

• VIRAL HEPATITIS •

Autoantibodies and hepatitis C virus genotypes in chronic hepatitis C patients in Estonia

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Abstract

AIM: To determine the prevalence of several autoantibodies in chronic hepatitis C patients, and to find out whether the pattern of autoantibodies was associated with hepatitis C virus (HCV) genotypes.

METHODS: Sera from 90 consecutive patients with chronic hepatitis C were investigated on the presence of anti-nuclear (ANA), anti-mitochondrial (AMA), anti-smooth muscle (SMA), anti-liver-kidney microsomal type 1 (LKMA1), anti-parietal cell (PCA), anti-thyroid microsomal (TMA), and anti-reticulin (ARA) autoantibodies. The autoantibodies were identified by indirect immunofluorescence. HCV genotypes were determined by a restriction fragment length polymorphism analysis of the amplified 5' noncoding genome region.

RESULTS: Forty-six (51.1%) patients were positive for at least one autoantibody. Various antibodies were presented as follows: ANA in 13 (14.4%) patients, SMA in 39 (43.3%), TMA in 2 (2.2%), and ARA in 1 (1.1%) patients. In 9 cases, sera were positive for two autoantibodies (ANA and SMA). AMA, PCA and LKMA1 were not detected in the observed sera. HCV genotypes were distributed as follows: 1b in 66 (73.3%) patients, 3a in 18 (20.0%), and 2a in 6 (6.7%) patients.

CONCLUSION: A high prevalence of ANA and SMA can be found in chronic hepatitis C patients. Autoantibodies are present at low titre (1:10) in most of the cases. Distribution of the autoantibodies show no differences in the sex groups and between patients infected with different HCV genotypes.

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Key words: Chronic hepatitis C; Autoantibodies; HCV genotypes

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INTRODUCTION

Autoimmune diseases are characterized by the loss of tolerance

against self-antigens, activation of autoreactive lymphocytes and pathological damage of single or multiple organs^[1]. As a secondary event, autoreactivity is detected in liver disorders associated with a variety of etiological factors (e.g., drug- and chemical-induced autoimmunity, viral and microbial infection-induced autoimmunity)^[2,3]. Among them, viral infections play the most relevant role, in particular hepatitis C virus (HCV). A broad spectrum of extrahepatic syndromes has been observed in chronic hepatitis C. Autoimmune manifestation ranges from non-organ-specific autoantibody seropositivity and cryoglobulins to immunological diseases such as glomerulonephritis, vasculitis, lichen planus, and mixed cryoglobulinemia^[4-7]. The relationship between such autoimmune disorders as autoimmune gastritis and celiac disease, and HCV infection has been indicated but remains speculative^[8,9].

Many organ- and non-organ-specific autoantibodies are commonly found in the sera of HCV-infected patients. Antibodies against smooth muscles (SMA) are a heterogeneous group of antibodies of different specificity that reacts with cytoskeleton antigens of smooth muscle cells. In chronic HCV infection, SMA are found in 10-66% of cases. The prevalence of antinuclear antibodies (ANA) ranges between 6% and 22%, and they are usually presented at low titer (1:40-1:80). Anti-liver-kidney microsomal antibodies (LKMA) are found in the cytoplasm of hepatocytes and proximal renal tubules. They are directed against different epitopes on cytochrome P450. LKMA type 1 was detected in low titre in up to 10% of chronic hepatitis C patients^[10-15]. Anti-asialoglycoprotein receptor, anti-liver membrane antigen, anti-liver cytosol antigen, anti-hepatocyte plasma membrane, anti-thyroglobulin, anti-thyroid peroxidase, anti-thyroid microsome, anti-phospholipid, anti-neutrophil cytoplasmic, and many other autoantibodies have also been described in patients with HCV infection^[1,2,7]. Each antibody is directed against a certain intracellular antigen released during cell death and presented to the immune system. Their pathogenic role and clinical significance remain unclear.

Most of the previous studies showed that there was no significant difference in clinical and biochemical parameters between chronic C hepatitis patients with and without positive serum autoantibodies^[12,16,17].

HCV isolates have been classified into 6 major genotypes and more than 70 subtypes (Simmonds' classification)^[18]. Particular genotypes are associated with different courses and outcome of liver diseases, and also with different responsiveness to interferon therapy. It was hypothesized that various amino acid sequences of each genotype might elicit different autoantibodies or other immune reactions (or both)^[16]. Results of the studies to clarify the relationship between HCV genotype and autoimmune manifestations are controversial. A majority of them failed to confirm the association between clinical course of HCV infection, autoimmune disorders and particular HCV genotypes.

The aims of the present study were to determine the prevalence of several autoantibodies in chronic hepatitis C patients, and to find out whether the pattern of autoantibodies was associated with HCV genotype.

