)	11 (35.5%)	20 (64.5%)
GROUP III	26 (30.2%)	45.0 ± 9.10	18 (69.2%)	8 (30.8%)	20 (76.9%)	6 (23.1%)

Based on clinical exam 22(75.9%) and 10 (32.3%) of our patients in group I & II respectively presented with morning stiffness while no patients experienced morning stiffness in group III (p<0.001). 23 (79.3%) and 5 (16.1%) experienced symmetric arthritis in group I & II respectively while one patients(2.7%) experienced symmetric arthritis in group III(p<0.001). Regarding to the X ray finding 19 (65.5%)and 8 (25.8%)patients in group I & II respectively showed erosions on X ray finding while no patients with erosions were found in group III (p<0.001). (Table 2)

Table (2)Comparison Between The Studied Groups As Regard Clinical Manifestation And X Ray Finding

	Group I No. 29	Group II No. 31	Group III No. 26	*p* Value
Morning Stifness				
Present	22(75.9%)	10 (32.3%)	0 (.0%)	0.001
Absent	7 (24.1%)	21 (67.7%)	26 (100.0%)	0.001
Symmetric Arthritis				
Present	23 (79.3%)	5 (16.1%)	1 (2.7%)	0.001
Absent	6 (20.7%)	26 (83.9%)	25 (96.2%)	0.001
X Ray Finding				
Erosion	19 (65.5%)	8 (25.8%)	0 (.0%)	0.001
No Erosion	10 (34.5%)	23 (74.2%)	26 (100.0%)	0.001

As regard to laboratory investigation the mean Hg level were 10.7±1.5 g/dL 11.3± 1.5 g/dL and 11.7± 1.4 g/dL in group I,II, and III respectively. The mean WBCs count were 7.4 ±1.7 ×10"3,7.5±1.7 ×10"3 and 7.1±1.6 ×10"3 in group I,II, and III respectively. The mean platelet count were 164.8 ± 40.8,175.1± 63.7 and159.8± 38 in group I,II, and III respectively, with no statistically significant differences among the three groups regarding to Hg, WBCs, and platelet count. The mean level of ALT were 36.96 ±14.6 U/L, 43.3±21.2 U/L and 56.7±19.9 U/L in group I,II, and III respectively. The mean s. bilirubin were 1.3±0.5 mg/dL, 1.4±0.52 mg/dL and 1.35±0.53 mg/dL in group I,II, and III respectively, with no statistically significant differences among the three groups (p=0.78).Also the mean prothrombin concentration were 86.2±10.3 %, 87.1±10.3% and 87.13±10.46% in group I,II, and III respectively, with no statistically significant differences among the three groups(p=0.92). The mean ESR 1st hour were 40.55 ± 12.6 mm/h, 13.90 ± 3.5mm/h and 13.19 ± 5.6 mm/h in group I,II, and III respectively. There was a statistically significant increases in the level of ESR 1st hour in group I compared to group I and II (p=0.001).Regarding to the rheumatoid

factor (RF) level the mean level were 32.2±19.6 U/L, 28.9±16.7 U/L and 26.1±15.5 U/L in group I, II and III respectively with no statistical significant differences between the groups (p0.4).The mean level of anti-CCP antibodies was significant higher (55.8±6.3 U/mL) in group I, compared to (21.28±3.4 U/mL) in group II and (15.5±2.7 U/mL) in group III (p 0.001)Table (3)

Table (3)Comparison Between The Studied Groups As Regard laboratory investigations

PARAMETER	Group I No. 29	Group II No. 31	Group III No. 26	*p* Value
ALT (U/L)	36.96 ±14.6	43.3±21.2	56.7±19.9	0.001
S.BILIRUBIN (mg/dl)	1.33±0.5	1.4±0.52	1.35±0.53	0.78
PROTHROMBIN conc. (%)	86.2±10.3	87.1±10.3	87.13±10.46	0.92
HCV RNA (IU)	$7.5\pm1.02\times10^5$	$6.9\pm7.7\times10^5$	$5.2\pm7.8\times10^5$	0.61
ESR 1st (mm/h)	40.55 ± 12.6	13.90 ± 3.5	13.19 ± 5.6	0.001
ESR 2nd (mm/h)	65.3±12.5	28.06±7.4	23.3±7.3	0.001
RF (U/L)	32.2±19.6	28.9±16.7	26.1±15.5	0.4
AccP (U/mL)	55.8±6.3	21.28±3.4	15.5±2.7	0.001

4.Discussion

Chronic HCV is associated with immunological abnormalities including circulating immune complexes. production of auto antibodies. Both viral and host factors may contribute to the development of auto antibodies.[9]Differentiation between patients

with RA and those with HCV related arthropathy has great relevance in clinical practice to establish the aggressive treatment. to prevent joint erosions in patients with true RA and to reduce the risk of immunosuppression therapy in patient with HCV - related anthropopathy. [10]

Rheumatoid factor test may be positive in other rheumatic diseases and infectious diseases as chronic hepatitis infection. Also. RF has two important limitation firstly patients with other diseases may express RF positive yielding a limited specificity for RA. and secondly RF is not stable. So usefulness of RF to differentiate between RA patients and HC'V related arthritis patients is of limited value.[11]. Anti-CCP were also described in some infectious diseases, especially chronic hepatitis C (CHC). CHC can be associated with many extra hepatic manifestations including rheumatologic. The most common rheumatologic one is polyarthralgia. Moreover, RF is commonly detected in CHC. Therefore, distinction between CHC and RA at onset may be difficult. [12]

Antibodies were largely overcome after the discovery of citrulline residues and subsequent development of an immune enzymatic tests using anti CCP antibodies. The assay of anti-CCP has been developed in recent years. Anti CCP is useful in pre-clinical and early diagnosis of RA. It is also important for prediction of disease severity and radiographic joint damage. The development of test for anti CCP has increased the possibility of distinguishing between RA and other causes of arthritis(13).

