Original Article

HCV-RNA and Anti-HCV IgM in Egyptian Subjects Bearing IgG Anti-HCV Antibodies

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SUMMARY: The hepatitis C virus (HCV) infection is associated with a wide spectrum of clinical entities ranging from asymptomatic carriage to severe forms of chronic hepatitis. In Egypt, HCV infection has been shown to be highly prevalent. The aim of this study was to determine the frequency and significance of anti-HCV IgM in the sera of clinically healthy blood donors and chronic HCV patients, whose sera were also positive for anti-HCV IgG. Anti-HCV IgM was detected in the sera of 7 (46%) of the blood donors (n=15), of whom 5 (71%) had a positive HCV-RNA. The corresponding results in patients with a chronic hepatitis C (CHC) infection (n=19) were 8 (42%) and 5 (62%) respectively. The detection of anti-HCV IgM did not correlate with a positive test for HCV-RNA ($R=0.2$) in the CHC patients. However, the levels of anti-HCV IgM in CHC patients were associated significantly with the level of serum transaminase, a finding that can be used in monitoring disease activity in such a group of patients. On the other hand, a significant association was evident between the detection of anti-HCV IgM and HCV-RNA in the sera of blood donors. Thus among the blood donors, viraemia correlates well with the detection of HCV-IgM Ab, but it cannot be excluded in its absence. The presence of HCV-IgM in some patients with CHC infection indicates that the antibody as a viral marker may not be unique to acute HCV infection.

INTRODUCTION

The detection of IgM antibodies directed to viral proteins has been demonstrated to be a useful serological marker of recent viral infections. During the chronic course of an infection, IgM antibodies may become detectable in the "re-activation" or "flare-up" phase. In chronic infections such as chronic hepatitis B (HB), it has been demonstrated that IgM antibodies to the nucleocapsid of hepatitis B virus (HBV) (anti-HBc) persist in circulation, and this persistence correlates with viral replication, the biological activity of chronic HB, and a more aggressive state of the disease (1).

The IgM antibody has a similar prognostic value in a chronic hepatitis delta virus (HDV) infection (2), where it has been found that the presence of IgM anti-HDV correlates with an underlying liver inflammation and is also predictive of cirrhosis (3). Furthermore, the rate of disease progression in the human immunodeficiency virus (HIV) infection has been related to a specific IgM anti-HIV response, suggesting that individuals at risk of rapid disease progression can be identified through measurements of specific IgM concentrations (4).

For the hepatitis C virus (HCV) infection, HCV IgM has been described as a marker of disease activity (5,6) and appears to be associated with HCV-RNA (7). It is well known that HCV is responsible for a number of chronic liver diseases (8). However, some HCV-RNA positive subjects have been reported to have normal alanine aminotransferase (ALT) levels together with normal liver histology (9).

In Egypt, hepatitis C is a major health problem with 14.5%

seroprevalence among urban blood donors. The seroprevalence in a rural community-based study was 15.5%, 70.4% for haemodialysis patients, 75.6% in thalassaemic multi-transfused children, and 7.7% in health care workers (10).

The aim of this study was to test the hypothesis that the IgM anti-HCV core might help in monitoring disease activity in the same way that anti-HBc IgM helps in chronic HBV infection. Also, the aim was to test whether the IgM anti-HCV core could help in discriminating between HCV-RNA positive subjects with and without liver disease, among apparently healthy blood donors and chronic hepatitis C (CHC) patients.

MATERIALS AND METHODS

Fifteen serum samples (13 males and 2 females) from the blood bank of the Suez General Hospital, Suez City, Egypt which had been taken from apparently healthy volunteer blood donors, all with a negative history for jaundice, hospital admission due to fever, and blood transfusion within the past 6 months were used. The medical examination of the donors excluded cardiac diseases, hypertension, and clinical signs of anemia. These donors also tested negative for hepatitis B surface antigen (HBsAg), tested normal ALT, and all of them were positive for IgG anti-HCV antibodies. Their ages ranged from 35 to 59 years with a mean age of 46.3. Additionally, 19 consecutive CHC patients attending the hepatology clinic at Suez Canal University Hospital, Ismailia, Egypt, and who showed no evidence of autoimmune or drug-induced liver diseases were used as subjects. A liver histology previously performed on all 19 chronic hepatitis cases showed the features of chronic persistent hepatitis in 8 cases and chronic active hepatitis in 11 cases. The ages ranged from 29 to 48 years with a mean age of 39.6. None of this group of patients had received anti-viral

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treatment.

