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ORIGINAL ARTICLE

The frequency of non-organ-specific autoantibodies in patients with chronic hepatitis C and its relation with disease severity and response to therapy

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ABSTRACT

Background: Increased levels of non-organ-specific autoantibodies are frequently seen in patients suffering from chronic hepatitis C (CHC); however, the etiology and its effects on the course of the disease and response to therapy are largely undetermined. Particularly, it seems of utmost importance to define whether this increase is solely an insignificant coincidence or a major finding which have an impact on the course of the disease.

Materials and methods: Fifty-two patients with CHC (case group) and 52 aged- and sex-matched IBS patients (controls) were enrolled. The sera of all subjects were checked for non-organ-specific autoantibodies, including anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-mitochondrial antibody (AMA), and anti-liver/kidney microsomal antibody (ALKM). All cases underwent a liver biopsy and treated with a 12-month course of combination therapy with interferon and ribavirin.

Results: The mean age of cases and controls was 32.8±12.7 and 31.6±14.1 years, respectively. The overall frequency of non-organ-specific antibodies was significantly higher in anti-HCV positive patients in comparison with controls (36.5% vs 7.7%, $p<0.001$). Seropositivity of ANA and ASMA was significantly higher in patients with CHC than in controls (11.5% vs. 1.9%, $p<0.05$ and 13.5% vs. 1.9%, $p<0.027$, respectively). There was no significant relationship between seropositivity of different autoantibodies and patients' age and sex, duration of disease and serum aminotransferases levels. Nor this seropositivity had significant relationship with grade and stage of the liver disease and response to treatment, while serum globulin level was significantly higher in ANA positive patients.

Conclusion: Seroprevalence of ANA and ASMA seems to be higher in patients with CHC but its impact on the severity of disease and response to therapy is the subject for further investigations.

Keywords: *Hepatitis C, Autoantibody, Response to treatment, Aminotransferases.*
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INTRODUCTION

Immunological alterations are quite common entities in patients with chronic hepatitis C infection. These immunological alterations could be manifested as non-organ-specific autoantibodies (NOSAs) (1), anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA) (2), anti-liver/kidney microsomal type-1 antibodies (LKM1)

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(3), cryoglobulins, or anti-thyroid antibodies in the blood of patients with glomerulonephritis, Lichen planus, lymphocytic sialadenitis, or mixed cryoglobulinaemia (1-19). These associations could in part, explain the immune modulation induced by hepatitis C virus (HCV) (20) or, as in the case of NOSAs, be a manifestation secondary to the hepatocellular damage in relation to the genetic background of the host (21).

It is not clear whether this coincidence is the result of an immunological response to HCV or a random co-presentation. Disease activation in HCV-infected patients positive for autoantibodies during interferon therapy suggests the possibility that the drug may activate an autoimmune reaction, and the presence of autoantibodies is the hallmark of subclinical autoimmune disease (22-24).

PATIENTS and METHODS

Fifty-two patients with chronic hepatitis C, who had been referred to the Gastroenterology Clinic of Imam Khomeini hospital in Tehran were included. We considered the following baseline inclusion criteria: positive HCV-Ab (ELISA) for more than 6 months, positive HCV- RNA (PCR), elevated serum ALT, as well as features of chronic HCV infection on liver biopsy with or without cirrhosis. However, the following exclusion criteria were observed: pregnancy, intention for forthcoming pregnancy, spouse of a pregnant woman, coinfection with hepatitis B or HIV virus, comorbidities (i.e. ulcerative colitis, idiopathic thrombocytopenic purpura, lupus erythematous, autoimmune hemolytic anemia, scleroderma, autoimmune thyroiditis, psoriasis, or rheumatoid arthritis), hemoglobin level of less than 13g/dl in males and 12g/dl in females, neutrophil count of less than 1,500/dl and platelet count of 90,000/dl or less.

All patients were investigated for serum anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), anti-liver/kidney microsomal antibodies, and anti-mitochondrial antibodies (AMA).

