Cryoglobulinaemia in hepatitis C-positive patients in Iran

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ABSTRACT

Introduction: Cryoglobulins are found in a wide spectrum of disorders but are often transient and without clinical implications. The so-called essential mixed cryoglobulinaemia shows a striking association with hepatitis C virus (HCV) infection (greater than 90 percent). Type II cryoglobulinaemia is the major extrahepatic manifestation of HCV infection. This study aims to investigate the frequency of cryoglobulinaemia in HCV-positive patients in central Iran.

Methods: 50 HCV-positive patients referred to the Shaheed Sadoughi Hospital in Yazd, Iran from May 2004 to December 2005, were included in the study. Their sera were assessed for cryoglobulins. The sera were separated by centrifugation at 37 degrees Celsius and placed in a four degrees Celsius refrigerator in two tubes, one simple and the other, a Wintrobe tube, to see if precipitation occurs during a 48-hour up to a seven-day period.

Results: In this study, 50 HCV positive patients were evaluated. Only two patients were female. Mean age was 32 years (ranging from 17 to 52 years). In eight patients, the cryoglobulin test was positive. In seven patients, the test became positive less than 72 hours after sampling. Only one patient who was positive for cryoglobulinaemia, had clinical manifestation of frank vasculitis.

Conclusion: The prevalence of cryoglobulinaemia in Iran may be lower than other areas, and it may be due to a higher prevalence of cryoglobulinaemia in females and lower prevalence of cryoglobulinaemia in HCV genotype-1a. Most of the cases in our study were male and the more prevalent genotype in Iran is genotype-1a.

Keywords: cryoglobulinaemia, hepatitis C, vasculitis

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INTRODUCTION

Cryoglobulinaemia refers to the presence of single or mixed immunoglobulins (Ig) in the serum, which precipitate at temperatures below 37°C and re-dissolve at higher temperatures. This *in vitro* phenomenon can be observed in a wide spectrum of haematological, infectious, and immunorheumatological disorders. Meltzer et al presented the first report of mixed cryoglobulinaemia (MC) in 1966.[1-3] Cryoglobulins are classified into three subgroups according to their immunochemical composition, namely:[4]

Type I cryoglobulinaemia is composed of a single monoclonal Ig, usually IgM. This type accounts for 10%–15% of people with cryoglobulinaemia. It is mainly found in patients with lymphoproliferative disorders (multiplemyeloma, Waldenström macroglobulinaemia).

Type II cryoglobulinaemia is seen in hepatitis C virus (HCV) and with chronic inflammatory states, such as systemic lupus erythematosus, Sjögren syndrome, and viral infections (particularly HCV).[2-5]

HCV infection affects crucial mechanisms of the immune system predisposing to autoimmune and lymphoproliferative disorders. HCV type II cryoglobulinaemia is the major extrahepatic manifestation of HCV infection. In fact, the virus is able to escape immune elimination, leading to a chronic infection, accumulation of circulating immune complexes, and autoimmune phenomena. Furthermore, HCV stimulates the production of monoclonal rheumatoid factors (RF).[6] Cryoglobulinaemic vasculitis is an immune-complex-mediated systemic vasculitis involving small- to medium-sized vessels. A causative role of HCV in over 80% of patients has been definitively established, with a heterogeneous geographical distribution.[7]

The main clinical features of MC are purpura, arthralgias, weakness (the typical clinical triad reported by the majority of patients at diagnosis), liver involvement, renal involvement, peripheral neuropathy and widespread vasculitis. The vasculitic lesions of the skin and of other organs are the consequence of vascular deposition of circulating immune complexes and complements. There is generally no relationship between the severity of vasculitic manifestations and the serum levels of cryoglobulins or complements. Patients with type II MC may develop a malignant B-cell lymphoproliferative disorder.[7,8] In this study,

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Table I. Clinical and laboratory characteristics of HCV patients with cryoglobulinaemia.

Cryoglobulinaemia	Positive n (%)	Negative n (%)	Total n	p-value
Gender				
Male	7 (14.6)	41 (85.4)	48	0.181
Female	1 (50)	1 (50)	2	
ALT level (IU/L)				
< 50	3 (23.1)	10 (76.9)	13	
50–100	5 (14.7)	29 (85.3)	34	0.577
100–400	0	3 (100)	3	
Co-infection				
HBsAg	0	3 (100)	3	0.436
HIV	0	2 (100)	2	0.529
Acquisition route				
IV drug	5 (15.2)	28 (84.4)	33	
Transfusion	0	2 (100)	2	0.749
Unknown	3 (20)	12 (80)	15	
Age (years)				
≤ 32	3 (20)	12 (80)	15	0.614
> 32	5 (14.3)	30 (85.5)	35	

the prevalence of cryoglobulinaemia in patients with HCV was evaluated and the relationships between age, gender, route of disease acquisition and co-morbidity of other organs were investigated.

METHODS

50 HCV-positive patients referred to the Shaheed Sadoughi Hospital in Yazd, Iran from May 2004 to December 2005, were included. There was no exclusion criteria for age and gender, or underlying diseases, for this study. Their sera were assessed for cryoglobulins, where 10 ml of blood was drawn while the patient was in the fasting state (since lipids may interfere with the test), into a tube not treated with an anticoagulant. The syringe was also preserved in an incubator at 37ºC. The tubes were placed in warm water (37ºC) and transported to the laboratory. The serum was separated by centrifugation at 37ºC and placed in a 4ºC refrigerator in two tubes, one simple and another Wintrobe tube, to see if precipitation occurs over a 48-hour to a seven-day period.

