

Predictive value of rheumatoid factor titre in cryoglobulinemia in Hepatitis C positive patients

Background/objective: HCV viremia has been known to provoke a plethora of autoimmune syndromes as well as nonspecific rheumatologic manifestations. HCV is the most frequent cause of mixed cryoglobulinemia, which is characterized by endothelial deposition of rheumatoid factor containing immune complexes and endorgan vasculitis. Rheumatoid factor positivity is found to be more prevalent among patients with HCV infection compared to the general population. The aim of the study was to ascertain the relationship of rheumatoid factor titer with cryoglobulinemia in hepatitis C virus positive patients and to assess its relation with different disease characteristics.

Methods: A cross sectional study was carried out through one year. Fifty patients known to suffer from HCV were subjects of the study. Patients were interviewed and demographic, clinical and serologic data were recorded. All patients were tested for cryoglobulins by crude method and rheumatoid factor titer was determined in all patients. Child-Pugh classification was used for assessment of liver cell failure. Data were analyzed by the Statistical Package for the Social Sciences (SPSS, version 17). Data analysis is done by using Chi-Squared test (χ^2) test and Fisher's exact test as appropriate. Associations between interval, ordinal and dichotomous variables were tested by Pearson's Product Moment Correlation Coefficients (r).

Results: Cryoglobulinemia was detected in 36 (72%) patients out of the 50 HCV patients. Rheumatoid Factor (RF) was positive in 38 patients (76%). All HCV patients who were positive for cryoglobulin had a positive RF. Presence of RF found to be positively significantly correlated with the presence of cryoglobulins in HCV patients.

Conclusions: HCV infection is a major contributing factor of mixed cryoglobulinemia with elevation in RF titre. Positive anti-HCV antibodies together with highly positive RF titre in the presence of musculoskeletal, neurological and cutaneous manifestations strongly suggest the diagnosis of mixed cryoglobulinemia.

Keywords: Hepatitis C • cryoglobulin • rheumatoid factor

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Introduction

Hepatitis C Virus (HCV) is one of the major globally cause of death and morbidity [1]. Recent estimates showed an increase in its seroprevalence over the last decade to 2.8% infections worldwide [2]. Extrahepatic manifestations are frequently encountered among patients with HCV infection. Many of these manifestations are autoimmune disorders, with added mortality and morbidity due to involvement of multiple organ systems [3].

Mixed cryoglobulinemic vasculitis is known to be the most frequently encountered extrahepatic disease that HCV infection can trigger [4]. Mixed Cryoglobulinemia (MC) is a relatively rare disease associated with many infections or immunological diseases, whose etiology is not yet fully explained. However, HCV infection plays an important role and the contribution of genetic factors and/or environmental factors is still unknown [5,6].

MC is characterized by the aberrant clonal expansion of B cells that produce rheumatoid factor like IgM [5,6]. This forms Immune Complexes (ICs) containing polyclonal HCV-specific IgG and HCV RNA; these complexes in turn deposit on the vascular endothelium of organs such as skin, kidneys, and peripheral nerves, eliciting a complement C1q-mediated vasculitis [7]. Prevalence of MC has been reported to vary from 1% to 56% of the HCV infected individuals. The prevalence of MC increases with the duration of the hepatic illness [8,9].

The aim of the study was to ascertain the relationship of rheumatoid factor titer with cryoglobulinemia in HCV positive patients and to assess its relation with different disease manifestations.

Patients and methods

The current research represents a cross sectional

study conducted over a period of one year. The study included fifty patients with chronic HCV infection. All patients were either outpatients or inpatients of tropical, internal medicine and rheumatology departments of Minia University Hospital, Egypt. The study carried out with the approval of local ethical committee and in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all patients.

All patients were subjected to full history taking and thorough clinical examination. Laboratory investigations included complete blood picture, first hour erythrocyte sedimentation rate (Westergren), C-reactive protein, serum transaminases, serum bilirubin, total proteins, serum albumin, prothrombin time and concentration, international normalization ratio (INR), serum urea and creatinine and complete urine analysis.