All serum samples which tested negative for HBsAg and tested positive for the antibodies to HCV by a second generation enzyme immunoassay were tested for anti-HCV IgM (anti-HCV EIA, Abbott Laboratories, North Chicago, Ill.), transaminases levels (ALT, AST) and for HCV-RNA.

Detection of IgM Anti-HCV: Antibodies of the IgM subclass directed against the recombinant HCV core antigen were assayed with a specific EIA. Both EIA tests, anti-HCV IgG and IgM, were used according to the manufacturer's instructions.

Detection of HCV-RNA: All of the sera, 15 from the blood donors and 19 from CHC patients, were tested for the presence of HCV-RNA by the reverse transcriptase polymerase chain reaction (RT-PCR).

Viral RNA extraction: A QIAamp viral RNA purification kit (QIAGEN Ltd., Crawley, England) was used for viral RNA extraction by combining the selective binding properties of a silica-gel-based membrane with the speed of microspin technology. The samples were first lysed under highly denaturing conditions to inactivate the RNases and to ensure isolation of the intact RNA. The samples were then adjusted to the appropriate binding conditions and applied to a spin column (QIAamp Spin Column Ltd., Crawley, England) where RNA binds while contaminants are washed away and the RNA is eluted in water ready for amplification. Briefly, 140 μ l of serum was pipetted into a 1.5 ml microfuge RNase free tube, and 560 μ l of freshly prepared buffer containing carrier RNA was added, mixed thoroughly by vortexing, and incubated at room temperature for 10 min. Then, 560 μ l of 96% ethanol was added to the sample and mixed. After mixing, 630 μ l of the solution was placed in the spin column in a 2 ml collection tube and centrifuged at 6000 $\times$ g (8000 rpm) for 1 min. The tube containing the filtrate was properly discarded. The previous step was repeated, and 500 μ l of wash buffer was added to the spin column and centrifuged for 1 min. Another 500 μ l of wash buffer was added, and then it was centrifuged for 3 min. The RNA was eluted with 50 μ l of RNase-free water, preheated to 80°C, and centrifuged at 6000 $\times$ g for 1 min. The eluted RNAs were used for cDNA synthesis.

cDNA synthesis and amplification of HCV-RNA: The samples were tested for HCV-RNA by reverse transcription of the extracted RNA, followed by double PCR using nested primers from the 5'-untranslated region (UTR) of the HCV genome (Sorin Biomedica Diagnostics, Saluggia, Italy). The cDNA was synthesized by the addition of the reverse transcriptase enzyme (Moloney leukemia virus transcriptase) and antisense 319R. Amplification was performed following the nested primers protocol (11,12), with two sets of primers located in the 5'UTR of the viral genome. The first round of PCR (35 cycles) was carried out using primer 1 CH (5'GGTGCACGGTCTACGAGACCTC-3') and 2 CH (5'AACTACTGTCTTCACGCAGAA-3'), generating a PCR product of 289 bp. For the second round (25 cycles), we used primers 1TS (5'GCGACCCAACACTACTCGGCT-3') and 4CH (5'ATGGCGTTAGTATGA-GTG-3'), generating a PCR

product of 187 bp. The PCR cycles were as follows: denaturation at 94°C for 1 min, annealing of primers at 55°C for 1 min, and elongation at 72°C for 2 min. The amplification products were analyzed by agarose gel electrophoresis and visualized under UV after staining with ethidium bromide.

RESULTS

HCV-RNA could be detected in 6 (40%) of the 15 asymptomatic HCV carriers and in 15 (79%) of the 19 chronic HCV patients (Fig. 1). Among the 15 asymptomatic anti-HCV-positive subjects without active liver disease, 7 (46%) had detectable IgM anti-HCV, but 5 (71%) of them were HCV-RNA positive. On the other hand, 8 (42%) of the 19 patients with CHC were seropositive for IgM anti-HCV, of whom 5 (62%) had a positive HCV-RNA in their sera (Table 1).

The sensitivity and the specificity of IgM anti-HCV as a marker of hepatitis C viraemia in all patients with HCV infection was found to be low (50% and 62%, respectively). Meanwhile, the sensitivity and the specificity of IgM anti-HCV testing in asymptomatic patients was found to be moderate (83% and 78%, respectively).

Patients with chronic hepatitis who tested negative for IgM anti-HCV ($n=11$) had mean ALT values that were significantly lower than those of the IgM anti-HCV-positive patients (67 ± 18.4 IU/L vs. 129.2 ± 14.7 IU/L, $p>0.02$). In addition, there was a positive correlation between the raised level of the serum ALT and that of the IgM anti-HCV in patients who were positive for IgM anti-HCV (Fig. 2).