RESULTS

In this study, 50 HCV positive patients were evaluated. Two patients were female. Mean age was 32 years, ranging from 17 to 52 years. Two patients were positive for HIV and three patients were also positive for hepatitis B virus (HBV) as co-infections. 13 out of 50 patients (26%) had alanine aminotransferase (ALT) less than 50 U/ml, 68% had ALT 50–100 U/ml and 6% had ALT 100–400 U/ml. 66% of patients acquired HCV from intravenous drug abuse and 4% from transfusion (one patient was thalassaemic and another one was haemophilic). No known risk factor was found in 30% of the patients. In eight patients, the cryoglobulin test was positive. In seven patients, the test became positive within 72 hours of sampling. Unfortunately, the duration of acquired hepatitis C stage and grade of the HCV diseases were not available.

No relationship was found between age, gender, ALT level, HBsAg, HIV antibodies and presence of cryoglobulins in the serum (Table I). Only one patient who was positive for cryoglobulinaemia had the clinical manifestation of overt vasculaitis, such as artheralgia, palpable purpurae, kidney involvement, proteinuria and haematuria.[9] Seven other patients with positive cryoglobulinaemia had no clinical and biochemical stigmata of vasculitis or cryoglobulinaemia syndrome.

DISCUSSION

Chronic hepatitis is frequently observed during the clinical course of mixed MC. A possible role for hepatotropic viruses in the pathogenesis of the disease

has long been suggested.[10-12] HCV infection affects crucial mechanisms of the immune system, and predisposes the patient to autoimmune and lymphoproliferative disorders. HCV type II cryoglobulinaemia is the major extrahepatic manifestation of HCV infection.[13-15] In this study, no correlation was found between cryoglobulinaemia and age or gender. There was also no relation between cryoglobulinaemia and co-infection with HBV, HIV, levels of liver enzyme, and route of disease acquisition. Since the number of cases studied here is less than optimal, the results must be viewed with caution.

The prevalence of cryoglobulinaemia differs from one geographic area to another. In spite of the relationship between HCV and cryoglobulinaemia, the frequency of HCV antibodies is different in these patients.[1,4,5] This difference is probably due to a different version of HCV antibodies tested during screening or due to co-infection of HBV or HIV, which themselves induce cryoglobulinaemia.[16] Age, gender and race may be important in these results. In one study of haemophilic patients, there was a close relationship between HCV duration and cryoglobulinaemia.[17] Different HCV genotypes are also important in the formation of cryoglobulinaemia.[18] Cryoglobulinaemia is more common in the 2a and 2c genotype, compared to other HCV genotypes. As HCV genotype-1a is more prevalent in Iran, this may explain the low prevalence of cryoglobulinaemia in our HCV patients.[16,19]

Another study that investigated the role of HCV, liver damage in the pathogenesis of cryoglobulinaemia and the prevalence of cryoglobulinaemia in 226 consecutive patients with chronic liver diseases (HCV in 127 patients) produced results which suggested that HCV is a major cause of cryoglobulinaemia. Besides the viral infection itself, multiple factors appear to be responsible for the production of cryoglobulins, including cirrhosis and duration of liver disease.[16] In another study of 19 patients with MC, 42% were tested positive for HCV antibodies and 84% positive for HCV RNA. Further study found a relationship between HCV and cryoglobulinaemia and lymphoma.[12,13] In another study of haemophilic HCV-positive patients, no relationship was found between cryoglobulinaemia and stage of liver disease, age, liver enzyme level and HBV-positivity.[17]

Diagnosis of cryoglobulinaemia needs a high index of suspicion, because the symptoms are very diverse and many subspecialties are involved in the management of these patients. Detection of cryoglobulinaemia is highly operator-dependent. Serum samples to be processed for detection of cryoglobulins must be collected carefully to ensure accurate quantisation. The laboratory that is to receive the sample must be notified in advance to prepare for the processing, because some cryoprecipitation may occur even at room temperature (22°C). In half of patients with MC, disease progression is slow; in the other half, progression is from moderate to severe, and leads to renal and liver failure. Older age, the male gender, and kidney involvement are associated with poor prognosis. In our series, only one patient had a full-blown clinical picture of MC. The patient was treated with Interferon and Ribaverin and he improved clinically. Subsequent HCV-PCR (polymerase chain reaction) tests yielded negative results.

Clinical presentation of cryoglobulinaemia was seen in 10%–50% of studies. Cryoglobulinaemia presents in a clinical spectrum, which includes cryoprecipitant and positive RF, sometimes with decreased C4 at one end, and at the other end, full-blown cryoglobulinaemia with serological involvement of multiple organs with leucocytoclastic vasculitis. The important issue is that cryoglobulinaemia may cause the HCV antibodies to be tested falsely negative. HCV-PCR test in these patients is recommended to confirm the HCV infection. Interestingly, another study showed that PCR assay for HCV may be falsely negative even in the case of cryoglobulinaemia.[20-22] The first study evaluating the prevalence of HCV infection by a PCR technique in a large series of Italian patients with MC showed a striking correlation between HCV seropositivity (91%) and HCV viraemia (86%).[20]

The low prevalence of cryoglobulinaemia in this study may be due to a higher prevalence of cryoglobulinaemia in females and a low prevalence of cryoglobulinaemia presenting with HCV genotype-1a. The majority of the patients in our study were male, and the more prevalent genotype in Iran is genotype-1a.[16,17,19] We also only used HCV-antibodies as a marker of HCV infection and as this test is not sensitive enough, some patients may be missed.[20]

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