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Role of Interferon-gamma and Immune Response Biomarkers in Predicting IFN-alpha Responsiveness and Treatment Outcome in Patients with Hepatitis C Virus

¹Amira F. Barakat, ¹Asmaa Hegazy, ²Raghda E. Farag, ³Azza Abdul Baky, ⁴Lamiaa F. Arafa and ³Amal Farouk

¹Department of Internal Medicine, ²Department of Tropical Medicine, Mansoura Faculty of Medicine, El-Mansoura, Egypt

³Department of Clinical Pathology, Ain Shams University Hospital, Cairo, Egypt

⁴Department of Medical Biochemistry, Mansoura Faculty of Medicine, El-Mansoura, Egypt

Corresponding Author: Amira F. Barakat, Department of Internal Medicine, Mansoura Faculty of Medicine, El-Mansoura, Egypt

ABSTRACT

The aim of this study was to examine interferon-gamma (IFN- γ), anti-C1q antibodies, myeloperoxidase antineutrophil cytoplasmic antibodies (MPO-ANCA) and anticardiolipin antibodies in the serum of biopsy-proven chronic hepatitis C patients before and after interferon (IFN)-alpha and ribavirin therapy to address whether or not viral clearance is related to these biomarkers and to explore a possible association between the pattern of these immune response parameters with the virological and biochemical status of Hepatitis C Virus (HCV). The serum levels of IFN- γ , anti-C1q, myeloperoxidase ANCA and anticardiolipin antibodies were assayed on 64 patients with chronic hepatitis C virus infection before and 48 weeks after treatment with pegylated IFN- α plus ribavirin and compared with sera from 20 normal control subjects. Serum levels of IFN- γ , anti-C1q, myeloperoxidase ANCA and anticardiolipin antibodies were significantly higher in HCV patients in comparison to healthy controls ($p < 0.0001$). IFN- γ levels were significantly increased after 48 weeks of antiviral treatment when compared to pretreatment serum levels and it was significantly more elevated in responder HCV patients than non responders. While as, both anti-C1q antibodies and myeloperoxidase ANCA levels were significantly decreased after 48 weeks antiviral treatment in the HCV patients. Moreover, IFN- γ levels (but not other studied biomarkers) in HCV patients correlated significantly with high alanine aminotransferase (ALT) levels as well as with high viral load ($r = 0.675$, $p \leq 0.05$, $r = 0.912$, $p \leq 0.001$, respectively). In conclusion, IFN-gamma might be useful in predicting the clinical outcome of the combination therapy of pegylated-IFN alpha and ribavirin, as well as responsiveness to IFN-alpha-based therapy may be improved by using easily assessed immune response biomarkers such as interferon gamma, anti-C1q antibodies. Furthermore, early treatment in HCV patients with multiple serological abnormalities will prevent further autoimmune response which eventually could prevent marked extrahepatic complications.

Key words: Interferon-gamma, immune response biomarkers, predicting IFN- α responsiveness, hepatitis C virus

INTRODUCTION

Infection with hepatitis C virus (HCV) may be associated with a wide spectrum of immunological abnormalities. Hepatitis C virus tends to induce nonspecific autoimmune reactions,

as demonstrated by the high prevalence of various autoantibodies. In addition, interferon-gamma (IFN- γ), as one of the T helper related cytokine, has elicited great interest in chronic viral infections because it is abundantly produced and has direct antiviral activity. The pathogenesis of viral chronic liver disease that leads to liver damage is suggested to be immune-mediated (Feng *et al.*, 2009). The immunopathogenesis of chronic Hepatitis B (HBV) or HCV infection occur through the participation of CD8 T cells, CD4 T cells and Natural Killer (NK) cells as well as cytokines (Kondo *et al.*, 2004). A previous study indicates that HBV and HCV could induce both early and late immune responses (Feng *et al.*, 2009). The immunological and genetic aspects of patient defenses which determine the outcome of virus/host interaction will lead to persistent viraemia as have been suggested by previous studies (Khakoo *et al.*, 2004; Cooper *et al.*, 1999; Thimme *et al.*, 2001). Moreover, innate immune mechanisms are involved also in the development of chronic persistent replication of intracellular pathogens (Lodoen and Lanier, 2005; Ahmad and Alvarez, 2004).

