ORIGINAL ARTICLE

Frequency of serological markers of rheumatoid arthritis in patients with chronic hepatitis C

Fréquence des marqueurs sérologiques de la polyarthrite rhumatoïde chez des patients ayant une hépatite C chronique

Mariam Ghozzi[1,2,3]
Amani Mankai[4,5]
Ouafa Kallala[3,6],
Sarra Melayah[1,2,7]
Zeineb Chedly[2]
Fatma Mechi[2]
Abdelhalim Trabelsi[3,6]
Ibtissem Ghedira[1,2]

1 Laboratory of Immunology, Farhat Hached University Hospital, Sousse, Tunisia
2 Faculty of Pharmacy, Department of Immunology, University of Monastir, Monastir, Tunisia
3 Research Laboratory for «Epidemiology and Immunogenetics of Viral Infections» (LR14SP02), Sahloul University Hospital, University of Sousse, Sousse, Tunisia
4 High School of Sciences and Techniques of Health, Tunis El Manar University, Tunis, Tunisia
5 Research Unit «Obesity: Etiopathology and Treatment, UR18ES01», National Institute of Nutrition and Food Technology, Tunis, Tunisia
6 Faculty of Pharmacy, Department of virology, University of Monastir, Monastir, Tunisia
7 LR12SP11, Biochemistry Department, Sahloul University Hospital, Sousse, Tunisia

Corresponding author :
Mariam Ghozzi, Associate Professor in Immunology,
Address : Laboratory of Immunology, Farhat Hached Hospital, Rue Ibn El Jazzar, 4000 Sousse, Tunisia.
Tel : (216) 97305444, Fax: (216) 73226702,
E-mail: mariam_80@hotmail.fr

Abstract
Background : Viral hepatitis C is a chronic liver disease caused by hepatitis C virus (HCV) which can trigger autoimmune responses. We aimed to investigate the frequency of rheumatoid factor (RF) and anti-cyclic citrullinated peptides autoantibodies (CCP-Ab) in Tunisian patients with chronic hepatitis C (CHC).
Materials and Methods : Sera of 88 patients with CHC were collected over a period of two years. RF and CCP-Ab were performed by indirect ELISA. Ninety healthy blood donors (HBD) served as control group.
Results : Serological markers of RA were more frequent in CHC patients than in HBD (50% versus 7.8%, $p<10^{-6}$). CCP-Ab were significantly more frequent in patients with CHC than in HBD ($p=0.03$). RF were significantly more frequent than CCP-Ab in patients with CHC ($p<10^{-5}$). CCP-Ab or RF were significantly more frequent in female patients than in male patients ($p=0.03$). RF-IgA and CCP-Ab levels were positively correlated with age (r=0.317, $p=0.01$ and r=0.353, $p=0.005$ respectively). RA serological markers were significantly more frequent in patients with cirrhosis than in patients without cirrhosis ($p=0.03$).
Conclusion : The current study has shown that CHC is associated with a high frequency of serological markers of rheumatoid arthritis.
Keywords : chronic hepatitis C, rheumatoid arthritis, rheumatoid factor, anti-cyclic citrullinated peptides autoantibodies

Résumé
Introduction : L'hépatite virale C est une maladie chronique du foie causée par le virus de l'hépatite C (VHC) qui peut déclencher des réponses auto-immunes. Dans ce travail, nous avons cherché à étudier la fréquence des auto-anticorps de la polyarthrite rhumatoïde (PR) (le facteur rhumatoïde (FR) et les anticorps anti-peptides cycliques citrullinés (Ac anti-CCP)) chez des patients tunisiens atteints d'hépatite C chronique (HCC).
Matériel et Méthodes : Les sérums de 88 patients atteints d'HCC ont été collectés sur une période de deux ans. Le FR et les Ac anti-CCP ont été réalisés par ELISA indirecte. Quatre-vingt-dix donneurs de sang sains (DSS) ont servi de groupe témoin.
Résultats : Les marqueurs sérologiques de la PR étaient plus fréquents chez les patients atteints d'HCC que chez les DSS (50 % contre 7,8 %, $p < 10^{-6}$). Les Ac anti-CCP étaient significativement plus fréquents chez les patients que chez les sujets sains (p = 0,03). Le FR était significativement plus fréquent que les Ac anti-CCP chez les patients atteints d'HCC ($p < 10^{-5}$). Les Ac anti-CCP ou le RF étaient significativement plus fréquents chez les femmes que chez les hommes (p = 0,03). Les taux de FR-IgA et d'Ac anti-CCP étaient positivement corrélés à l'âge (r = 0,317, p = 0,01 et r = 0,353, p = 0,005 respectivement). Les marqueurs sérologiques de la PR étaient significativement plus fréquents chez les patients atteints de cirrhose que chez les patients sans cirrhose (p = 0,03).
Conclusion: L'étude actuelle a montré que l'HCC est associée à une fréquence élevée de marqueurs sérologiques de la PR.
Mots-clés : hépatite C chronique, polyarthrite rhumatoïde, facteur rhumatoïde, anticorps anti-peptides cycliques citrullinés.

INTRODUCTION

Autoimmunity is a pathophysiological phenomenon that occurs when the immune system mistakenly targets and attacks the body's own tissues and cells (1). Viral hepatitis C is a chronic liver disease caused by hepatitis C virus (HCV) which can trigger autoimmune responses (2). Patients with HCV infection have higher levels of autoantibodies than healthy people (3). These autoantibodies can target various self-antigens, such as the smooth muscle cells, liver-kidney microsomal antigen, mitochondrial antigens, nuclear antigens... (2). In fact, it is believed that viral proteins, such as the core protein and nonstructural proteins, can trigger an immune response against self-antigens because of the structural mimicry (4). In addition, chronic hepatitis C (CHC) is associated with decreased Treg counterbalancing Th17. Treg/Th17 imbalance is known to be associated with inflammatory conditions and various autoimmune diseases (5).