MATERIALS AND METHODS

Patients

Ninety consecutive patients (male 58, 64.4%, female 32, 35.6%) with established chronic hepatitis C from the two hospitals of Estonia (Tallinn Central Hospital and Tartu University Clinics) were studied. The diagnosis of chronic hepatitis C was based on the presence of elevated aminotransferase level associated with HCV RNA positivity for more than 6 mo and the presence of chronic inflammation in liver biopsy. None of the patients had received antiviral treatment before the study. Percutaneous liver biopsy was performed in 72 patients, who were advised to undergo interferon monotherapy or the combination of interferon and ribavirin therapy. The results of the biopsies were consistent with a diagnosis of chronic hepatitis alone or with liver cirrhosis.

HCV RNA and genotypes

Serum samples were frozen and stored at -20 °C until use. The presence of HCV RNA in the serum samples was determined using reverse transcription-PCR protocol with conservative primers from 5' noncoding region of HCV genome (Amplicor Roche hepatitis C virus test). HCV genotypes were determined by the restriction fragment length polymorphism (RFLP) analysis of the amplified 5' noncoding genome region as described previously^[19].

Autoantibodies

Sera were investigated for the presence of IgG type ANA, AMA, SMA, LKMA, PCA, TMA, and ARA antibodies. For autoantibody detection, standard indirect immunofluorescence tests on unfixed 4 µm cryostat sections from a composite block of mouse stomach, rat kidney and liver as well as from hyperplastic human (blood group 0) thyroid gland were used. The sera were diluted 1:10 and 1:100. Rabbit anti-human IgG fluorescein isothiocyanate (FITC) - conjugated secondary antibody was used^[20].

Statistical analysis

A statistical analysis (χ^2 test) was performed to determine the relationship between the presence of autoantibodies and HCV genotypes, and also the differences in the distribution of autoantibodies between men and women. $P < 0.05$ was considered statistically significant.

RESULTS

Presence of autoantibodies (Table 1)

Among 90 patients, 46 (51.1%) were positive for at least one autoantibody. SMA and ANA were detected most frequently in the observed sera. SMA was present in 39 (43.3%) and ANA in 13 (14.4%) sera. SMA in dilution 1:100 was detected in 4 males and 1 female, and SMA in dilution 1:10 in 25 male and 9 female patients. ANA 1:100 was positive in 1 male and ANA 1:10 in 8 male and 4 female patients. In 9 cases, sera were positive for two autoantibodies (ANA and SMA at different dilutions). TMA (1:10 and 1:100) was found in 2 female patients. ARA 1:100 was found in 1 male patient. AMA, PCA and LKMA1 were not detected in the observed sera.

HCV genotypes

The most prevalent HCV genotype in the study population was 1b (66 % or 73.3% patients). Other genotypes were distributed as follows: 3a in 18 (20.0%) patients, and 2a in 6 (6.7%) patients.

Relationship between autoantibodies and HCV genotypes (Table 2)

The data demonstrating the relationship between the presence

of different autoantibodies and HCV genotypes, and also the differences in the distribution of autoantibodies between men and women are shown in Table 2. Significance level was $P > 0.05$ in all cases. Thus, there were no differences in the distribution of autoantibodies between the patients infected with different HCV genotypes and between the sex groups.

Table 1 Distribution of autoantibodies in chronic hepatitis C patients

Autoantibodies	Patients, total (n = 90) n%	Males (n = 58) n%	Females (n = 32) n%
ANA 1:10	12 (13.3)	8 (13.8)	4 (12.5)
ANA 1:100	1 (1.1)	1 (1.7)	0 (0)
SMA 1:10	34 (37.8)	25 (43.1)	9 (28.1)
SMA 1:100	5 (5.5)	4 (6.9)	1 (3.1)
ANA+SMA	9 (10.0)	7 (12.1)	2 (6.3)
ARA 1:100	1 (1.1)	1 (1.7)	0 (0)
TMA 1:10	1 (1.1)	0	1 (3.1)
TMA 1:100	1 (1.1)	0	1 (3.1)
Total	46 (51.1)	32 (55.2)	14 (43.7)

Table 2 Comparison of distribution of HCV genotypes among patients positive for different autoantibodies and between sex groups

	Total (n = 90) n (%)	1b (n = 66) n (%)	3a (n = 18) n (%)	2a (n = 6) n (%)	Men (n = 58) n (%)	Women (n = 32) n (%)
ANA	13 (14.4)	10 (15.2)	2 (11.1)	1 (16.6)	8 (13.8)	5 (15.6)
SMA	39 (43.3)	30 (45.5)	8 (44.4)	1 (16.6)	29 (50.0)	10 (31.3)
TMA	2 (2.2)	2 (3.0)	-	-	-	2 (6.3)
ARA	1 (1.1)	-	1 (5.6)	-	1 (1.7)	-
Total	46 (51.1)	37 (56.1)	9 (50.0)	1 (16.6)	32 (55.2)	14 (43.8)

DISCUSSION

Our study demonstrated a high prevalence of serological markers of autoimmunity among the patients chronically infected with HCV. In other similar studies, these serological features have been found to characterize HCV-infected patients independent of their clinical status, i.e., patients with chronic hepatitis C, patients with mixed cryoglobulinemia or other autoimmune manifestations or symptom-free HCV-infected individuals^[17]. In the majority of patients, autoantibodies were found low titre.