Infection with hepatitis C virus may be associated with a wide variety of immunological abnormalities that could be demonstrated by high prevalence of various autoantibodies (Kisiel and Kryczka, 2007) as well as the presence of circulating immune complexes (Jablonska *et al.*, 2008). Most extrahepatic manifestations of the hepatitis C virus infection occur through the influence of virus on the host's immune system. From the commonly encountered serological immune response to HCV infection is the development of cryoglobulinemia, rheumatoid factor, anticardiolipin, antinuclear antibodies (Mouelhi *et al.*, 2008).

Antiphospholipid antibodies (aPL) (lupus anticoagulant and anticardiolipin antibodies) are a heterogeneous family of immunoglobulins that react with complexes of phospholipids and plasma proteins (Kisiel and Kryczka, 2007). Previous reports have mentioned the increased prevalence of aPL antibodies in several bacterial, parasitic and viral infections (Sene *et al.*, 2009). Most of the previous studies confirms that anticardiolipin antibodies are frequently found in patients with chronic HCV infection but mostly of no clinical importance (Kisiel and Kryczka, 2007; Sene *et al.*, 2008, 2009). Some studies however, have found an increased incidence of thrombotic disorders in patients with chronic hepatitis C virus who manifest aPL positivity (Rafai *et al.*, 2006; Cojocaru *et al.*, 2005). Thus, the clinical significance of antiphospholipid antibodies in patients with chronic hepatitis C virus and some other viral infections is controversial (Habibagahi *et al.*, 2007). Moreover, their amelioration under antiviral therapy and correlation with the virological load remains to be determined (Sene *et al.*, 2009).

Autoantibodies against a variety of self-antigens can be detected in the sera of patients with HCV infection. C1q is the first component of the classical pathway of complement activation and its main function is to clear immune complexes from tissues and self-antigens generated during apoptosis (Walport, 2001). Autoantibodies against C1q have been described in many immune-complex diseases including hypocomplementemic urticarial vasculitis and Systemic Lupus Erythematosus (SLE) (Tsirogianni *et al.*, 2009; Wisnieski *et al.*, 1995). Few previous studies had focused on the presence of anti-C1q antibodies in hepatitis C virus infection and aimed at evaluation of the prevalence of anti-C1q antibodies in HCV infection (Saadoun *et al.*, 2006; Lienesch *et al.*, 2006). However, assessing the role of antiviral treatment of HCV patients on anti-C1q and its correlation with the viral load and liver status was not yet studied.

Regarding the role of Anti-neutrophil Cytoplasmic Antibodies (ANCA) in HCV patients, a previous study by DeRiva and colleagues suggests that the finding of ANCA by ELISA is common not only in autoimmune Chronic Liver Disease (CLD) but, also in viral-related CLD

(Valentina *et al.*, 2009). Furthermore, the positivity for ANCA might have a prognostic value in patients with viral-related as well as autoimmune-related cirrhosis (Valentina *et al.*, 2009). Recently, a high prevalence of ANCA has been reported in patients with HCV (Yasuda *et al.*, 2011). But again, assessing the role of antiviral treatment of HCV patients on ANCA needs further assessment.