On studying the articular manifestations in our patients. we found that 22(75.9%) patients of group I were presented with morning stiffness of more than one year,10 (32.3%) patients of group II were presented with the morning stiffness of more than one year. While no patients presented with the same complaint in group III (0%), the results is in agreement with Elbordeny*etal.,*2008 who studied who studied 60 HCV infected patients with different rheumatological manifestations, 80% was presented with HCV and rheumatoid arthritis, while 65% of HCV patient presented with rheumatoid like manifestation due to chronic HCV infection [14]. The difference in our results in group II due to different age group. Jadali, *et al.,*2010 reported that HCV-related arthritis usually manifests as rheumatoid-like, symmetrical inflammatory polyarthritis. The joints involved in HCV-related arthritis are similar to RA. In patients with chronic HCV infection, there is a well-defined picture of arthritis accompanied by the presence of mixed cryoglobulinemia that may produce an intermittent mono or oligoarticular, nondestructive arthritis involving large and medium size joints. 2% to 20% of HCV-infected patients experience arthritis and as 50% experience arthralgia. [15]

Positive Xray finding in the form of joint erosion was highly significant in the group I 19 (65.5%) patients compared to 8 (25.8%) patients in group II while no positive Xray finding in group III, This was in agreement with Zucherman. *et al.,*who studied 185 HCV infected patients with different rheumatological manifestations. Nearly the same results were reported by Keren*et al.,*who found 50% of HCV related arthropathy patients had 4 ACR criteria for diagnosis of RA. [16]

RF was higher in group I (32.2±19.6) compared to (28.9±16.7),and (26.1±15.5) in group II and III respectively, with no significant differences between the groups, the results agree with several studies indicated a high prevalence of HCV infection in patients with rheumatoid arthritis (RA) as a prototype for rheumatic diseases. For example in one study 23 (7.6%) of RA patients had HCV antibodies, and 7 (2.3%) had active infection by HCV. However, opposite results were also reported. [17] Some of these investigations indicated that HCV may trigger the progression of RA particularly in genetically susceptible individuals.[18] In addition, elevations of RF have been described in patients infected with the HCV. In particular, a positive IgM RF was detected frequently (>60%) in patients with HCV-related arthropathy.[19]. 2% to 20% of HCV-infected patients experience arthritis and as 50% experience arthralgia.Differentiation between true RA and HCV-related arthritis may be complex. HCV-related arthritis usually shows a fairly benign course that, in contrast to true RA, is characteristically non deforming and is not associated with articular bony erosions. Furthermore, unlike classic RA [15]. The mean ESR (1st and 2nd hour) was significantly high in group I compared to groups II and III. Based on different studies, 40-74% of HCV infected patients may experience other complications during the course of the disease that are principally immunological. [20, 21]

Sene*et al.*(2006)[22] detected that RF is not specific tool, as many cases of recent onset RA were seronegative while many cases of rheumatoid like conditions such as HCV infection may be seropositive. Koga,2007[23] also reported that RF can be detected in from 50~80% of RA patients, however, it is also detected in patients with either other autoimmune diseases or HCV infection, as well as even in normal healthy subjects.[23].

In our study. there was a statistically significant increased level of anti CCP among patients of group I (55.8±6.3)compared to patients in groups II and III, 21.28±3.4 and 15.5±2.7 respectively. Anti-CCP was

not detected in any of the 26 sera from patients of group III.

Anti-CCP antibodies were detected in all patients with group I and the mean values of anti-CCP antibodies in that group were markedly elevated, the results agree with that is reported with KOGA *et al.,*2007 who reported that No sera with elevated anti-CCP were found in the patients with HCV infection without articular manifestation, The seropositivity for anti-CCP in autoimmune disease patients was associated with a high frequency of RA association [23]

Bizzaro*et al.,*2013 reported that the presence of anti-CCP antibodies, at both low and high concentration, is significantly associated with RA development in subjects with recent onset undifferentiated arthritis. However, time interval from the onset of the first symptoms to the fulfillment of the classification criteria appears to be directly related to the initial anti-CCP level. [24]

In our study we demonstrated a significant higher titer of anti-CCP in RA with HCV patients, while in HCV patients with and without articular manifestation (groups I& II) there was no significant elevation in the anti-CCP titer while RF level was higher (non-significant) in group II. It appears that anti-CCP positivity was better than RF to predict the progression of rheumatoid arthritis, the results agree with Kroot*et al.,*in a study of patients with rheumatoid arthritis, found that anti-CCP positive patients at follow up had developed significantly more radiological damage than patients with this antibody [25]. in concordance with Meyer *et al.,*who found that higher predictive values for radiological damage in patients with positive tests for both anti-CCP and RF than those in patients who were positive for only one of two tests [26].

Cacoub*et al.,*reported that chronic HCV infection is associated with a number of extra hepatic manifestations, of which rheumatologic complaints are the most common. In a large, prospective study of 1,614 HCV-infected patients, arthralgia's were the most common complaint, with a reported prevalence of 23%.3 A symmetric, inflammatory polyarthritis primarily involving small joints, which resembles rheumatoid arthritis (RA), has been described in association with HCV infection. [27] To conclude the distinction between HCV associated arthropathy and rheumatoid arthritis has great relevance for clinicians. Our results showed that anti - CCP antibodies were a significant marker for diagnosis of HCV associated with RA and rarely present in HCV infected patients with rheumatoid like manifestations and were a reliable serological marker to discriminate between patients with HCV associated rheumatological manifestations and patients with rheumatoid arthritis.

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