In the other hand, arthritis is one of the most common extrahepatic manifestations of HCV infection. In fact, up to 23% of patients with chronic hepatitis C developed arthritis at some point in their disease course (6). The type of arthritis associated with HCV infection is usually a symmetric polyarthritis. The symptoms of this arthritis may include pain, stiffness, swelling, and redness in the joints, and it can be quite debilitating for some patients (6). It is hard to distinguish arthropathy seen in HCV infection from an initial presentation of rheumatoid arthritis (RA) (7-9). Rheumatoid factor (RF) and anti-cyclic citrullinated peptides autoantibodies (CCP-Ab) are produced by the immune system and are commonly associated with RA.

In the literature, only one study reported the frequency of serological markers of RA (CCP-Ab and RF-IgM, RF-IgG and RF-IgA) in CHC (10).

Taking into account all of the above, we aimed to perform a serological screening of RA in patients with CHC and to try to explain this association.

MATERIALS AND METHODS

Study participants

Sera of eighty-eight patients with CHC were collected over a period of two years from a hospital of the center of Tunisia. Laboratory tests performed for diagnosis of CHC included HCV RNA evaluation and anti-HCV antibodies detection. Ninety age- and sex-matched healthy blood donors (HBD) served as control group. None of study participants had autoimmune disease. All serum samples were stored at -80°C until use. The study was approved by the ethics committee of our hospital.

Methods

HCV serological markers detection

Electrochemiluminescence immunoassay automated on Cobas Elecsys (Roche Diagnostics®, GmbH, Mannheim, Germany) was performed to detect anti-HCV antibodies. Recombinant nucleocapsid peptides, antigens and NS3 and NS4 proteins, were used in the elecsys anti-HCV II test to measure anti-HCV antibodies. Positive results were confirmed by Monolisa™ HCV Ag-Ab ULTRAV2 (BioRad®, Marnes-La-Coquette, France) which is a qualitative immunoenzymatic test for the detection of HCV infection based on the detection of anti-HCV antibodies using the most common HCV genotypes such as NS3, NS4A, NS4B and NS5A from genotypes 1a and 1b and NS3 and NS4 regions of genotypes 2 and 3a.

Quantification of plasmatic viral load

We used Cobas®AmpliPrep/Cobas® TaqMan HCV test (Roche Diagnostics®, GmbH, Mannheim, Germany) to determine RNA levels. It is a highly sensitive test that accurately detects HCV genotypes 1 through 6 with fully automated sample extraction and real-time PCR amplification, detection and quantification. The detection limit was 15 international units (IU)/mL.

Serological markers of RA detection

Rheumatoid factor

RF was performed using a commercial ELISA kit (Orgentec Diagnostika). Sera diluted at 1/101 were incubated in the wells coated with Fc fragment of human immunoglobulin (Ig) G1 for 30 minutes. RF in positive sera was bound to the antigen. After a washing step, peroxidase-labeled anti–human IgM, IgG, or IgA was added. A second washing step was performed after 15 minutes of incubation. Finally, the addition of an H2SO4 solution stopped the reaction, generating a yellow color whith an intensity proportional to the concentration of RF the serum. Optical density of the color intensity was measured photometrically, at a wavelength of 450 nm. The cutoff values for positivity were 49.5 U/ mL for RF-IgG, 45.5 U/mL for RF-IgA, and 31 IU/mL for RF-IgM (11).

Anti-cyclic citrullinated peptide autoantibodies

Serum levels of CCP-Ab (IgG) were evaluated by a second generation ELISA test (Orgentec Diagnostika®, Mainz, Germany). Wells of microplate were sensitized by highly purified cyclic citrullinated vimentin peptides. CCP-Ab present in sera of patients bind to the antigen coated on the surface of wells. A washing step was performed after incubation. Then enzyme conjugate was added and binds to the formed immune complexes. After 30 minutes incubation step, a second washing step removes unbound enzyme conjugate. The addition of substrate solution causes a blue color to appear. To stop

the reaction, an acid solution was added. The intensity of the obtained yellow color was measured photometrically at 450 nm. The cut off for positivity was 20 RU/ml.

Statistical analysis

Frequencies of CCP-Ab and RF in patients and healthy blood donors were compared using open EPI version 3 as software. Chi-square test or Fisher's exact test were used. A *p*-value lower than 0.05 was considered statistically significant. Comparison of the means of the quantitative variables was carried out using the Student's test. 95% confidence interval and odds ratio were determined for significant differences by WinPepi software. Correlations were determined by Spearman's test using IBM® SPSS® Statistics.

RESULTS

The present study included 88 patients with CHC (mean age: 56.67±13.4 years; range: 27-84 years), 51 (58%) were women and 37 (42%) were men. Forty-seven patients were under treatment. Forty-one patients were not treated yet. Twenty-one patients were in stage of hepatic cirrhosis.

Serological markers of RA were more frequent in CHC patients than in HBD. In fact, 44 (50%) CHC patients had RF or CCP-Ab compared to only seven (7.8%) HBD ($p<10^{-6}$). CCP-Ab were significantly more frequent in patients with HCV infection than in healthy subjects (11.4% vs 3.3%, p=0.03). In patients, RF-IgG were present in 25%, RF-IgA in 18.2% and RF-IgM in 30.6% of cases. All these frequencies were higher than those found in HBD and the difference were statistically significant ($p<10^{-4}$ for RF-IgG and RF-IgA and $p<10^{-6}$ for RF-IgM). RF were significantly more frequent than CCP-Ab in patients with HCV infection (42% vs. 11.4%; $p<10^{-5}$) (**Table 1**).