Natural Killer (NK) cells are innate immune cells known for their immediate effectors functions against virus-infected cells and tumor cells. These effectors functions include the destruction of target cells via the production of cytokines, such as tumor necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ) (Vivier *et al.*, 2011). IFN-gamma, was particularly studied because it is abundantly produced and has direct antiviral activity (Billiau and Matthys, 2009). It has been recently shown that patients with chronic hepatitis C virus (HCV) infection display a polarized NK cell phenotype with increased cytotoxicity and IFN- γ production (Ahlenstiel *et al.*, 2010; Ohviero *et al.*, 2009; Stegmann *et al.*, 2010). Furthermore, other studies stated that the kinetics of the *in vivo* responsiveness of NK cells, with IFN- γ production, to the IFN- α therapy in human are not yet known and it will be important for the therapeutic use of IFN- α in chronic HCV infection (Edlich *et al.*, 2012). It is well documented that imbalance of T helper 1 (Th1) and T helper 2 (Th2) may exert a critical influence on the inflammatory environment of the host as well as the final outcome of infection (Sobue *et al.*, 2001; Bertoletti *et al.*, 1997). Previous reports have shown that IFN- γ may directly inhibit virus replication and mediate liver injury (Shin *et al.*, 2005; Penna *et al.*, 1997; Byrnes *et al.*, 2007; Jo and Thimme, 2010).

Combination therapy of pegylated interferon- α (Peg-IFN- α) plus ribavirin is standard treatment for patients with chronic hepatitis C nowadays. This combination has led to a Sustained Virological Response rate (SVR) of 50 to 80% depending on genotype. This percentage still unsatisfactory if we consider the side effects of the treatment, overall costs and the prolonged duration of therapy. So far, different strategies have been developed to predict SVR in HCV infected patients on antiviral therapy such as genotype, fibrosis stage, viral load and genetic polymorphism related to race, insulin resistance and viral kinetics (Moraes Coelho and Villela-Nogueira, 2010). Studying further predictive factors that are readily accessible from the peripheral blood and easily to be assessed in the laboratory might help the decision about starting or discontinuing therapy in chronic HCV infected patients.

Although, serological auto-immune manifestations in HCV infected patients were explained by the lymphotropism of HCV, interferon-based treatment of HCV infection where accused by some studies to precipitate or exacerbate the associated auto-immune disease (Mouelhi *et al.*, 2008). Thus, in patients with serological auto-immune disorders associated with HCV infection careful interpretation of clinical and biological features is necessary. Furthermore, using more specific antibodies can be helpful in differentiating whether these serologic autoimmune manifestations are induced by HCV infection or related to the use of antiviral treatment in such patients (Mouelhi *et al.*, 2008).

The aim of the study was to investigate the features of immune response in patients with HCV infection before and after IFN- α and ribavirin therapy, through assessing one of the T helper-related cytokines (interferon- γ), plus a variety of autoantibodies which are prevalent in HCV patients including; autoantibodies against C1q, myeloperoxidase ANCA and anticardiolipin antibodies. Moreover, we aimed to address whether or not viral clearance is related to the studied immune mediators and to explore the possible association between these immune biomarkers and both virological and biochemical status of HCV patients.

MATERIALS AND METHODS

This study was conducted on 64 chronically viraemic HCV genotype 4 patients (M/F: 50/14; aged 36.71±14.12 years) from Internal Medicine and Tropical departments, Mansoura University Hospital, in the period between October, 2009 to November 2011. In addition to twenty age and sex matched healthy subjects served as controls.

Clinical and immunologic status of HCV patients were prospectively studied before and then 48 weeks after antiviral treatment with combination of pegylated IFN- γ and ribavirin.

Patients were selected according to Egyptian International Program Guideline Selection Criteria with the following inclusion criteria: Age 18-60 years, HCV RNA positive in serum compensated liver disease (total serum bilirubin <1.5 mg dl⁻¹; INR 1.5, serum albumin \geq 3.5, platelet count (not less than) \geq 90,000 mm with no evidence of hepatic decompensation (hepatic encephalopathy, esophageal varices or ascites) and acceptable hematological and biochemical indices (Hemoglobin \geq 13 g dl⁻¹ for men and 12 g dl⁻¹ for women; neutrophil count >1500 mm⁻³ and serum creatinine <1.5 mg dl⁻¹, with Body Mass Index \leq 30, liver biopsy findings compatible with chronic viral hepatitis in the preceding 12 months. The exclusion criteria were: Age less than 18 or more than 60 years; previous treatment with interferon or ribavirin or neutropenia (<than 1500 neutrophils mm⁻³), thrombocytopenia (<than 90. 000 platelets mm⁻³), anemia, serum creatinine more than 1.5 times above the upper limit of normal; history of alcohol or hemolytic disease; decompensated cirrhosis; autoimmune hepatitis; hepatitis B infection; HIV infection; current intravenous drug use; severe depressive illness; severe comorbid disease; organ transplant; pregnancy; unwilling to practice contraception and hepatocellular carcinoma. Informed consents were obtained from subjects included in the study.

