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ORIGINAL ARTICLE

Evaluation of the liver condition in chronic hepatitis C virus patients with and without vasculitis

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KEYWORDS

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Abstract *Aim of the work:* The association between hepatitis C virus (HCV)-related vasculitis and severe hepatic fibrosis is a controversial issue. In this study, we aimed to evaluate the liver affection in a group of patients with HCV-related vasculitis and a control group with chronic HCV infection without vasculitis.

Patients and methods: Twenty-six HCV associated vasculitis patients (22 females, 4 males) with a mean age of 51.9 ± 8.5 years (range 36–72 years) and a control group including 20 age- and sex matched HCV infected patients without any extra-hepatic autoimmune manifestations were recruited in this study. All patients and controls were evaluated by routine biochemical tests, conventional ultrasonography and Fibroscan.

Results: The mean disease duration in patients with vasculitis and the control group was 7.5 ± 7.3 and 4.1 ± 3.6 years, respectively ($p = 0.062$). Mean aspartate aminotransferase, bilirubin and international normalized ratio (INR) values were higher in the control group ($p = 0.036$, 0.041 and 0.017, respectively). Hepatomegaly was found in 11 (42.3%) vasculitis patients and 17 (85%) controls ($p = 0.006$), while portal hypertension was found in 4 (15.4%) vasculitis patients and 9 (45%) controls ($p = 0.046$). On Fibroscan, eleven vasculitis patients (42.3%) had mild to moderate liver fibrosis (*F*1–2), and 10 (38.5%) had severe liver fibrosis (*F*3–4), while only one patient (5%) of the control group had mild, and 17 (85%) had severe liver fibrosis ($p = 0.002$).

Conclusion: Patients with chronic HCV infection without vasculitis have worse liver functions and more advanced liver fibrosis than those with HCV related vasculitis.

1. Introduction

Chronic infection with the hepatitis C virus (HCV) is associated with a wide spectrum of clinical manifestations which include hepatic and extrahepatic disorders. Liver diseases may range from asymptomatic minimal histologic changes with normal or near normal liver enzymes to end-stage cirrhosis and hepatocellular carcinoma [1].

Mixed cryoglobulinemia (MC) is the most documented extrahepatic manifestation of HCV infection and presents a clinical triad of purpura, arthralgia and weakness with associated cryoglobulins. These are composed of different immunoglobulins, with a monoclonal component in type II and only

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polyclonal immunoglobulins in type III [1,2] in addition to viral core proteins and RNA, suggesting that cryoglobulin formation results from the host immune response against chronic HCV infection [3]. Although between 12% and 56% of HCV infected patients have circulating cryoglobulins in serum, only about 2–50% of those develop symptoms [3–5]. The development of cryoglobulinemic vasculitis has been associated with type II cryoglobulinemia. Eighty percent of the monoclonal rheumatoid factors (mRF) of type II cryoglobulinemia are unique to patients with HCV infection and are hypothesized to be driven by the HCV infection and are termed WamRF [6]. The pathological hallmark of MC is a leukocytoclastic vasculitis, involving small and medium-sized vessels responsible for cutaneous and visceral organ involvement [7]. In addition to the classic MC syndrome, systemic vasculitis related to HCV infection in the absence of detectable cryoglobulins [8] as well as a polyarteritisnodosa- like vasculitis have been described [9,10]. An epidemiological association between MC and severe liver damage has been reported [1,11]. On the other hand, Ferri [12] stated that chronic hepatitis in cryoglobulinemic vasculitis has usually a mild–moderate clinical course and seems to be less severe if compared to HCV-related chronic hepatitis without the MC syndrome. Similarly, hepatocellular carcinoma less frequently complicates MC syndrome compared to the whole population of HCV-positive individuals. Since corticosteroids and immunosuppressive drugs are frequently needed to treat HCV related vasculitis especially with major organ involvement [3], hepatotoxicity in the presence of underlying liver pathology remains of particular concern.

Liver biopsy has been the gold standard for staging of liver fibrosis, however, it is invasive and associated with some risks such as pain and bleeding. In addition, a liver biopsy sample is only a very small piece of the liver, which can lead to incorrect staging if this sample is not representative of the rest of the liver. Moreover, different pathologists can interpret the same sample differently, which can result in discrepancies in liver disease staging. Fibroscan (transient elastography) is a relatively recent non-invasive method useful for staging of hepatic fibrosis. Essentially, the technology measures the velocity of the sound wave passing through the liver and then converts that measurement into a liver stiffness measurement. The entire process is often referred to as liver ultrasonographic elastography [13,14]. In this study, we aimed to study the liver affection by conventional ultrasonography, Fibroscan and routine biochemical tests in a group of patients with HCV-related vasculitis and in a control group with HCV infection without vasculitis.