Serology included rheumatoid factor (latex agglutination slide test), cryoglobulin (crude method), hepatitis markers for hepatitis C by Enzyme Linked Immune Sorbent Assay (ELISA), and quantitative HCV-Polymerase Chain Reaction (PCR) to assess viral load. Abdominal ultrasonographic examination was done for all patients for assessment of ascitis, liver size and echogenicity, portal hypertension, spleen size and echogenicity, and kidney size and echogenicity. Child-Pugh classification and Child-Turcotte-Pugh (CTP) scoring system were also used for assessment of liver failure [10].

The diagnosis of chronic hepatitis C was based on the presence of anti-HCV antibodies by ELISA with history suggestive of exposure and or liver problems. Only patients who had their antibody profile positive for HCV were included. Patients having end-stage renal disease, other autoimmune disease, or coexisting viral infection like hepatitis B surface antigen positive were excluded from the study.

Statistical analysis

Data were analyzed by the Statistical Package for the Social Sciences (SPSS, version 17). Differences in frequencies were analyzed by Chi-Squared test (χ^2) test and Fisher's exact test as appropriate. Student's t-test was used to compare parametric data while the chi-square test was used when the data were nonparametric. Associations between interval, ordinal and dichotomous variables were tested by Pearson's Product Moment Correlation Coefficients (r).

Two-tailed tests were used throughout, with statistical significance set at the conventional 95% level.

Results

Demographic data and disease characteristics

Among the 50 studied HCV-infected patients, there were 31 females (62%) and 19 males (38%). The mean age of the patients was 51.8 ± 10.9 (ranging from 21-75 years old), the mean duration of illness with hepatitis C was 10.26 ± 4.8 (ranging from 1-21 years).

Among the different hepatic manifestations of HCV patients; ascitis was the commonest manifestation being detected in 34 patients (68%), followed by encephalopathy which was detected in 32 patients (64%), abdominal pain was found in 27 patients (54%) and hematemesis was present in 21 patients (42%). According to Child score; highest score was reported in 18 patients (36%) who were presented with mild degree of liver failure defined as group B. Other scores; group A (compensated liver cirrhosis) and group C (marked degree of liver failure) were represented equally being detected in 16 patients (32%) (Table 1).

Cryoglobulinemia was the main HCV related rheumatologic manifestation being detected in 36 patients (72%). Other rheumatic manifestations like arthralgia and arthritis was detected in 20 patients (40%) and 17 patients (34%) respectively. Raynaud's phenomenon was the least detected rheumatologic manifestation being represented in 12 patients (24%).

One or more neurological manifestations were found in 43 HCV-infected patients (86%), while 7 (14%) had no neurological manifestation. Peripheral neuropathy was the most commonly detected neurologic manifestation in our patients with 22 patients (44%) having parathesia, 12 patients (24%) having mononeuritis, 5 patients (10%) having mononeuritis multiplex and 4 patients (8%) having polyneuritis.

Table 1. Hepatic manifestations of HCV patients (n=50).

	Frequency	Percentage (%)
Ascitis	34	68
Encephalopathy	32	64
Abdominal pain	27	54
Hematemesis	21	42
Child score		
A (5-6)	16	32
B (7-9)	18	36
C (10-15)	16	32

In addition, one or more cutaneous manifestation was detected in 35 HCV patients (70%). These manifestations included palpable purpura in 29 patients (58%), leg ulcers in 4 patients (8%), pruritis in 3 patients (6%), and urticarial rash in only 2 cases (4%).

Comparison between Cryoglobulin +ve & cryoglobulin -ve groups of HCV patients regarding different demographic, clinical and laboratory parameters

By comparing HCV patients who were positive for cryoglobulin (n=36) with those who were negative for the same (n=14); we found that HCV patients who were positive for cryoglobulin had a significantly longer disease duration ($p<0.05$), higher bilirubin, raised ESR and rheumatoid factor titer than HCV patients with negative cryoglobulin ($p<0.01$) (Table 2).