CCP-Ab or RF were significantly more frequent in female patients than in male patients (60.8% vs. 37.8%; p=0.03). The frequency of RF-IgM was higher in females than in males (39.2% vs. 18.9%; p=0.04) (**Table 2**).

Table 1. Frequency of serological markers of rheumatoid arthritis in patients with chronic hepatitis C and in control group

	Patients (n=88) n (%)	Control group (n=90) n (%)	*P*	95% Confidence Interval	Odds ratio
CCP-Ab or RF	44 (50)	7 (7.8)	**$<10^{-6}$**	[4.96-28.37]	11.86
CCP-Ab and RF	3 (3.4)	0 (0)	0.23	-	-
CCP-Ab	10 (11.4)[a]	3 (3.3)	**0.03**	[0.99-13.9]	3.72
CCP-Ab only	7 (7.9)	3 (3.3)	0.3	-	-
RF	37 (42)[a]	5 (5.5)	**$<10^{-6}$**	[4.58-33.22]	12.33
RF-IgG	22 (25)	2 (2.2)	**$<10^{-4}$**	[3.36-64.05]	14.67
RF-IgA	16 (18.2)	0 (0)	**$<10^{-4}$**	-	-
RF-IgM	27 (30.6)	2 (2.2)	**$<10^{-6}$**	[4.5-84.26]	19.48

CCP-Ab; anti-cyclic citrullinated peptides antibodies; CI; confidence interval; OR: odds ratio; RF: rheumatoid factor.

a: RF vs CCP-Ab: $p<10^{-5}$

Table 2. Frequency of serological markers of rheumatoid arthritis according to sex

	Female Patients (n=51) n (%)	Male patients (n=37) n (%)	*P*	95% Confidence Interval	Odds ratio
CCP-Ab or RF	31 (60.8)	14 (37.8)	**0.03**	[1.08-6.02]	2.55
CCP-Ab and RF	2 (3.9)	1 (2.7)	NS	-	-
CCP-Ab	8 (15.7)[a]	2 (5.4) [b]	0.2	-	-
CCP-Ab only	6 (11.7)	1 (2.7)	0.2	-	-
RF	24 (47)[a]	13 (35.1) [b]	0.2	-	-
RF-IgG	12 (23.5)	10 (27)	0.7	-	-
RF-IgA	10 (19.6)	5 (13.5)	0.4	-	-
RF-IgM	20 (39.2)	7 (18.9)	**0.04**	[1.03-7.4]	2.76

CCP-Ab: anti-cyclic citrullinated peptides antibodies;
RF: rheumatoid factor.
a: RF vs CCP-Ab in female patients : $p<10^{-3}$
b: RF vs CCP-Ab in male patients : $p=10^{-3}$

The correlation study between RF and CCP-Ab levels with age showed a significant positive correlation between age and CCP-Ab levels (r=0.359, *p*=0.005) (**figure 1**) on one hand and between age and RF-IgA levels (r=0.317, *p*=0.01) (**figure 2**) in the other hand. There was no correlation between age and RF-IgM levels (r=0.135, *p*=0.29) nor between age and RF-IgG levels (r=0.194, *p*=0.128).

RA serological markers were significantly more frequent in patients with cirrhosis than in patients without this complication (71.4% vs 44.8%; *p*=0.03). RF (IgG and/or IgM and/or IgA) and RF-IgM were also more frequent in patients with cirrhosis than those without cirrhosis (61.9% vs 37.3%, *p*=0.04 and 52.4% vs 23.9%, *p*=0.01 respectively) (**Table 3**).

Out of 88 patients, 47 received CHC treatment. Serological markers of RA (CCP-Ab or RF) were significantly more frequent in untreated patients than in treated patients (63.4% vs. 40.4%; *p*=0.03). RF-IgG was significantly more frequent in untreated patients than in treated ones (36.6% vs.14.9%; *p*=0.01) (**Table 4**).

Our CHC population was divided, according to the account viral load quantification, in two groups: 38 (43.2%) patients with detectable viral load and 50 (56.8%) patients with undetectable viral load. Among patients with detectable viral load, 24 (63.1%) had CCP-Ab or RF. This frequency was significantly higher than that of patients with undetectable viral load (63.1% vs. 42%, *p*=0.04). Frequencies of RF and of CCP-Ab between the two groups were not statistically different (52.6% vs. 36%; *p*=0.1 and 15.8% vs. 8%; *p*=0.2 respectively). Mean level of CCP-Ab was significantly higher in patients with detectable viral load than in those with undetectable viral load (14.53±10.53 vs. 10.51±8.2; *p*=0.04) (**Table 5**). There was no correlation between RA serological markers levels and viral load.