Chronic HCV patients with determined genotype 4 were selected for the study while as other genotypes were excluded from the study.

Patients were given pegylated interferon alpha-2a in a fixed dose of 180 μ g weekly by subcutaneous injection. All of them received ribavirin in an adjusted dose according to body weight; patients <75 kg were given 1000 mg and those >75 kg were given 1200 mg. The safety was assessed by clinical evaluation and laboratory tests at week 1, 2, 4 and monthly thereafter during treatment. Stepwise reductions in the interferon and ribavirin dosages were allowed according to international guidelines to manage adverse events or laboratory abnormalities that had reached predetermined thresholds of severity. Patients were given growth factors (erythropoietin hormone and macrophage colony stimulating factor when possible) to avoid dose reduction in IFN and/or ribavirin.

Complete physical examination and laboratory investigations including liver function tests (ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin; PT, prothrombin time) and complete blood count were done for all participants.

Serologic evidence of chronic HCV infection was determined by the detection of antibodies to HCV (anti-HCV) using fourth generation enzyme immunoassay (HCV antibody by ELISA using Cobas/Core supplied by Roch Germany). While active viral replication was defined by the detection of HCV RNA using a quantitative assay based on real-time PCR using Tag PCR master kit, Qiagen, Hilden, Germany. Viral load was assessed by HCV-RNA at baseline and 24 weeks after treatment. Subjects were considered to have a Sustained Virological Response (SVR) if they had negative HCV RNA 24 weeks after completing the 48 weeks of treatment. Subjects were considered relapsers if they had positive HCV RNA at week 72 after negative HCV RNA at 48 weeks of treatment. Subjects who failed to attain a negative HCV RNA at week 24 from the start of treatment or a

decline of HCV RNA of $>2 \log_{10}$ IU ml⁻¹ at week 12 of treatment were considered Non Responders (NR) and all were enrolled in our study.

Immunological study was done for all participants and includes: Interferon-gamma (IFN- γ) cytokine, serum anti-C1q antibodies, anticardiolipin IgG antibodies (ACL) and myeloperoxidase anti-neutrophil cytoplasmic antibodies MPO-ANCA.

Serum IFN- γ was assayed by a sandwich enzyme linked immunosorbent assay technology supplied by Boster Biological Technology Co. Results were expressed as pg ml⁻¹.

Anti-C1q was assayed by immunometric enzyme immunoassay supplied by Orgentec Diagnostika GmbH (Germany) (Walport *et al.*, 1998). Results were expressed as unit ml⁻¹ (U ml⁻¹) and positive anti-C1q was considered if the serum level was more than 10 U ml⁻¹, as recommended by the manufacturer as the cutoff value.

Anticardiolipin IgG antibodies, was assayed by immunometric enzyme immunoassay, using Demeditec Diagnostics GmbH (Germany). Results were expressed as GPL unit ml⁻¹. Normal serum levels were considered normal if 10 GPL U ml⁻¹.

Serum myeloperoxidase anti-neutrophil cytoplasmic antibodies (MPO-ANCA) was assayed by immunometric enzyme immunoassay (IgG against myeloperoxidase using Orgentec Diagnostika GmbH (Germany). Results were expressed as U ml⁻¹. Normal serum levels were considered normal if <5.0 U ml⁻¹.