Moreover, all patients received a combination therapy with interferon (3,000,000 units 3 times weekly) and ribavirin (1000-1200 mg/day) for 12 months. Meanwhile, 52 age- and sex-matched patients with irritable bowel syndrome (IBS) who had normal liver enzymes levels were randomly selected from the same clinic as the control group. The baseline exclusion criteria were also applied for controls. Response to therapy was defined as negative HCV-RNA PCR and normal liver enzymes levels after a 12-month combination treatment; however, if these changes persisted during a further 6-month follow up period, then the patient was considered to have a sustained virologic response. All patients were requested to complete an informed consent. A checklist of demographic, laboratory, and pathologic features was completed prior to the commencement of therapy and during follow up. Data were analyzed by SPSS for Windows (version 10.5, USA) and student t-test, Mann-Withney U-test, and chi-square tests were used, when appropriate.

RESULTS

The mean age of the patients was 32.8±12.7 years with male to female ratio of 2.5:1. Of 52 cases, 19 (36.5%) were positive for non-organ-specific antibodies. However, ANA, ASMA, AMA, and ALKM were positive in 6(11.5%), 7(13.5%), 5(9.6%), and 1(1.9%) chronic hepatitis C patients, respectively. The mean age of the control group was 31.6±14.1 years. Totally, autoantibodies were detected in 4 control subjects (7.7%); ANA with the titer of 1/40, ASMA with the titer of 1/40, and AMA with the titers of 1/20 and 1/40 in different cases. The overall frequency of non-organ-specific antibodies (NOSAs) was significantly higher in anti-HCV positive cases than in controls ($p<0.001$). Seropositivity of ANA and ASMA were also significantly higher in cases

Table 1. *Distribution of serum aminotransferases (AST, ALT), albumin, globulin, and liver pathologic grades and stages in chronic hepatitis patients according to autoantibodies status*

	Anti-nuclear antibody (ANA)		Anti-smooth muscle antibody (ASMA)		Anti-mitochondrial antibody (AMA)	
	Positive (n=6)	Negative (n=46)	Positive (n=7)	Negative (n=45)	Positive (n=5)	Negative (n=47)
Age (year)	28.3±9.2	34.2±13.2	32.3±11.7	34.0±13.3	30.8±20.6	34.1±12.2
Duration of disease (year)	17.0±9.5	29.0±22.9	29.5±20.3	26.5±22.7	43.0±26.6	25.1±21.2
ALT (mg/dl)	85.5±20.4	87.5±28.3	129.3±110.0*	80.8±41.0	126.4±57.5	82.2±55.0
AST (mg/dl)	63.5±14.5	70.8±30.8	108.1±75.6*	64.4±27.5	93.4±37.1	67.8±39.2
Globulin(mg/dl)	4.7±0.7*	3.4±1.0	3.6±1.3	3.5±1.0	3.6±0.8	3.5±1.1
Albumin(mg/dl)	4.6±0.2	4.4±0.6	4.1±0.8	4.4±0.5	4.5±0.6	4.4±0.5
Grade	5.0±2.5	6.5±2.5	6.2±2.2	6.4±2.6	5.4±2.0	6.4±2.6
Stage	2.8±1.0	3.3±1.7	2.8±2.3	3.3±1.6	2.0±0.7	3.4±1.7

* Differences were statistically significant between groups.

than in controls ($p<0.05$ and $p<0.027$, respectively). Groups have shown differences in the frequency of AMA and ALKM, however, these differences did not reach a statistically significant level. The mean serum levels of aminotransferases, albumin, and globulin are summarized in table 1.