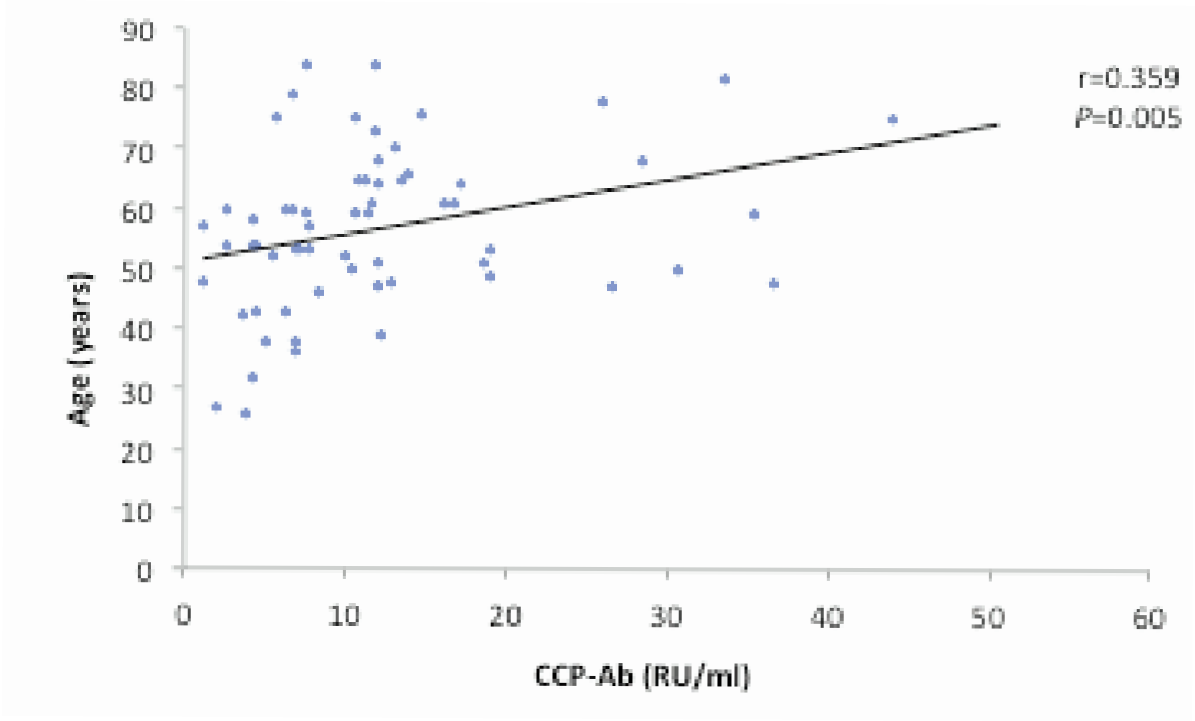

Figure 1. Correlation between CCP-Ab levels and age

CCP-Ab: Anti-cyclic citrullinated peptide

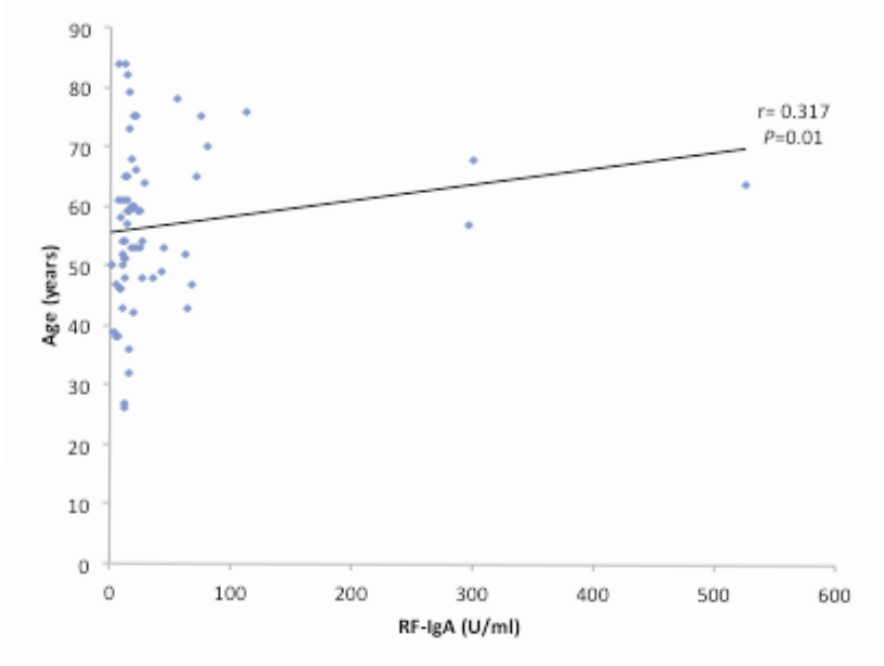

Figure 2. Correlation between RF-IgA levels and age

RF: rheumatoid factor, Ig: immunoglobulin

Table 3. Frequency of serological markers of rheumatoid arthritis in chronic hepatitis C patients with and without cirrhosis

	Patients with cirrhosis (n=21) n (%)	Patients without cirrhosis (n=67) n (%)	*P*	95% Confidence Interval	Odds Ratio
CCP-Ab or RF	15 (71.4)	30 (44.8)	**0.03**	[1.09-8.37]	3.08
CCP-Ab and RF	1 (4.7)	2 (3)	NS	-	-
CCP-Ab	3 (14.3)	7 (10.4)	0.8	-	-
CCP-Ab only	2 (9.5)	5 (7.5)	NS	-	-
RF	13 (61.9)	25 (37.3)	**0.04**	[1.01-7.35]	2.73
RF-IgG	6 (28.6)	16 (23.9)	0.6	-	-
RF-IgA	5 (23.8)	10 (14.9)	0.5	-	-
RF-IgM	11 (52.4)	16 (23.9)	**0.01**	[1.28-9.57]	3.51

CCP-Ab: anti-cyclic citrullinated peptides antibodies; RF: rheumatoid factor.