Statistical analysis: The statistical analysis of data was done using SPSS (SPSS, Inc. Chicago, IL), program statistical package for Social Science (version 16). To test the normality of data distribution K-S (Kolmogorov-Smirnov) test was done only significant data revealed to be nonparametric. The description of the data was done in form of mean \pm standard deviation (mean \pm SD) for quantitative data. Nonparametric data were expressed as median and range. For quantitative data student t-test was used to compare between two groups. Mann-Whitney test and Kruskal-Wallis were used for non parametric data. To test the association between variables Pearson correlation co-efficient test was used. The p-value is considered significant if ≤ 0.05 at confidence interval 95% (Munro, 2002).

RESULTS

In the present study serum levels of IFN- γ , anti-C1q, myeloperoxidase ANCA and anticardiolipin antibodies were significantly higher in HCV patients at baseline (before treatment) in comparison to healthy controls ($p < 0.0001$). Data are shown in Table 1.

IFN- γ levels were significantly increased after 48 weeks antiviral treatment when compared to pretreatment serum levels and it was significantly more elevated in responder (SVR) HCV patients than Non Responders (NR). While as, both anti-C1q antibodies and myeloperoxidase ANCA levels were significantly decreased after 48 weeks antiviral treatment in the HCV patients Table 2.

Regarding anticardiolipin antibodies (ACL), there was reduction in the serum ACL levels after antiviral treatment but it was not statistically significant Table 2.

In this study IFN- γ levels (but not other studied immune biomarkers) in HCV patients correlated significantly with high ALT levels as well as with high viral load ($r = -0.675$, $p < 0.05$, $r = -0.912$, $p < 0.001$, respectively).

All the pretreatment serum levels of the studied immunological parameters were significantly correlated with their post treatment serum levels (Table 3).

Table 1: Comparison between serum levels of immunologic parameters in HCV patients at baseline (before treatment) versus controls

Immunologic parameter	HCV (n = 64)(Median-range)	Controls (n = 20)(Median-range)	p-value
Interferon- γ (pg ml ⁻¹)	240 (160-380)	16.4 (9.1-46.3)	<0.0001
Anti-C1q antibodies (U ml ⁻¹)	4.3 (0.5-24.9)	2.4 (1.1-4.9)	<0.001
Anticardiolipin antibodies (GPL unit ml ⁻¹)	10.7 (5.4-22.5)	3.32 (2-6)	<0.0001
Myeloperoxidase ANCA (U ml ⁻¹)	0.8 (0.2-11.5)	0.23 (0.11-3)	<0.0001

Table 2: Comparison of serum levels of immunologic parameters in HCV patients before (at baseline) and after treatment

Immunologic parameter	Before treatment (Median-range)	After treatment (Median-range)	p-value
Interferon- γ (pg ml ⁻¹)	240 (160-380)	287.5 (170-400)	<0.0001
Anti-C1q antibodies (U ml ⁻¹)	4.3 (0.5-24.9)	1.2 (0.3-11.7)	<0.0001
Anticardiolipin antibodies (GPL unit ml ⁻¹)	10.7 (5.4-22.5)	10.2 (2.2-15.9)	NS
Myeloperoxidase ANCA (U ml ⁻¹)	0.8 (0.2-11.5)	0.45 (0.1-9)	<0.0001