2. Patients and methods

Twenty-six HCV associated vasculitis patients and 20 age- and sex matched chronically infected HCV control subjects without any extra-hepatic autoimmune manifestations were recruited in this study. The protocol of the research project has been approved by the institution within which the work was undertaken and it conforms to the provisions of the World Medical Association's Declaration of Helsinki.

The data recorded for every patient included the age, the age of onset and the duration of HCV infection. The disease duration for patients and controls was calculated from the time of diagnosis of HCV infection; for patients in whom the clinical manifestations of vasculitis preceded the diagnosis of HCV infection, the disease duration was calculated from the onset of vasculitis related symptoms.

The disease manifestations recorded for every patient were as follows: the presence of arthralgia, arthritis, skin manifestations, neuro-psychiatric affection, cardiac, pulmonary, renal, gastrointestinal, hepatic, ear, nose and throat (ENT), or ophthalmologic involvement. According to the clinical presentation, patients were classified as having medium or small sized vessel vasculitis [15], (group A and group B, respectively).

Liver function tests included aspartate aminotransferase (AST), alanine aminotransferase (ALT) (reflecting the micro-inflammatory changes of the liver), serum albumin, total bilirubin, prothrombin concentration (PC) and international normalized ratio (INR) (reflecting the synthetic function of the liver). All patients were evaluated for rheumatoid factor (RF), antinuclear antibodies (ANA) and antibodies to anti-double stranded nucleic acid (anti-dsDNA). Antineutrophil cytoplasmicantibodies (ANCA) test was performed using indirect immunofluorescence assay on ethanol-fixed neutrophils. Cryoglobulins were isolated by centrifugation of refrigerated patient's serum in Wintrobe's tubes. Positive cryoglobulinemia was considered in patients who had a measurable cryocrit level $>1\%$. Complement components 3 and 4 (C3 and C4) were assessed.

Exclusion criteria included: (1) hepatitis B virus (HBV) patients by performing hepatitis B surface antigen (HbsAg) and hepatitis B core antibody (HbcAb) tests using the enzyme linked immunosorbent assay (ELISA) technique, (2) Human Immunodeficiency virus (HIV) by performing anti-HIV-I and -II antibody testing by ELISA technique, (3) coexistence of autoimmune, lymphoproliferative or other infectious disease (except HCV infection), (4) presence of any other cause of vasculitis, (5) any subject with other associated liver pathology as Bilharziasis, (6) patients with conditions that interfere with the reliability of Fibroscan measurement (morbidly obese subjects, those with a large amount of chest wall fat or ascites) [13].

Real time ultrasound scanning was done using Toshiba, Aplio MX device with convex probe, 3–5 MHz to measure the liver size, to detect the presence of liver texture changes; brightness, or cirrhosis (irregular surface, coarse texture, attenuated hepatic veins), signs of portal hypertension (presence of abdominal collaterals, splenomegaly), ascites and to exclude hepatic focal lesions.

Liver stiffness was measured by using Fibroscan. Up to ten successful acquisitions were performed on each patient. Success rate was calculated as the ratio of the number of successful acquisitions over the total number of acquisitions. The median value of successful measurements was kept as the representative of liver stiffness .Only the liver stiffness measurement obtained with 10 successful acquisitions and a success rate of at least 60% were considered reliable [16].

The relation between Fibroscan reading in K Pascal and the stage of fibrosis was considered as follows: $F0 = 0{:}2.9$, $F1 = 3{:}5.9$, $F2 = 6{:}8.9$, $F3 = 9{:}16.9$ and $F4 = 17{:}75$ [17].

Statistical analysis was performed by SPSS (version 11) for windows. The means and standard deviation (SD) were computed for the continuous variables. The difference between the means was tested by the standard t test. For comparison of percentages, chi-squared (χ^2) test was used with Fisher's exact test. Mann–Whitney U test was used for comparison of

non-parametric data. Differences were considered to be significant when p value was less than 0.05.

3. Results

This study included 26 HCV vasculitis patients, 22 females (84.6%) and 4 males (15.4%) with a male to female ratio of 0.182. The mean age was 51.9 ± 8.5 years with a range between 36 and 72 years. Twenty age- and sex matched HCV infected patients without any extra-hepatic autoimmune manifestations were included as controls. They were 17 females (85%) and 3 males (15%) with male to female ratio of 0.176.