Table 4. Frequency of serological markers of rheumatoid arthritis according to treatment

	Untreated Patients (n=41) n (%)	Treated patients (n=47) n (%)	*P*	95% Confidence Interval	Odds ratio
CCP-Ab or RF	26 (63.4)	19 (40.4)	**0.03**	[0.17-0.92]	0.39
CCP-Ab and RF	2 (4.9)	1 (2.1)	0.8	-	-
CCP-Ab	6 (14.6)	4 (8.5)	0.5	-	-
CCP-Ab only	4 (9.7)	3 (6.4)	0.8	-	-
RF	22 (53.6)	16 (34)	0.06	-	-
RF-IgG	15 (36.6)	7 (14.9)	**0.01**	[0.1-0.85]	0.3
RF-IgA	9 (21.9)	6 (12.8)	0.1	-	-
RF-IgM	14 (34.1)	13 (27.6)	0.5	-	-

CCP-Ab: anti-cyclic citrullinated peptides antibodies; RF: rheumatoid factor.

Table 5. Mean levels of serological markers of rheumatoid arthritis according to viral load

	Patients with detectable viral load (n=38)	Patients with undetectable viral load (n=50)	*P*
CCP-Ab (RU/ml)	14.53±10.53	10.51±8.2	**0.04**
RF-IgG (U/ml)	55.73±50.9	65±125.24	0.6
RF-IgA (U/ml)	45.87±93.8	25.4±42.75	0.2
RF-IgM (IU/ml)	38±47.4	54.91±146	0.4

CCP-Ab: anti-cyclic citrullinated peptides antibodies; RF: rheumatoid factor.

DISCUSSION

We demonstrated, herein, a significantly higher frequency of serological markers of RA in CHC patients than in the general population (50% vs 7.8%; $p<10^{-6}$). The frequency of CCP-Ab (11.4%) was higher in our study than in those previously described (10,12-14) (Table 6). The three isotypes of RF (IgG, IgA and IgM) have been determined previously in only one study (10). Our frequency of RF-IgG was similar to that of Ferucci *et al.,*. (25% and 15% respectively) (10). The frequency of RF-IgA was also similar to that of Ferucci *et al.,*. (18.2% and 13% respectively) (10). The frequency of the double positivity (CCP-Ab and RF) in our study (3.4%) was comparable to the prevalence of RA reported in a meta-analysis of 646228 patients with CHC (4.5%) (15). Among the three RF isotypes, IgA was found to be the most specific in our previous study on RA (11). In the present study, the frequency of RF-IgA in female patients reached 19.6%, which was significantly higher than in the control group (19.6% vs. 0%).

CCP-Ab is the most specific serological marker of RA (11). In the current study, the frequency of CCP-Ab was 11.4% in the whole group of CHC patients, reaching 15.7% in female patients. This frequency (15.7%) was similar to that of RA (14.7%) determined from medical records about 1,020 CHC patients (16).

It has been demonstrated that CCP-Ab and RF-IgA predict the development of RA (17). So, our patients who had CCP-Ab and RF-IgA could develop RA in the future. The significantly high frequency of RF in comparison with CCP-Ab could in part be due to the probability for our patients to have cryoglobulinemia which was described to be very frequent (34%) in CHC patients (18). HCV is the most common etiology of type II and type III cryoglobulinemia which are usually IgM and IgG.

Untreated patients had significantly higher frequency of RA serological markers than treated patients (p=0.03). CCP-Ab level was significantly higher in untreated patients than in CHC treated patients (p=0.0004). Over a 6-year follow-up of a large cohort of CHC patients in Taiwan (19), RA events occurred in 3.1% of patients. The incidence rate was significantly lower in those who received treatment compared to those who remained untreated (1.4% vs. 3.5%, *p*=0.018). Patel *et al.,* (20) and AbdelHamid Gohar *et al.,* (21), by comparing the characteristics of RA in patients with CHC and those without CHC, found that CHC patients had higher pain scores and higher disease activity score DAS 28. CHC patients with RA had higher disease activity scores, poorer response to treatment, and a higher risk of joint destruction compared to patients with RA (20).

We found that female sex was associated with higher risk of developing serological markers of RA. In fact we found that there was a significant correlation of CCP-Ab and RF-IgA with age and that autoantibodies of RA were more frequent in females than males (*p*=0.01) which is in concordance with the results of Tung (19) and of Lin *et al.,* (14) who found that female patients with CHC had higher prevalence of RF than males patients. It is well known that RA is more frequent in females (22). This could be explained by X-chromosomes related genes and sex hormones which play a role in the sex differences in T and B cell responses (4).

In our patients' group, we found that there were positive correlations between CCP-Ab levels and age and between RF-IgA levels and age. These results agree with those of Tung (19) who found that patients with older age had a greater risk to develop RA.

In the present study, we found that CCP-Ab mean level in patients with detectable viral load was significantly higher than in those with undetectable viral load (*p*=0.04). Wang *et al.,* (23) found that increased HCV

RNA levels were associated with high frequency of RF. However, Mahmoud *et al.,* (24) did not find a statistically significant difference in CCP-Ab and RF levels between CHC patients with undetectable, mild, moderate and severe levels of viremia. This could be explained by the small size of patient's group with CHC in their study (n=22). RF were significantly more frequent in patients with cirrhosis than in patients without this complication (61.9% vs 37.3%, *p*=0.04). Wang *et al.,* (23) found, in their study, that 60% of CHC patients with RF had liver cirrhosis compared to 13% of those without RF. A study comparing intestinal microbiota in CHC patients with and without liver cirrhosis demonstrated that gut dysbiosis is more pronounced in those with cirrhosis (25). This ascertainment could explain the higher frequency of RA seropositivity in our CHC patients with cirrhosis comparing with those without this complication. Another study performed by Preveden *et al.,* (26) showed that treatment with probiotics improved clinical symptoms and decreased inflammatory markers in patients with HCV-related cirrhosis, suggesting that modulation of the gut microbiota may have therapeutic potential in this population.