NS: Non significant

Table 3: Correlations between the studied immunologic parameters in HCV patients

Immunologic parameter	IFN- γ after (U ml ⁻¹)	Anti-C1q before (U ml ⁻¹)	Anti-C1q after (U ml ⁻¹)	ACL-Abs before (GPL unit ml ⁻¹)	ACL- Abs after (GPL unit ml ⁻¹)	MPO-ANCA before after (U ml ⁻¹)	MPO-ANCA (U ml ⁻¹)
IFN- γ before (U ml ⁻¹)							
r	0.402	0.045	0.014	0.123	-0.012	0.057	0.045
p	<0.001**	ns	ns	ns	ns	ns	ns
IFN- γ after (U ml ⁻¹)							
r		0.119	0.219	0.303	0.083	0.089	0.128
p		ns	ns	<0.01 *	ns	ns	ns
Anti-C1q Abs before (U ml ⁻¹)							
r			0.609	0.11	0.149	0.069	-0.071
p			<0.0001**	ns	ns	ns	ns
Anti-C1q Abs after (U ml ⁻¹)							
r				0.13	-0.051	0.074	0.08
p				ns	ns	ns	ns
ACL-Abs before (GPL unit ml ⁻¹)							
r					0.457	-0.04	0.018
p					<0.0001**	ns	ns
ACL-Abs after (GPL unit ml ⁻¹)							
r						0.243	-0.258
p						<0.05*	<0.04*
MPO ANCA before (U ml ⁻¹)							
r							0.96
p							<0.0001**

IFN- γ : Interferon- γ , ACL-Abs: Anticardiolipin antibodies, MPO: Myeloperoxidase, treatment, after: after treatment, ns: Non significant, *p<0.05, **p<0.001, ***p<0.0001, r: Correlation coefficient, Abs: Antibodies

Interestingly, anticardiolipin antibodies were the main immune response parameter that significantly correlated with other studied parameters. Firstly, ACL serum levels after antiviral treatment was significantly negatively correlated with MPO -ANCA serum levels before and after treatment (r = -0.234, p<0.05, r = -0.258, p<0.04, respectively). Secondly, ACL serum levels before antiviral treatment was positively correlated with IFN- γ levels after treatment (r = 0.303, p<0.01). Data are shown in Table 3.

DISCUSSION

In spite of recent progress for HCV treatment, there remains significant room for improvement. To date, a variety of viral factors and host factors that correlate with SVR in the combination

therapy have been noted. In order to establish the better treatment, the detail mechanism of HCV elimination should be elucidated (Bertoletti *et al.*, 1997).

In the present study it was found that interferon- γ levels were significantly higher in HCV patients when compared to healthy controls which was in agreement with recent studies by Zhang *et al.* (2011) and Fathy *et al.* (2011). On the other hand, a previous study by Li and colleagues stated that there were no significant differences between IFN- γ in both HCV patients and controls (Li *et al.*, 2010). Interestingly, we found that after a 48 weeks treatment by combination interferon alpha and ribavirin the serum IFN- γ was significantly increased in comparison to its serum levels at baseline (before start of treatment) and in responders to treatment more than non responders. Moreover, there was a significant correlation between serum IFN- γ and HCV viral load as assessed by PCR as well as with liver condition as assessed by high ALT levels. These results were in accordance with that mentioned by Zhang *et al.* (2011) but not in agreement with Fathy *et al.* (2011) who stated that there are significant reduction in IFN-gamma serum levels after 3 months of IFN alpha and ribavirin therapy and it also stated that IFN-gamma was not correlated with high viral load. These discrepancies in results could be attributed to the differences in numbers; which was relatively small in Fathy *et al.* (2011) (26 patients), as well as to the time of assessment of IFN-gamma serum levels which was earlier (12 weeks) than the usual proper duration to have the expected antiviral response. Thus, from the present study we can suggest that IFN- γ can be regarded as an attempt by the immune system to inhibit viral replication and to eradicate the infection as well as a predictive outcome factor for the responsiveness of HCV patients to IFN alpha therapy, thus reducing treatment costs and decrease side effects of long duration of therapy.