Vasculitis was the presenting symptom in 24/26 patients (92.3%). In 7 patients the diagnosis of HCV infection was made during the laboratory work up of vasculitis. In 2 patients the diagnosis of HCV infection preceded the onset of vasculitis by 6 and 9 years. In the remaining 17 patients, HCV infection was diagnosed after the onset of vasculitis by a mean of 5.7 ± 4.5 years (range from 1 to 18 years). Comparisons between all vasculitis patients and controls and between group A (medium-sized vessel vasculitis) and group B (small sized-vessel vasculitis) concerning the demographic data are shown in Table 1.

HCV was attributed to previous operation in 2 patients, to blood transfusion in 1 patient, and to a dental procedure in 3 patients. Six patients (23.1%) were diabetic. Ten patients (38.5%) were hypertensive. Patients were classified into two groups according to the size of the vessel involved: group A; patients with medium sized vessel vasculitis, they were 8 patients (30.8%), 3 of them had small vessel vasculitis in addition, and group B; patients with only small-sized vessel vasculitis, they were 18 patients (69.2%). Thirteen patients received 1–3 pulses of IV methylprednisolone (0.5–1 g/pulse) during their illness. Sixteen patients had a history of receiving oral prednisone (starting by 0.5 mg/kg with gradual tapering to 5–10 mg/day maintenance dose at the time of the study). Eight patients started at low dose oral prednisone (5–10 mg/day). Six patients received azathioprine (50–100 mg/day) during their illness. Ten patients received cyclophosphamide for 6 monthly pulses with mean total dose 5500 ± 2387.5 mg (6 patients in group A and 4 patients in group B).

The details of clinical and laboratory data of vasculitis patients (all patients, group A and group B are shown in Table 2. Symmetric poly-arthritis was seen in 3 patients of group A and in 10 patients of group B. One patient (group A) had asymmetric polyarthritis, and 2 other patients had mono-arthritis (1 in group A and 1 in group B). Gangrene occurred in the upper limbs in three patients (group A). Nerve conduction velocity studies of the patients with peripheral neuropathy revealed that 6 patients had axonal-demyelinating lesions (group A). One patient had brain vasculitis (group A) and another had multiple infarcts (group B) as diagnosed by magnetic resonance imaging (MRI). Cardiac involvement was diagnosed in 4 patients; pericardial effusion was detected in one patient (group A), valvular lesions and diastolic dysfunction in one patient (group B) and ischemic heart disease in 2 patients (group B). None of the patients had mesenteric or retinal vasculitis.

In five patients that had positive ANA, one had a homogeneous pattern (group B), and 4 patients had a speckled pattern (one patient in group A and 3 patients in group B). None of the patients had anti DNA antibodies or ANCA.

The details of liver function tests, sonographic features and Fibroscan results are shown in Table 3. Elevated liver enzymes were found in 10/26 patients with HCV vasculitis and in 12/20 of the control group at the time of the study. Eleven vasculitis patients (42.3%) had mild to moderate liver fibrosis ($F1$–2), and 10 patients (38.5%) had severe liver fibrosis ($F3$–4), while only one (5%) of the control group had mild, and 17 (85%) had severe liver fibrosis ($p = 0.002$). Fibroscan images of a patient with $F1$ and another with $F4$ fibrosis stage are shown in Figs. 1 and 2.

On comparing Fibroscan results between cryoglobulin positive ($n = 17$) and cryoglobulin negative ($n = 9$) vasculitis patients, those with cryoglobulins had a median fibrosis stage of $F2$ (range: $F0$–4), while those without cryoglobulins had a median fibrosis stage of $F1$ (range: $F1$–4), $p = 0.990$. Ten patients with cryoglobulinemia (58.82%) had normal–mild liver fibrosis ($F0$–2) while 7 (41.12%) had severe fibrosis ($F3$–4), while among patients without detectable cryoglobulins 6 (66.67%) had normal–mild and 3 (33.33%) had severe liver fibrosis, respectively with no statistically significant difference ($p = 0.327$).