While the exact mechanisms linking the two pathologies remain unclear, several etiological factors have been proposed (27). In fact, molecular mimicry between viral antigens and self antigens could play a role in autoimmunity in CHC patients (28). It has been shown that antibodies against HCV are found in the synovial fluid of RA patients (29), suggesting that HCV may trigger an autoimmune response that causes joint inflammation (30, 31). In the other hand, chronic inflammation is a hallmark of both HCV infection and RA. It has been suggested that chronic HCV infection may contribute to the development of RA by promoting systemic inflammation and oxidative stress, which can damage joint tissues and lead to joint destruction (24). HCV infection is known to activate toll-like receptors, leading to the production of proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) (31). These cytokines can stimulate the production of RF and CCP-Ab by B cells and promote the survival of RF and CCP-Ab producing cells (32). Moreover, environmental factors, such as smoking and exposure to certain chemicals, have been implicated in the development of both HCV infection (20) and RA (33). In addition, it was proved that patients with HCV infection had a significantly decrease in gut bacterial diversity and that this decrease was associated with the severity of clinical stage (34,35). Indeed, patients had decrease in commensal bacteria, higher levels of *Bacteroidetes* and decreased levels of *Firmicutes* in their gut microbiota compared to healthy controls (36). This alteration in the gut microbiota composition was associated with increased production of pro-inflammatory cytokines, which are known to be involved in the pathogenesis of RA (36). In fact, The impairment of intestinal barrier functions in CHC patients exposes liver to bacteria and fungi which leads to hepatic inflammatory followed by accumulation of fibrin (37). Peptidyl arginine deiminase (PAD) may citrullinate the fibrin in liver cytosol. The citrullinated fibrin could then stimulate the production of CCP-Ab. In the other hand, in CHC, there is high production of IL-17, a pro-inflammatory cytokine (38,39). This inflammation promotes the accumulation of fibrin and consequently its citrullination (40). N-acetyl glucosamine-6-sulfatase (GNS) expressed in both joints and in liver and filamine A (FLNA), an ubiquitus protein, are two newly identified autoantigens of RA (41). These two self-antigens could be also citrullinated as a result of the liver inflammation. In the other hand, it is well known that intestinal *Prevotella copri* correlated with the disease in patients with RA (42). As a consequence of the increase of intestinal permeability, *Prevotella copri* could translocate from gut to liver. GNS and FLNA had sequence homology with T cell epitopes of *Prevotella copri* (54). Hence, *Prevotella copri* could activate immune response against cross-reactive epitopes.

Dysbiosis of the intestinal microbiota and the resulting increase in intestinal permeability can also lead to yeast translocation, with *Saccharomyces cerevisiae (S. cerevisiae)* being the most prevalent species. This translocation triggers an immune response characterized by the production of anti-*S. cerevisiae* antibodies (ASCA). Elevated ASCA levels have been observed in patients with CHC (44) and with RA (45). *S. cerevisiae* can migrate to the liver via the portal circulation, where it binds to ASCA, forming immune complexes that activate the complement system and exacerbate hepatic inflammation. While ASCA specifically target the mannose on *S. cerevisiae*, they can also recognize mannose on HCV, leading to recognition of HCV by ASCA upon invasion of hepatocytes (46,47). Furthermore, ASCA can bind to beta-2 glycoprotein I (β2GPI), a protein synthesized in the liver. Cross-reactivity between *S. cerevisiae* and β2GPI has been documented (48), and we have previously shown that patients with CHC have a significantly higher frequency of anti-β2GPI antibodies (aβ2GPI) compared to healthy controls (49). Given that β2GPI is a ubiquitous glycoprotein found even in joints, ASCA could bind to β2GPI in the joints, leading to complement activation and inflammation. Additionally, shared sequences between autoantigens in RA and mannan from the *S. cerevisiae* cell wall were identified (48).

This study was limited by the small number of patients and by the insufficient relevant information in patients' medical records particularly

regarding articular manifestations.

In conclusion, HCV infection can contribute to the development of autoimmune conditions. Differentiating between patients with CHC related arthropathy and those with associated RA has a great relevance in the patient care especially since the most frequently used disease modified antirheumatic drugs are hepatotoxic and may exacerbate liver damage in HCV-infected patients (43) and since anti-CD20 (Rituximab), used as a treatment of RA, is associated with immunosuppression and reactivation of HCV (14).

Conflict of interest : The authors declare no conflict of interest

Research funding: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

REFERENCES

1. Pisetsky DS. Pathogenesis of autoimmune disease. Nat Rev Nephrol. 2023:1–16.

2. Deshpande P, Bundell C, McKinnon E, Hellard M, Ffrench R, Wilkinson AL, et al. Frequent occurrence of low-level positive autoantibodies in chronic hepatitis C. Pathology. 2020;52(5):576-583.

3. Vergani D, Mieli-Vergani G. Autoimmune manifestations in viral hepatitis. Semin. Immunopathol. 2013;35(1):73-85.

4. Johnson D, Jiang W. Infectious diseases, autoantibodies, and autoimmunity. J Autoimmun. 2023;137: 102962.

5. Paquissi FC. Immunity and Fibrogenesis: The Role of Th17/IL-17 Axis in HBV and HCV-induced Chronic Hepatitis and Progression to Cirrhosis. Front. Immunol. 2017;8:1195.