The mean globulin level was significantly higher among ANA-positive cases ($p<0.048$). Our results failed to show any significant association between ANA positivity and serum liver enzymes levels, however, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly higher among ASMA-positive cases as compared with ASMA-negative cases ($p<0.006$ and $p<0.037$, respectively). Finally, positive AMA was not associated with serum aminotransferase and globulin levels. Meanwhile, levels autoantibodies were not correlated with patient's age, sex, the duration of the disease, and pathologic findings of the liver. Of 5 AMA-positive cases, four appeared to have lower and one moderate stage disease, but elevated level of alkaline phosphatase was detected in one subject. Following a 12-month combination therapy, 28 cases (53.8%) showed a favorable response as documented with negative HCV-RNA PCR and normal liver enzymes levels. Nevertheless, sustained virologic response was reported in 21 cases (46.6%). Our findings highlighted that autoantibodies have not altered the profile of response in our patients.

DISCUSSION

Hepatitis C virus (HCV) has been implicated in the development of a variety of autoimmune phenomena, some of which are well documented and include a panel of auto-antibodies shared with autoimmune hepatitis (AIH). ANA and SMA have been demonstrated in 6-21 and 14-55 percent of cases, respectively (1-8,25). Lower titers of ANA and ASMA might be detected in 41-76% of chronic hepatitis C patients (26,27). However, ANA titers of 1:160 and higher can be detected in 22% of HCV-infected cases. Moreover, ANA might be present with titers of 1:640 or higher without any features of autoimmune diseases (15). On the other hand, anti-LKM is definitely rare, with the prevalence of zero to 5% in different studies (1-3,5,9,10,25). These controversies could be explained by different selection criteria and laboratory methods in different studies. Lenzi and colleagues have reported a frequency of 25% for

non-organ-specific antibodies (NOSAs) among anti-HCV positive patients, which was higher than HbsAg-positive cases and normal controls (6 and 7 percent, respectively) (1). We have detected 19 (36.5%) patients with positive NOSAs that in accordance with Lenzi and many other previous studies which agree on the prevalence rate of 33% for positive non-organ-specific antibodies among chronic hepatitis C patients (2,3,6,9). The frequency of ANA, ASMA and ALKM was the same as many other studies, however, we have reported positive AMA more frequently than others (2-4%) (6,28-30). Prior investigators have explained this controversy by the presence of primary biliary cirrhosis in hepatitis C patients, however as mentioned earlier, none of our AMA-positive patients were cirrhotic, but one had elevated serum alkaline phosphatase. Thus, this hypothesis seems to be inapplicable for this group of Iranian patients. Differed laboratory methods could probably explain this discrepancy; however, future studies may shed further insight into the possible mechanisms. Autoantibodies are often detected in low titers. It can explain the immunomodulatory effect of hepatitis C. In our study, titers of autoantibodies were quite low; however, some studies have reported higher titers. In the latter condition, the differentiation between chronic hepatitis C and autoimmune hepatitis and treatment could be a challenging situation (26). Interferon therapy may result in progression of autoimmune hepatitis. On the other hand, steroids may cause replication of HCV. Bellary et al. reported two such cases that have been treated with prednisolone and azathioporin (26). Liver biopsy might be beneficial in differentiation of hepatitis C and autoimmune hepatitis. Portal lymphoid aggregation, mild macrovesicular steatosis, and the damage to biliary ducts are mostly present in hepatitis C (30,31), whereas, severe piecemeal necrosis, lobular hepatitis, and giant cells are the dominant features of autoimmune hepatitis (7,31). Corticosteroid therapy offered to hepatitis C patients may result in normalization of aminotransferases levels; however, HCV viremia may increase in such cases. Although prior studies did not find a relationship between virus count and the severity of the disease, further studies are strongly recommended in this field (32).