In addition, ascitis and hepatic encephalopathy were significantly more prevalent in the first group than other group ($p<0.01$). Moreover, HCV-infected patients with B Child score were more prevalent in the first group than second one and this difference was statistically significant. ($p<0.05$) (Table 3).

Correlation between cryoglobulin and different disease parameters

Among different laboratory parameters detected in HCV patients; the presence of cryoglobulin was negatively significantly correlated with albumin ($r=-0.47$, $p=0.003$), positively significantly correlated with rheumatoid factor

and ESR ($r=0.38$, $p=0.001$), ($r=0.49$, $p=0.001$) respectively.

Meanwhile and for different hepatic manifestations detected in our patients, presence of cryoglobulin was positively significantly correlated with presence of bilirubin, ascitis, and encephalopathy ($r=0.43$, $p=0.003$), ($r=0.44$, $p=0.001$), ($r=0.64$, $p=0.01$) respectively (Table 4).

Rheumatoid factor was detected in 38 patients (76%). All patients who were RF positive (100%) had cryoglobulinemia with absolute specificity and positive predictive value of RF of 1 for the presence of cryoglobulinemia in HCV-infected patients. Moreover, titre of RF was strongly significantly higher in cryoglobulin positive group than cryoglobulin negative group ($p<0.01$) (Table 2).

Discussion

HCV is a major cause of liver-related morbidity and mortality worldwide and represents a major public health problem [11]. Autoimmune manifestations are common in patients chronically infected by HCV. These manifestations can be dominant, whereas the hepatic disease can be quiescent or mild. Depending on the pathogenic and epidemiological evidence provided by different studies; it is found that MC has a very strong association with hepatitis C virus infection [12].

Since the identification of HCV in 1989, it has been recognized as the cause of about 90% of

Table 2. Comparison between Cryoglobulin +ve & cryoglobulin -ve groups of HCV patients regarding different demographic and laboratory parameters.

	HCV-infected patients with +ve cryoglobulin (n=36)	HCV-infected patients with -ve cryoglobulin (n=14)	t	p-value
Age (years)	52.06 ± 10.8	51.36 ± 11.7	0.2	0.84
Duration of HCV infection (years)	11.94 ± 4.19	5.93 ± 3.5	4.7	0.01*
Articular index	8.11 ± 8.16	6.2 ± 7.84	0.7	0.46
Swollen joint	4.03 ± 4.78	2.36 ± 4.23	1.2	0.24
Hb (gm%)	8.87 ± 1.23	9.4 ± 0.85	-1.5	0.09
Wbcs (mm ³)	4177.8 ± 1475	4964 ± 1331	-1.7	0.08
Platelets (mm ³)	135.5 ± 72.4	146.2 ± 54.2	-0.5	0.62
ESR	60.17 ± 24.20	33.14 ± 13.64	3.9	0.001**
RF titer	252.5 ± 266.5	50.29 ± 38.1	4.4	0.001**
AST	68.03 ± 46.09	63.14 ± 28.42	0.4	0.71
ALT	69.11 ± 48.3	63.43 ± 30	0.5	0.62
Bilirubin	3.05 ± 1.15	1.95 ± 0.71	4.1	0.001**
Albumin	2.81 ± 0.71	3.47 ± 0.58	-3.07	0.71

Hb: Haemoglobin; Wbcs: White Blood Cells; ESR: Erythrocyte Sedimentation Rate; RF: Rheumatoid Factor; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase

* $P<0.05$, significant difference.

** $P<0.01$, significant difference.

Table 3. Comparison between Cryoglobulin +ve & cryoglobulin -ve groups regarding different manifestations in patients with hepatitis C patients.