6. Lormeau C, Falgarone G, Roulot D, Boissier MC. Rheumatologic manifestations of chronic hepatitis C infection. Joint Bone Spine. 2006;73(6):633-8.

7. Su FH, Wu CS, Sung FC, Chang SN, Su CT, Shieh YH et al. Chronic hepatitis C virus infection is associated with the development of rheumatoid arthritis: a nationwide population-based study in taiwan. PLoS One. 2014;9(11):e113579. Erratum in: PLoS One. 2016;11(11):e0166508.

8. Masuko-Hongo K, Kato T, Nishioka K. Virus-associated arthritis. Best. Pract. Res. Clin. Rheumatol. 2003;17(2):309-18.

9. Jadali Z, Alavian SM. Autoimmune diseases co-existing with hepatitis C virus infection. Iran. J. Allergy. Asthma. Immunol. 2010;9(4):191-206.

10. Ferucci ED, Choromanski TL, Varney DT, Ryan HS, Townshend-Bulson LJ, McMahon BJ et al.. Prevalence and correlates of hepatitis C virus-associated inflammatory arthritis in a population-based cohort. Semin. Arthritis. Rheum. 2017;47(3):445-450.

11. Sghiri R, Bouajina E, Bargaoui D, Harzallah L, Fredj HB, Sammoud S et al. Value of anti-mutated citrullinated vimentin antibodies in diagnosing rheumatoid arthritis. Rheumatol. Int. 2008;29(1):59-62.

12. Bombardieri M, Alessandri C, Labbadia G, Iannuccelli C, Carlucci F, Riccieri V, et al. Role of anti-cyclic citrullinated peptide antibodies in discriminating patients with rheumatoid arthritis from patients with chronic hepatitis C infection-associated polyarticular involvement. Arthritis. Res. Ther. 2004;6(2):R137-41.

13. Orge E, Cefle A, Yazici A, Gürel-Polat N, Hulagu S. The positivity of rheumatoid factor and anti-cyclic citrullinated peptide antibody in nonarthritic patients with chronic hepatitis C infection. Rheumatol. Int. 2010;30(4):485-8.

14. Lin KM, Chen WM, Tung SY, Wei KL, Shen CH, Chang TS, et al. Prevalence and predictive value of high-positive rheumatoid factor and anti-citrullinated protein antibody levels in nonarthritic patients with chronic hepatitis C infection. Int. J. Rheum. Dis. 2019;22(1):116-120.

15. Younossi ZM, Henry L, P Ong J, Tanaka A, Eguchi Y, Mizokami M, et al. Systematic review with meta-analysis: extrahepatic manifestations in chronic hepatitis C virus-infected patients in East Asia. Aliment. Pharmacol. Ther. 2019;49(6):644-653.

16. Ramos-Casals M, Muñoz S, Medina F, Jara LJ, Rosas J, Calvo-Alen J, et al. Systemic autoimmune diseases in patients with hepatitis C virus infection: characterization of 1020 cases (The HISPAMEC Registry). J. Rheumatol. 2009;36(7):1442-8.

17. Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 2003;48(10):2741-9.

18. Ramos-Casals M, Jara LJ, Medina F, Rosas J, Calvo-Alen J, Mañá J, et al. Systemic autoimmune diseases coexisting with chronic hepatitis C virus infection (the HISPAMEC Registry): patterns of clinical and immunological expression in 180 cases. J. Intern. Med. 2005;257(6):549-57.

19. Tung CH, Lai NS, Li CY, Tsai SJ, Chen YC, Chen YC. Risk of rheumatoid arthritis in patients with hepatitis C virus infection receiving interferon-based therapy: a retrospective cohort study using the Taiwanese national claims database. BMJ. Open. 2018 ;8(7):e021747.

20. Patel R, Mikuls TR, Richards JS, Kerr G, Cannon GW, Baker JF. Disease characteristics and treatment patterns in veterans with rheumatoid arthritis and concomitant hepatitis C infection. Arthritis Care Res. (Hoboken) 2015;67(4):467-74.

21. Abd EI-Hamid Gohar N, Ali Aballa M, Khairy Mehasseb M, Abd El-Rahman Saleh W. Impact of hepatitis C virus infection on disease activity, functional status and ultrasonography findings in Egyptian rheumatoid arthritis patients. The Egyptian Rheumatologist. 2018 ;40(2) :79-83.