4. Discussion

The association between cryoglobulinemia and severe liver affection is a controversial issue [1,11,18]. Most studies reporting an association between cryoglobulinemia and liver cirrhosis have depended on the presence of cryoglobulins in serum irrespective of the presence of vasculitis, possibly due to the small number of symptomatic patients [5,11,18], with few studies specifically mentioning the association between cryoglobulinemic vasculitis and liver damage [12,18,20,21]. Agnello [22] suggested that these differences in patient selection may be responsible for the controversies regarding the association between cryoglobulinemia and severe liver damage. Cryoglobulinemia associated with chronic HCV infection has been associated with female sex, older age, longer disease duration and presence of liver cirrhosis in several studies [19,23–25]. It has been suggested that liver cirrhosis may be the cause for the excess production of cryoglobulins in chronic HCV infection

Table 1 Comparisons between patients and controls and between group A and group B concerning the demographic data.

	HCV with vasculitis patients $n = 26$	Controls $n = 20$	P	Group A $n = 8$	Group B $n = 18$	P
Age (years)	51.9 ± 8.5	53.4 ± 10.5	0.601	57.9 ± 6.9	49.3 ± 7.9	0.014*
Disease duration (years)	7.5 ± 7.3	4.1 ± 3.6	0.062	8.4 ± 9.6	7.1 ± 6.3	0.691
Age at disease onset (years)	43.9 ± 10.7	49.3 ± 11.5	0.112	49.5 ± 8.9	41.5 ± 10.8	0.079

A: medium-sized vessel vasculitis; B: small-sized vessel vasculitis.
* Statistically significant.

Table 2 The clinical and laboratory manifestations of all, group A and group B patients.

	All patients (26 patients) *n* (%)	Group A (8 patients) *n* (%)	Group B (18 patients) *n* (%)	*P*
Arthralgia	16(61.5)	5(52.5)	11(61.1)	1.000
Arthritis	15(57.7)	4(50)	11(61.1)	0.683
Raynaud's phenomenon	1(3.8)	1(12.5)	0	0.308
Purpura	17(65.4)	1(12.5)	16(88.9)	0.001*
Gangrene	3(11.5)	3(16.7)	0	0.022*
Depression	2(7.7)	1(12.5)	1(27.8)	0.529
Cognitive impairment	1(3.8)	1(12.5)	0	0.308
Brain MRI findings	2(7.7)	1(12.5)	1(5.6)	0.529
Peripheral neuropathy	9(34.6)	2(25)	7(38.9)	0.667
Mononeuritis multiplex	6(23.1)	6(75)	0	0.001*
IPF	3(11.5)	1(12.5)	2(11.1)	1.000
Cardiac involvement	4(15.4)	1(12.5)	3(16.7)	1.000
Nephritis	3(11.5)	1(12.5)	2(11.1)	1.000
RF positive	11(42.3)	4(50)	7(38.9)	0.683
ANA positive	5(19.3)	1(12.5)	4(22.2)	0.653
Cryoglobulin positive	17(65.4)	5(62.5)	12(66.7)	1.000
Decreased C3	8(30.8)	2(25)	6(33.3)	1.000
Decreased C4	7(26.9)	3(16.7)	4(22.2)	0.149

A: medium-sized vessel vasculitis; B: small-sized vessel vasculitis; IPF: interstitial pulmonary fibrosis; RF: rheumatoid factor; ANA: antinuclear antibodies, C3: complement component 3; C4: complement component 4.
* Statistically significant.

Table 3 The comparison between patients and controls and between group A and group B concerning the laboratory, sonographic and Fibroscan findings.