In Estonia, autoantibody studies have been made in unselected adult populations. According to one of these studies, the prevalence of AMA (when tested by immunoblotting against beef heart mitochondria) was <1% (13 positive sera out of 1 461 samples), that is in agreement with the reported incidence of less than 1% AMA in a mixed hospital population^[21]. In our study, AMA was not detected in the chronic hepatitis C patients (this may be due to the relatively small study group). In the other investigations in Estonia the prevalence of common tissue autoantibodies was studied in 448 healthy adults^[20]. Standard indirect immunofluorescence tests on tissue and *Chritidia lucilia* antigenic preparations were used. According to these papers ANA was found in 3% among the male persons and 11% among the female persons, showing a significantly higher prevalence of ANA among females. Other autoantibodies in this study were presented as follows (males/females): SMA 11%/10%, ARA <1%, PCA 2%/6%, TMA 2%/4%, and the total population being 22%. None of the serum samples was positive for LKMA in both studies. Compared with these findings, the presence of different autoantibodies in the chronic hepatitis C

patients in our study was significantly higher (total prevalence 51.1% vs 22%, $P < 0.05$). ANA autoantibodies in the female patients made an exception, their prevalence did not differ significantly (12.5% vs 11%). The differences in the distribution of different autoantibodies between the men and women in our study were also insignificant, although in the total population the prevalence of autoantibodies usually tended to be higher in women^[20]. The absence of LKMA antibodies in the investigated hepatitis C patients as well as in the study of Uibo *et al*^[20], could be a reflection of real low prevalence of these autoantibodies in Estonian population.

The genome of HCV is very variable, having an extremely high spontaneous mutation rate. On the basis of the degree of variability, HCV isolates were classified into genotypes and subtypes^[18]. Different HCV genotypes have been shown to have a varying impact on the severity of chronic diseases, effectiveness of interferon treatment, consequences of liver transplantation, and diagnostic procedures. A HCV genotyping study with 242 patients has been recently conducted in Estonia. The most prevalent (determined by the restriction fragment length polymorphism) was HCV subtype 1b (64.2%) and subtypes 3a and 2a, and other subtypes were presented respectively in 22.3%, 5.6% and 7.9% of the cases^[22]. The distribution of HCV genotypes in the present study group (1b, 73.3%, 3a, 20.0%, and 2a - 6.7%) is very similar to the previous investigation. This fact could indicate an independent selection of the patients, but also could be an indirect evidence for the absence of association between the serological markers of autoimmunity and HCV genotypes. It was hypothesized that the viral antigens of different genotypes might elicit different autoantibodies or other immunological reactions in the particular host. Several studies in this field have shown that the serological pattern of autoantibodies does not correlate with the particular genotype of HCV^[12,16,23]. Our study also failed to find any association between the pattern of autoantibodies and HCV genotypes. One of the reasons could be a real absence of such an association, another a relatively small study group, which did not allow making a statistical analysis (e.g., there was only one patient with HCV genotype 2a, who was positive for autoantibodies).

The clinical significance of the serological markers of autoimmunity is still an object of discussions. But it seems that there are no significant differences in clinical and biochemical parameters between chronic hepatitis C patients with and without autoimmune features^[12,13]. A recent study on the general population showed that in the absence of active liver disease the prevalence of non-organ specific autoantibodies was similar in HCV positive individuals and negative controls^[23]. The presence of non-organ-specific autoantibodies is more likely associated with the patient's age and duration and severity of chronic liver disease. Thus, reactivity against self-antigens can be related to the severity of liver damage without any independent pathogenic role.

A variety of environmental and host-related predisposing factors play a role in the pathogenesis of HCV infection determining the course of the disease, including autoimmune manifestations. The mechanisms of the development of autoimmune disturbances in HCV infection are mainly unknown. In genuine autoimmune liver disease autoantibody titres are high, restricted linear autoantigen epitopes are involved, and B-cell response is homogeneous. In contrast, virus-induced autoimmunity is represented by low autoantibody titre, multiple linear and conformational autoepitopes, and B-cell response is heterogeneous^[3]. In the case of chronic hepatitis C the polymorphism and nonspecificity of autoimmune manifestations, usually low autoantibody titre, the absence of association

between the clinical course of liver disease and viral genotype with the pattern of autoimmune reactions, could likely indicate generalized nonspecific activation and alteration of reactivity of the host immune system. The present study supports this hypothesis. Further investigations are required to better understand the interactions between HCV and the host immune system and the mechanisms of HCV-related autoimmunity.

In conclusion, a high prevalence of ANA (14.4%) and SMA (43.3%) is found in chronic hepatitis C patients in Estonia, although in the majority of the cases autoantibodies are presented in the sera at a low titre (1:10). Distribution of autoantibodies does not differ significantly in the sex groups and between the patients infected with different HCV genotypes. Compared to the total Estonian population the presence of different autoantibodies in chronic hepatitis C patients is significantly higher, with the exception of ANA autoantibodies, whose prevalence in female patients differs insignificantly.

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