22. Cush JJ. Rheumatoid Arthritis: Early Diagnosis and Treatment. Rheum. Dis. Clin. North. Am. 2022;48(2):537-547.
23. Wang CH, Flehmig B, Tschen SY. Hepatitis C virus, rheumatoid factors, and disease progression. Lancet. 1998;351(9098):294.
24. Geilan AM, Hania SZ, Mai MS, Mervat MM. Characteristics of rheumatoid arthritis patients with concomitant hepatitis C virus infection. The Egyptian Rheumatologist. 2011;33(3):139-145.
25. Heidrich B, Vital M, Plumeier I, Döscher N, Kahl S, Kirschner J, et al. Intestinal microbiota in patients with chronic hepatitis C with and without cirrhosis compared with healthy controls. Liver Int. 2018;38(1):50-58.
26. Preveden T, Scarpellini E, Milić N, Luzza F, Abenavoli L. Gut microbiota changes and chronic hepatitis C virus infection. Expert Rev Gastroenterol Hepatol 2017;11(9):813-819.
27. Strassburg CP, Vogel A, Manns MP. Autoimmunity and hepatitis C. Autoimmun. Rev. 2003;2(6):322-31.
28. Bogdanos DP, Muratori L, Bianchi FB, Vergani D. Hepatitis C virus and autoimmunity. Hepatology. 2000;31(6):1380.
29. Ueno Y, Kinoshita R, Tsujinoue H, Kato M. A case of hepatitis C virus (HCV)-associated arthritis. Quantitative analysis of HCV RNA of the synovial fluid and the serum. Br. J. Rheumatol. 1995;34(7):691-2.
30. Zignego AL, Ferri C, Pileri SA, Caini P, Bianchi FB, Italian Association of the Study of Liver Commission on Extrahepatic Manifestations of HCV infection. Extrahepatic manifestations of Hepatitis C Virus infection: a general overview and guidelines for a clinical approach. Dig. Liver. Dis. 2007;39(1):2-17.
31. Kivity S, Agmon-Levin N, Blank M, Shoenfeld Y. Infections and autoimmunity--friends or foes? Trends. Immunol. 2009;30(8):409-14.
32. Mohamed Raafat Hamed R, Aref Mohamed S, Ali Dwedar R, Samy Elkohly Y, Talaat Elgengehy F. Association of interleukin-6 and its -174G/C promoter polymorphism with clinical and laboratory characteristics of non hepatitis C virus rheumatoid arthritis patients. Egyptian Journal of Medical Human Genetics. 2018 (9):235-240.
33. Ruzzon F, Adami G. Environment and arthritis. Clin. Exp. Rheumatol. 2024;42(7):1343-1349.
34. Inoue T, Nakayama J, Moriya K, Kawaratani H, Momoda R, Ito K, et al. Gut Dysbiosis Associated With Hepatitis C Virus Infection. Clin. Infect. Dis. 2018;67(6):869-877.
35. Milosevic I, Vujovic A, Barac A, Djelic M, Korac M, Radovanovic Spurnic A, et al. Gut-Liver Axis, Gut Microbiota, and Its Modulation in the Management of Liver Diseases: A Review of the Literature. Int. J. Mol. Sci. 2019;20(2):395.
36. Aly AM, Adel A, El-Gendy AO, Essam TM, Aziz RK. Gut microbiome alterations in patients with stage 4 hepatitis C. Gut. Pathog. 2016;8(1):42. d
37. Iwadare T, Kimura T, Tanaka N, Yamazaki T, Wakabayashi SI, Okumura T, et al. Circulating thrombospondin 2 levels reflect fibrosis severity and disease activity in HCV-infected patients. Sci Rep 2022;12(1):18900.
38. Paquissi FC. Immunity and Fibrogenesis: The Role of Th17/IL-17 Axis in HBV and HCV-induced Chronic Hepatitis and Progression to Cirrhosis. Front. Immunol. 2017;8:1195.
39. Rios DA, Casciato PC, Caldirola MS, Gaillard MI, Giadans C, Ameigeiras B, et al. Chronic Hepatitis C Pathogenesis: Immune Response in the Liver Microenvironment and Peripheral Compartment. Front. Cell. Infect. Microbiol. 2021;11:712105.
40. Anstee QM, Dhar A, Thursz MR. The role of hypercoagulability in liver fibrogenesis. Clin. Res. Hepatol. Gastroenterol. 2011;35(8-9):526-33.
41. Pianta A, Arvikar SL, Strle K, Drouin EE, Wang Q, Costello CE, et al. Two rheumatoid arthritis-specific autoantigens correlate microbial immunity with autoimmune responses in joints. J. Clin. Invest. 2017;127(8):2946-2956.
42. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife. 2013;2:e01202.
43. Cabrera Villalba SR, Victoria Hernández Miguel M, Sanmartí Sala R. How does one manage patients with rheumatoid arthritis and positive serology to hepatitis B, hepatitis C, human immunodeficiency virus? Reumatol. Clin. 2011;7(3):203-7.
44. Ghozzi M, Mankaï A, Mechi F, Ben Chedlya Z, Kallala O, Melayah S, et al. High frequency of anti-Saccharomyces cerevisiae antibodies in chronic hepatitis C. Arab. J. Gastroenterol. 2024 (in press).
45. Melayah S, Ghozzi M, Jemni M, Sakly N, Ghedira I, Mankaï A. Anti-Saccharomyces cerevisiae Antibodies in Rheumatoid Arthritis. Lab. Med. 2022 Nov 3;53(6):585-589.
46. Dent M, Hamorsky K, Vausselin T, Dubuisson J, Miyata Y, Morikawa Y, et al. Safety and Efficacy of Avaren-Fc Lectibody Targeting HCV High-Mannose Glycans in a Human Liver Chimeric Mouse Model. Cell. Mol. Gastroenterol. Hepatol. 2021;11(1):185-198.
47. Urbanowicz RA, Wang R, Schiel JE, Keck ZY, Kerzic MC, Lau P, et al. Antigenicity and Immunogenicity of Differentially Glycosylated Hepatitis C Virus E2 Envelope Proteins Expressed in Mammalian and Insect Cells. J. Virol. 2019;93(7):e01403-18.
48. Rinaldi M, Perricone R, Blank M, Perricone C, Shoenfeld Y. Anti- Saccharomyces cerevisiae autoantibodies in autoimmune diseases: from bread baking to autoimmunity. Clin. Rev. Allergy. Immunol. 2013;45:152–161.
49. Melayah S, Kallala O, Ben Ahmed M, Fodha I, Yacoub Jemni S, Ghedira I,et al. IgA anti-beta-2 glycoprotein I antibodies in chronic hepatitis C. Arab J Gastroenterol. 2022;23(1):26-31.