The association between ALKM-1 and hepatitis C is presented in European studies. This could imply a genetic basis. Positive ALKM-1 in anti-HCV positive cases was mostly detected among older male patients with a positive history of blood transfusion (26). On the other hand, type 2 autoimmune hepatitis patients are often females with higher titers of autoantibodies (10, 15). During recent decades, treatment with interferon and its effect on autoantibodies were subjects of interest for researchers. Clifford et al. showed that autoantibody titers do not decrease during the treatment; also they are not predictive of weak response to treatment (27). Furthermore, some autoimmune diseases such as thyroiditis may develop in patients receiving interferon therapy (26), though autoimmune hepatitis is an exception (33). These patients may manifest with severely elevated levels of aminotransferase, among whom, cessation of therapy and introducing immunosuppressives may persistently control the disease. It is not clear whether this condition is an interferon-induced autoimmunity or an immunological condition in relation with hepatitis C (34). Elevated antiviral IgG level has been documented in acute autoimmune hepatitis patients despite the lack of active viral infection. This may result in false positive reports for viral infection (26). Polycolonal induction of humoral immunity might be the cause of this condition (35). On the other hand, autoimmune hepatitis-induced hypergammaglobulinemia could result in false positive ELISA test. In our study, HCV-RNA PCR was employed for all chronic hepatitis patients; therefore, this hypothesis may not be applicable for our cases. Prior investigators did not find a specific HCV genotype in NOSAs-positive cases (1,25).

We did not examine this issue due to our limitations. Our study agrees with previous studies implicating that autoantibodies are not associated with the severity of liver biopsy findings. In general, the prevalence of non-organ-specific antibodies is higher in anti-HCV positive subjects.

REFERENCES

1. Lenzi M, Bellentani S, Saccoccio G, et al. Prevalence of non-organ-specific autoantibodies and chronic liver disease in the general population: a nested case-control study of the Dionysos cohort. Gut 1999;45:435-41.

2. CliVord BD, Donahue D, Smith L, et al. High prevalence of serological markers of autoimmunity in patients with chronic hepatitis C. Hepatology l995;21: 613-19.

3. Meyer zum Buschenfeldc KH, Lohse AW, Gerken G, et al. The role of autoimmunity in hepatitis C infection. J Hepatol 1995;22(suppl 1):93-6.

4. Cassani F, Muratori L, Manotti P, et al. Serum autoantibodies and the diagnosis of type-1 autoimmune hepatitis in Italy: a reappraisal at the light of hepatitis C virus infection. Gut 1993;33:1260-3.

5. Abuaf N, Lund F, Giral P, et al. Non-organ specific auloantibodies associated with chronic C virus hepatitis. J Hepatol 1993;18:359-64.

6. Lenzi M, Johnson PJ, McFarlane LG, et al. Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogenecity. Lancet 1991;338:277-80.

7. Czaja AJ, Carpenter HA, Santrach PJ, et al. Evidence against hepatitis viruses as important causes of severe autoimmune hepatitis in the United States. J Hepatol 1993;18:342-52.

8. Fried MW, Draguesku JO, Shindo M, et al. Clinical and serological diVerentiation of autoimmune and hepatitis C virus-related chronic hepatitis. Dig Dis Sci 1993;38:631-6.

9. Czaja AJ, Manns MP, Homburger HA. Frequency and significance of antibodies to liver/kidney microsoine tYpe I in adult with chronic hepatitis. Gastroenterology 1992;103:1290-5.

10. Lund F, Abuaf N. Frangeul L, et al. Liver/kidney microsome antibody type 1 and hepatitis C virus infection. Hepatology 1992;16:630-6.

11. Tran A, Quaranta JF, Benzaken S, et al. High prevalence of thyroid autoantibodies in a prospective series of patients with chronic hepatitis C before interferon therapy. Hepatology 1993;18:253-7.

12. Cacoub P, Lunel-Fabiani F, Musset L, et al. Mixed cryoglobulinemia and hepatitis C virus. Am J Med 1994; 96:124-32.

13. Lund F, Musset L, Franjeul L, et al. Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. Gastroenterology 1994;106:1291-300.

14. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med 1992;327:1490-5.

15. Pawlotsky SM, Ben Hayia M, Andre C, et al. Immunological disorders in C virus chronic active hepatitis: a prospective case-control study. J Hepatol 1994;19:841-8.

16. Johnson JR, Gretch DR, Yamabe H, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. N EngI J Med 1993; 328:465-70.