	HCV with vasculitis patients *n* = 26	Controls *n* = 20	*P*	Group A *n* = 8	Group B *n* = 18	*P*
ALT (U/L)	42 ± 29.4	63.4 ± 46.2	0.08	51.1 ± 32.8	38.4 ± 0.4	0.363
AST (U/L)	38.9 ± 18.3	62.6 ± 44.8	0.036*	42.6 ± 19.7	37.9 ± 18.3	0.577
Total bilirubin (mg/dl)	0.4 ± 0.3	1.6 ± 2.2	0.041*	0.29 ± 0.12	0.45 ± 0.35	0.105
Serum albumin (g/dl)	3.3 ± 0.7	3.1 ± 0.6	0.514	3.0 ± 0.7	3.1 ± 0.7	0.256
PC (%)	92.2 ± 18.9	85.1 ± 14	0.146	82.5 ± 33.4	96.1 ± 5.2	0.288
INR	1.1 ± 0.2	1.3 ± 0.3	0.017*	1.2 ± 0.3	1.1 ± 0.1	0.099
Sonographic findings: n (%)						
Hepatomegaly	11(42.3)	17(85)	0.006*	5(62.5)	6(33.3)	0.091
Coarse liver	8(30.8)	19(95)	0.001*	4(50)	4(22.2)	0.197
Bright liver	7(26.9)	4(20)	0.732	2(25)	5(27.8)	1.000
Focal lesions	2(7.7)	6(30)	0.062	2(25)	0	0.086
Splenomegaly	7(26.9)	11(55)	0.072	4(50)	3(16.7)	0.149
Portal hypertension	4(15.4)	9(45)	0.046*	3(37.5)	1(5.6)	0.072
Fibroscanstage: n (%)						
*F*0	5(19.2)	2(10)	0.446	1(12.5)	4(22.2)	1.000
*F*1	6(23.1)	0	0.029*	1(12.5)	5(27.8)	0.628
*F*2	5(19.2)	1(5)	0.212	0	5(27.8)	0.281
*F*3	3(11.5)	6(30)	0.149	2(25)	1(5.6)	0.215
*F*4	7(26.9)	11(55)	0.072	4(50)	3(16.7)	0.149
Median	2	4	0.01*	3.5	1.5	0.225

A: medium-sized vessel vasculitis; B: small-sized vessel vasculitis; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PC: prothrombin concentration; INR: international normalized ratio.
* Statistically significant.

either directly due to immunological changes resulting from liver dysfunction or altered elimination of immune complexes by the reticuloendothelial system [24]. Supporting this hypothesis, cryoglobulins have been detected in different chronic liver diseases [26,27].

In the present study, there was no statistically significant difference between patients and controls in the mean age, sex distribution or estimated disease duration. The accurate calculation of disease duration in chronic HCV is difficult and sometimes impossible since many patients may be asymptomatic [28], moreover, in the present study, the source of infection was unknown in the majority of the studied patients. We chose to calculate the disease onset for patients and controls from the date of diagnosis of HCV infection and for those in whom the development of vasculitis preceded the diagnosis of HCV infection from the onset of vasculitic symptoms.

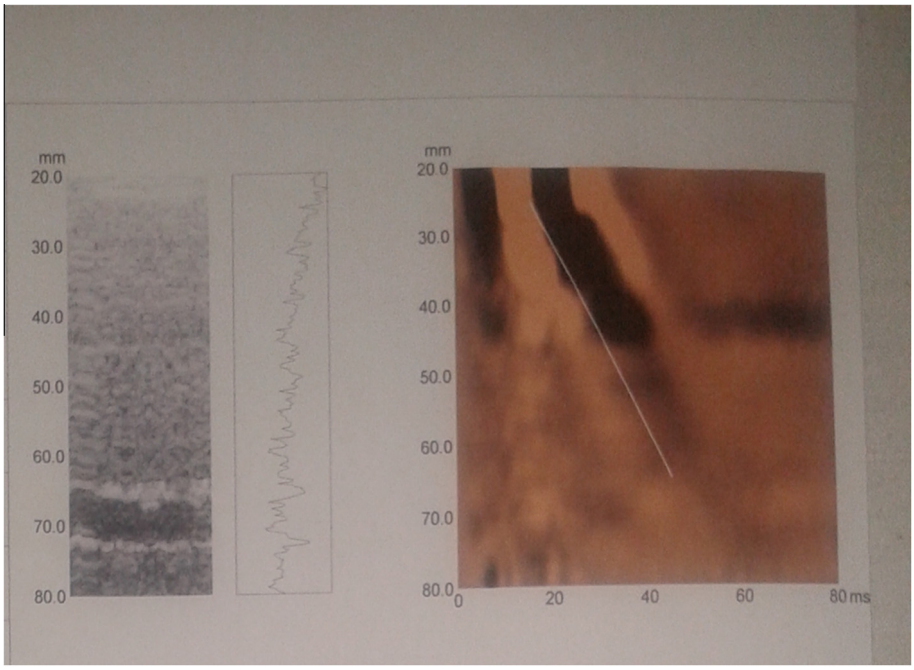

Figure 1 Fibroscan result of a patient with *F*1 hepatic fibrosis (5.8 kPa).