No correlation existed between anti-HCV IgM and hepatitis C viraemia ($R=0.2$) in CHC patients, meanwhile a significant correlation was evident between the detection of anti-HCV IgM and viraemia among the asymptomatic carriers ($R=0.5$).

DISCUSSION

In this study, the IgM anti-HCV antibody and HCV-RNA were tested in two groups of subjects, apparently healthy blood donors (asymptomatic carriers) and CHC patients. Our study demonstrated the presence of the IgM anti-HCV antibody in patients with CHC (8/19). Such a finding may support the role of the active host's immune response to HCV replication in the pathogenesis of liver injury in hepatitis C. The positive correlation between anti-HCV IgM levels and the high levels of liver enzymes may support this idea (Fig. 2). Also, the work of Brilliante et al. (7), who demonstrated that HCV-RNA levels did not correlate with ALT levels in patients with community-acquired hepatitis C, confirms our observation. They stated that a possible role could be present between the host immune response and liver injury.

High levels of IgM anti-HBc were detected in individuals with persistent HBV infection (13) and they reflected the activity of hepatitis (1,2). Likewise, the presence of anti-HCV IgM antibody in patients with CHC may be used for monitoring the disease activity in persons with persistent HCV infection. This study demonstrated a strong correlation between the

Table 1. Relationship between HCV-RNA and anti-HCV IgM in the two studied groups

Anti-HCV IgM	Asymptomatic Carriers		Chronic hepatitis C		Total
	HCV-RNA Positive	HCV-RNA Negative	HCV-RNA Positive	HCV-RNA Negative	
Positive	5	2	5	3	15
Negative	1	7	10	1	19
Total	6	9	15	4	34

Fig. 1. Nested PCR for HCV detection with an amplification product of 289 bp (upper arrow) and 187 (lower arrow).

levels of liver enzymes and the levels of anti-HCV IgM antibodies (Fig. 1), which means that it is possible to use this class of antibody in monitoring disease activity and the outcome of antiviral therapy. Many studies (14,15,16) have used anti-HCV IgM antibody as a marker in predicting the response to interferon therapy. Their results show that high or intermediate levels of anti-HCV IgM are usually associated with a poor response to interferon, while the disappearance of the antibody is usually associated with a sustained response.

Some previous studies have demonstrated the absence of anti-HCV IgM in asymptomatic patients with a hepatitis C infection (5,17). In this study, 46% of the asymptomatic subjects were found to be positive for anti-HCV IgM. We assume that the presence of this antibody in the asymptomatic group could be an early sign of chronicity or of an exacerbation of HCV-associated liver disease. In support of this view is the work of Quiroga et al. (18), who found that anti-HCV IgM reappears in serum preceding a biochemical relapse. They suggested that anti-HCV IgM testing could predict the exacerbation of HCV-associated liver disease. Also, Nagayama et al. (14) suggested that IgM anti-HCV antibodies could be particularly useful in following symptom-free carriers of HCV for the activity of liver disease.

IgM anti-HCV antibodies have been shown to be induced frequently during the acute phase of the disease (19,20). However, our results indicate that both asymptomatic and chronic hepatitis patients have circulating IgM antibody to the putative structural (core) protein of the HCV genome. This finding is in contrast to the common course in other virus infections, where IgM antibody usually represents an acute infection. This means that IgM anti-HCV cannot be used as a unique marker in the diagnosis of acute HCV infection.

In this study, IgM anti-HCV antibodies did not correlate with HCV-RNA in the CHC group of patients, but it was significantly correlated with the asymptomatic carrier's group. However, the work of Quiroga et al. (18) demonstrated that the IgM anti-HCV antibodies could be detected in most patients with ongoing viral replication and persistent liver disease, a feature that could not be resolved in our study.

Three conclusions may be drawn from this study: 1) the presence of IgM anti-HCV antibodies in asymptomatic and

Fig. 2. Relationship between IgM anti-HCV (C100-3 antigen) values, expressed as the enzyme immunoassay ratio of sample OD to negative control mean OD, and ALT values in patients with chronic hepatitis C ($R=0.7$, $p<0.001$).

chronic HCV groups indicate that this class of immunoglobulin cannot be considered as a unique marker for acute HCV infection; 2) the detection of anti-HCV IgM could be used as a marker of chronic liver disease; and 3) anti-HCV IgM is usually associated with HCV-RNA viraemia in asymptomatic blood donors, but its absence cannot exclude viraemia.

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