The interaction between the core protein of HCV and the C1q receptor has been shown to suppress the T cell immune response which may have implications in HCV persistence (Kittlesen *et al.*, 2000). C1q protein and C1q binding activity are enriched substantially in the cryoprecipitates of HCV infected patients (Sansonneo *et al.*, 2003). The wide expression of C1q receptor on the surface of blood cells and endothelial cells favors their specific binding to immune complexes containing HCV core protein. Efficient engagement of the C1q protein by cryoglobulins may represent an important pathogenic mechanism in the cryoglobulins-related pathway (Feng *et al.*, 2002). In the present study we found a significantly higher serum level of these autoantibodies in the studied HCV patients when compared to controls. Moreover, there was no significant correlation between anti-C1q antibodies and HCV viral load or ALT levels in the studies HCV patients. These results were in agreement with previous studies (Saadoun *et al.*, 2006; Lienesch *et al.*, 2006). Concerning the effect of interferon alpha plus ribavirin treatment over the serum levels of anti-C1q antibodies it was not yet probably evaluated in the previous studies. Thus, in the present study the effect of interferon alpha therapy on anti-C1q antibodies was assessed after 48 weeks treatment and interestingly it was found that serum levels were significantly decreased after interferon alpha and ribavirin combination therapy. But there were no significant differences in anti-C1q serum levels between responders or non responders HCV patients after 48 weeks treatment. These observations suggest the significant role of antiviral treatment in HCV patients in eliminating immune response autoantibodies such as those against C1q.

In the present study although, none of the participants had a previous history of thrombosis, higher serum levels of anticardiolipin antibodies was detected in HCV patients when compared to healthy controls. This was in agreement with previous studies (Sene *et al.*, 2009; Habibagahi *et al.*, 2007). Regarding the effect of antiviral treatment of HCV (combination of IFN- α

and ribavirin) on ACL serum levels, there were no statistically significant changes between pre and post treatment ACL antibodies. There were a controversial data about anticardiolipin antibodies (ACL Abs) serum levels of HCV patients in the previous studies, some found decrease in the ACL Abs after period of interferon therapy (Rajan and Liebman, 2001), others described increased in the positivity of ACL Abs after 6 month course of interferon alpha treatment (Leroy *et al.*, 1998). These controversies in results could be explained by the different patient population and probably genotypes of HCV studied.

Regarding MPO-ANCA serum levels in the present study, although none of the studied chronic HCV patients had clinical signs of vasculitis, MPO-ANCA was significantly higher in HCV patients when compared to healthy controls. This result was in accordance with that of Valentina *et al.* (2009) who stated that ANCA is common not only in autoimmune Chronic Liver Disease (CLD) but also in viral-related CLD. It was also in agreement with a recent study by Bonaci-Nikolic and colleagues who found increased serum levels of MPO-ANCA in chronic HCV patients and that in patients with positive ANCA, HCV infection should be excluded (Bonaci-Nikolic *et al.*, 2010). Furthermore, in the present study we found that MPO-ANCA was significantly reduced after 48 weeks treatment with interferon alpha plus ribavirin. It is found that a very small number of patients with HCV and positive ANCA, stated that with alpha interferon treatment for such patients the positivity of ANCA had decreased but still positive after end of treatment and that associated vasculitic symptoms and signs had been improved by using corticosteroids or immunosuppressive therapy but no sufficient data was available on alpha interferon therapy in such patients (Bonaci-Nikolic *et al.*, 2010). Thus, early diagnosis and therapy for patients with HCV infection and ANCA positivity considered a big challenge in preventing enhancement of the autoimmune response and induction of further inflammation and extrahepatic complications.

In conclusion, the present study confirms that presence of anticardiolipin antibodies has no pathologic significance in patients with HCV and that it is not affected by interferon alpha treatment in such patients. Moreover, we suggest that in patients with positive ANCA HCV infection should be excluded. Moreover, it was found that IFN-gamma might be useful in predicting the clinical outcome of the combination therapy of peg-IFN alpha and ribavirin. Thus the present study opens the interesting possibility that responsiveness to IFN-alpha-based therapy may be improved by using easily assessed immune response biomarkers such as interferon gamma, anti-C1q antibodies which could help in predicting responsiveness to IFN alpha therapy. Furthermore, early treatment in HCV patients with multiple serological abnormalities will prevent further autoimmune response which eventually could prevent marked extrahepatic complications.

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