	HCV-infected patients with +ve cryoglobulin (n=36)	HCV-infected patients with -ve cryoglobulin (n=14)	χ^2	p-value
Fever	11 (30.6%)	7 (50%)	1.65	0.17
Hematemesis	14 (38.9%)	7 (50%)	0.51	0.343
Abdominal pain	19 (52.9%)	8 (57.2%)	2.9	0.234
Ascitis	29 (80.6%)	5 (35.7%)	10.81	0.002**
Encephalopathy	28 (77.8%)	4 (28.6%)	10.35	0.006**
Child score				
A	7 (19.5%)	9 (64.3%)	-10.38	0.03*
B	14 (38.9%)	4 (28.6%)	10.51	0.04*
C	15 (41.7%)	1 (7.2%)	10.12	0.06
RF	36 (100%)	2 (14.3%)		

*p<0.05, significant difference.

**p<0.01, significant difference.

Table 4. Correlation between cryoglobulin and different disease parameters.

	Cryoglobulin	
	r	p
Hb	0.2	0.15
WBCs	0.24	0.62
platelet	-0.72	0.08
ALT	0.06	0.68
AST	0.05	0.71
Albumin	-0.47	0.003**
Bilirubin	0.43	0.003**
RF	0.38	0.001**
ESR	0.49	0.001**
CRP	0.06	0.71
Urea	0.13	0.36
Creatinine	0.04	0.78
Hematemesis	0.1	0.46
Abdominal pain	0.09	0.51
Ascitis	0.44	0.001**
Encephalopathy	0.64	0.01*
Child score	0.45	0.003**

Hb: Haemoglobin; Wbcs: White Blood Cells; ESR: Erythrocyte Sedimentation Rate; RF: Rheumatoid Factor; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase

*p<0.05, significant difference.

**p<0.01, significant difference.

cases of MC and less than 5% of cases are now considered essential, in which no causal agent can be determined [13]. MC can present with different clinico-serological patterns, varying from isolated serum mixed cryoglobulins to complete cryoglobulinemic syndrome that is characterized clinically by the presence of purpura, weakness, arthralgias and by a series of pathological conditions, including chronic hepatitis [14,15], membranoproliferative glomerulonephritis, peripheral neuropathy, skin ulcers, diffuse vasculitis, and less frequently by lymphatic and hepatic malignancies [16].

On the other hand, some patients may be seen with typical cryoglobulinemic syndrome,

but without serum cryoglobulins, which is the hallmark of the disease. This is generally a transient phenomenon due to the wide variability of cryoprecipitable immune-complex levels. Repeated cryoglobulin determinations are necessary for a correct diagnosis in these subjects [15,17].

Cryoglobulinemia is defined by the presence of circulating immunoglobulins that precipitate at cold temperature and dissolve with rewarming [14]. The majority of cryoglobulins are immune complexes that contain RF. Such cryoglobulins are known as "mixed" cryoglobulins [18]. IgM RF binds avidly to anti-HCV IgG or to the IgG-HCV immune complex leading to the presence

of cryoglobulin in the serum. These circulating immune complexes concentrate in capillaries of different tissues, where they deposit in the subendothelium and mesangium and initiate cellular proliferation and leukocyte infiltration causing vascular narrowing and occlusion [11].

Our present study included 50 patients with chronic HCV infection. Demographic data, clinical and serologic profiles were recorded. All patients were tested for cryoglobulins by crude method and rheumatoid factor titer was determined in all patients. Child-Turcotte-Pugh scoring system and Child-Pugh classification were used for assessment of liver cell failure.

Our results denoting that rheumatic, neurologic and skin manifestations are prevalent in our patients. These results are in agreement with Lee, Ji, Yeon, et al. [19] who conducted a study in 49 Korean patients with HCV infection and showed that arthralgia/arthritis was detected in (35%), and parenthesis in 44%. In accordance also with Schwaber and Zlotogorski [20] who found in their studies that skin manifestations are present in about 70% or more of cases with HCV related cryoglobulinemia. Our results also were in agreement with Ferri et al. [21] who concluded that up to 50% of patients with HCV infection can develop a variable combination of clinical or subclinical neuropsychiatric manifestations including peripheral polyneuropathy with variable CNS manifestations.