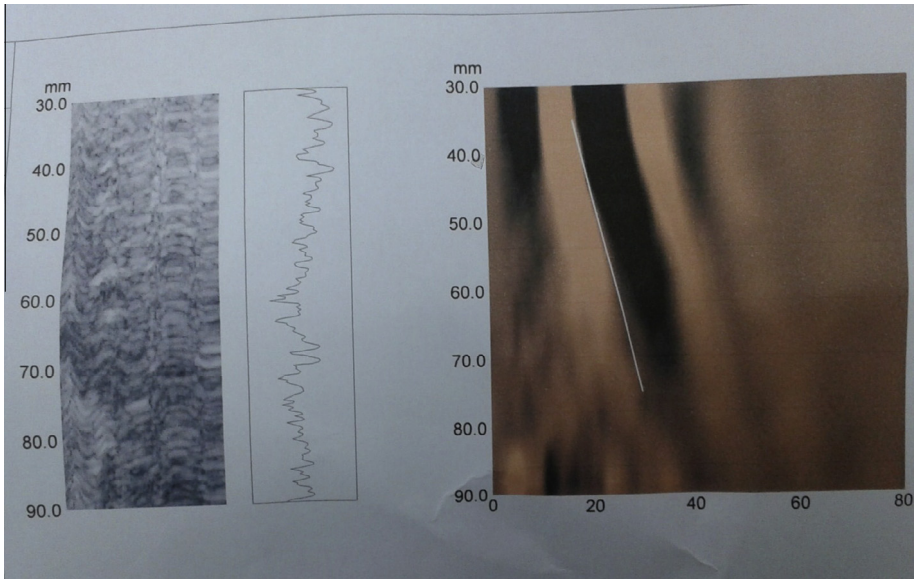

Figure 2 Fibroscan result of a patient with *F*4 hepatic fibrosis (30.3 kPa).

Hepatomegaly with coarse echogenic pattern and portal hypertension were more frequently found in HCV controls ($p = 0.0006$, 0.001 and 0.046, respectively). Liver function tests revealed significantly higher mean serum AST, total bilirubin and INR in the control group ($p = 0.036$, 0.041 and 0.017, respectively). Serum ALT levels were not significantly different between vasculitis patients and the control group, which is in agreement with Calleja et al. [29]. On Fibroscan, 42.3% of vasculitis patients and only one patient of the control group (5%) had mild liver fibrosis (*F*1–2), while 38.5% of patients with vasculitis had severe liver fibrosis (*F*3–4) as compared to 85% of the control group ($p = 0.002$). Among patients with vasculitis, no significant differences in liver function tests, ultrasonographic features and severity of liver fibrosis were found between those with small or medium sized vessel affection. Furthermore, the degree of liver fibrosis was not significantly different between cryoglobulin positive and negative vasculitis patients.

The frequency of advanced liver fibrosis in HCV vasculitis patients in this study was comparable to several studies which reported advanced fibrosis/cirrhosis in 25–44% of their patients with HCV vasculitis [20,21,29–31], however, in the present study, the frequency of severe liver affection was significantly higher in the control group without vasculitis. In contrast, Calleja et al. [29] found a much higher incidence of

cirrhosis in patients with cryoglobulinemic vasculitis as compared to those with chronic hepatitis without detectable cryoglobulins. Notably, in that study, vasculitis patients had a significantly longer disease duration than the control group with chronic hepatitis. Saadoun et al. [20] found cryoglobulinemia to be independently associated with advanced hepatic fibrosis. No significant differences in the severity of liver disease were found between symptomatic and asymptomatic cryoglobulinemia [20,21]. On the other hand, in a 15-year prospective study on 950 chronically infected HCV patients, the estimated progression rate of liver fibrosis was significantly lower in cryoglobulinemic syndrome (CS) positive than in MC negative patients. The 15-year cumulative probability of developing cirrhosis and/or hepatocellular carcinoma was higher in MC(−) than in CS(+) patients (24.9% vs. 14.2%, $p < 0.005$ and 20.3% vs. 7.5%, $p = 0.003$, respectively) [32]. It has been hypothesized that the Wa monoclonal RF (Wam-RF) of type II cryoglobulinemia may be protective against the progression of liver disease. The HCV, to evade the immune response, forms complexes with very low density lipoproteins and enters the hepatocytes through the low density lipoprotein receptor. By combining with the HCV–VLDL complex, Wam-RF prevents endocytosis of the virus into the liver cells, and thus may slow down the progress of liver damage [22].

In conclusion, patients with chronic HCV infection without vasculitis have worse liver functions and more advanced liver fibrosis than those with HCV-related vasculitis. Further studies are needed to identify the different protective mechanisms of the hepatocytes.

Disclosures

None.

Conflict of interest

We have no conflict of interest to declare.

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