17. Adinolfi LE, Utili R, Attanasio V, et al. Epidemiology, clinical spectrum and prognostic value of mixed cryoglobulinemia in hepatitis C virus patients a prospective study. J Hepatol Gastroenterol 1996;28: 1-9.

18. Wong VS, Egner W, Elsey T, et al. Incidence, character and clinical relevance of mixed cryoglobulinemia in patients. With chronic hepatitis C virus infection. Clin Exp Immunol 1996;104:25-31.

19. Haddad J, Deny P, Munz-Gotheil C, et al. Lymphocytic sialoadenitis of Sjogrcn's syndrome associated with chronic hepatitis C virus liver disease. Lancet 1992;339:321-3.

20. Muratori L, Gibelhini D, Lenzi M, et al. Quantification of hepatitis C virus-infected peripheral blood mononuclear cells by in situ reverse transcriptase polymerase chain reaction. Blood 1997;88:2768-74.

21. Czaja AJ, Herschel A, Carpenter A, et al. Immunological features and HLA association in chronic viral hepatitis. Gastroenterology 1995;108:157-64.

22. Muratori L, Lenzi M, Cataleta M, et al. Interferon therapy in liver/kidney microsomal antibody type 1 positive patients with chronic hepatitis C. J Hepatol 1994;21:199-203.

23. Papo T, Marcellin P, Bernuau J, et al. Autoimmune chronic hepatitis exacerbated by alpha-interferon. Ann Intern Med 1992:116:51-3.

24. Ruiz-Moreno M, Rua MJ, Carreno V. et al. Autoimnmne chronic hepatitis type 2 manifested during interferon therapy in children. JHepatol 1991;12:265-6.

25. Reddy KR, Krawitt EL, Homberg JC, et al. Absence of LKM 1 antibody in hepatitis C viral infection in the United States. J Viral Hepatitis 1995;2:175-9.

26. Bellary S, Schiano T, Hartman G, Black M. Autoimmune hepatitis and/or hepatitis C: How to decide. Hepatology 1996;23:647-9.

27. Clifford BD, Donahue D, Smith L, et al. High prevalence of serological markers of in autoimmunily in patients with chronic hepatitis C. Hepatology 1995;21:613-19.

28. Luo JC, Hwang SJ, Li CP, et al. Clinical significance of serum auto-antibodies in Chinese patients with chronic hepatitis C: negative role of serum viral titer and genotype. J Gastroenterol Hepatol 1998; 13(5):475- 9.

29. Garrido Palma G, Sanchez Cuenca JM, Olaso V, et al. Response to treatment with interferon-alfa in patients with chronic hepatitis C and high titers of-M2, -M4 and M8 antimitochondrial antibodies. Rev Esp Enferm Dig 1999;91(3):168-81.

30. Rolachon A, Pasquier D, Girard M, et al. Is there a relationship between the presence of autoantibodies or mixed cryoglobulinemia and the clinical and histological characteristics of chronic viral hepatitis C? Gastroenterol Clin Biol 1994;18:251-6.

31. Kalvenes MB, Haukenes G, Nysaeter G, et al. Raised levels of antibodies to human viruses at the clinical onset of autoimmune chronic active hepatitis. J Viral Hepatitis 1995;2:159-64.

32. Bach N, Thung S, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992;15:572-77.

33. Booth J, Foster GR, Kumar U, et al. Chronic hepatitis C virus infections: predictive value of genotype and level of viremia on disease progression and response to interferon alpha. Gut l995;36:427-32.

34. Papo T, Marcellin P, Bernuau J, Durand F, et al. Autoimmune chronic hepatitis exacerbated by alpha-interferon. Ann Intern Med 1992;116:51-3

35. GarcB a-Buey L, GarcB a-Monzo'n C, Rodriguez S, et al. Latent autoimmune hepatitis triggered during interferon therapy in patients with chronic hepatitis C. Gastroenterology 1995;108:1770-7.