There are multiple factors predisposing HCV-infected patients to develop rheumatologic manifestations. Chronic stimulation of B cells by HCV directly modulates B-cell and T-cell function that results in polyclonal activation and expansion of B-cell-producing IgM with RF activity. Under this trigger effect, oligoclonal or monoclonal IgM, which shares rheumatoid activity, is produced by a permanent clone of B cells, which favors the appearance of immune-complexes, formed by circulating HCV, anti-HCV polyclonal IgG, and the monoclonal IgM itself [22].

The prevalence of cryoglobulins in our study was (72%). This is in agreement with a prevalence of 70% in a Japanese study of 232 HCV-infected patients using diffusion method for detection of cryoglobulin [23]. A slightly lower than our prevalence, a study by Ferri et al. [24] and Landau et al. [25] who found that 50% to 60% of HCV-infected patients produce a mixed cryoglobulin that will lead to a cryoglobulinemic vasculitis in 15% of cases. In another study of 81 patients

with HCV, a lower prevalence of 45.7% was found [26]. Discrepancies between our study and others in the literature may have several factors: different genetic, environmental, and geographic factors between our study and others. Patients' recruitment at different specialist centers is variable; relative small number of the patients in the different studies. Another important factor is variability in the methods used to determine cryoglobulins in HCV-infected patients using different techniques.

The intrinsic mechanism by which HCV promotes cryoglobulin production is unclear. Virus persistence, therefore, may represent a continuous stimulus for host immune system unable to produce neutralizing antibodies [27,28]. In this context, cryoglobulins may represent the product of virus-host interactions in HCV-infected patients, whereas the production of IgM molecules with RF activity is a crucial event in the cryoprecipitating process [13].

Our results also showed a positive significant statistical correlation between HCV related cryoglobulinemia and RF titre ($p=0.01$) which show activity with increased manifestation of cryoglobulinemia. As cryoglobulin consists of immunoglobulin that has rheumatoid factor activity, it is logical that cryoglobulin and RF are correlated strongly.

Our results were in agreement with Pawlotsky et al., Odum, Sansonne et al. [29-31]; they were found RF activity with increasing titre in HCV associated cryoglobulinemia. On the contrary, Newkirk [32] found low levels of rheumatoid factor in cryoglobulinemia associated with HCV. Variation in such results may be due to different methods of detection of RF (latex or Rose-Waaler) and difficult and sometimes unavailable methods for detection of different genotypes of RF. Small sample size could be also a causative factor, so that RF was only detected in few patients, which may result in failure of association between RF and cryoglobulin to reach statistical significance, even when the association was strong (type II statistical error).

Upon reviewing previous researches, and to our knowledge, no other studies have addressed this association before. The current research was found to be the first one to evaluate the predictive value of rheumatoid factor titre in cryoglobulinemia in hepatitis C positive among Egyptian patients. Results showed a high proportion of the surveyed population having cryoglobulin (72%). The study found positive

RF in the whole number of HCV patients with positive cryoglobulin (100%) compared to 7% in those without ($p=0.001$). A significant correlation was found between seropositivity to rheumatoid factor and the presence of cryoglobulin. All patients who were RF positive (100%) had cryoglobulinemia with absolute specificity and positive predictive value of RF of 1 for the presence of cryoglobulinemia in HCV-infected patients.

Conclusion

We can conclude from this study that HCV infection is a major contributing factor of mixed cryoglobulinemia with elevation in RF titre. So, positive anti HCV antibody together with highly positive RF titre in the presence of musculoskeletal, neurological and cutaneous manifestations strongly suggest the diagnosis of mixed cryoglobulinemia.

The main limitation in our study was the small sample size so that further larger sample size could be recruited. In addition, inclusion of patients with advanced hepatic disease could be a minor cause of comorbid confounder in our study so exclusion of such cases is recommended.

Conflicts of interest

The authors have declared no conflicts of interest.

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