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VIRAL HEPATITIS

Serum autoantibodies positivity prevalence in patients with chronic HCV and impact on pegylated interferon and ribavirin treatment response

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Keywords
ALT – ANA – AST – HCV – histological activity – treatment response

Abbreviations
AFP, alpha fetoprotein; ALT, alanine aminotransferase; ANA, antinuclear antibodies; AST, aspartate aminotransferase; ETR, end of treatment response; EVR, early virological response; HCV, hepatitis C virus; IQR, inter quartile range; SD, standard deviation; SVR, sustained virological response; TSH, Thyroid stimulating hormone.

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Abstract

Background & Aims: Prevalence of serum autoantibodies in chronic hepatitis C (HCV) patients is higher than that in the general population. Interferon may induce autoimmune manifestations in patients treated with peg-interferon and ribavirin. Effect of autoantibody seropositivity and treatment response are limited and controversial. To detect the prevalence of serum autoantibodies in patients with chronic HCV and impact on histopathology and treatment response. *Methods:* Retrospective study including 3673 Egyptian chronic HCV naïve patients enrolled in the Egyptian national programme for HCV treatment with pegylated interferon and ribavirin in the years 2007–2010. Antinuclear antibody (ANA) was determined by ELISA considered positive with a titre ≥1:40 by indirect immunofluorescence. ANA-positive patients pre treatment workup including serum aminotransferases, thyroid profile and liver biopsy, follow-up during treatment and sustained virological response (SVR) were assessed compared to ANA-negative patients. *Results:* Serum ANA was positive in 1.6% of the studied patients. There were no statistically significant differences concerning the demographic, biochemical and histopathological data in ANA positive and negative patients. SVR was comparable between ANA-positive and ANA-negative patients (67.8% and 61.3% respectively). Follow-up treatment; ANA-positive patients' did not experience statistically significant haematological complications, flare-up of serum transaminases, thyroid dysfunction. No systemic autoimmune disorders developed during follow-up. *Conclusions:* ANA positivity is not a factor in chronic HCV disease progression and does not affect the treatment response. Pegylated interferon and ribavirin therapy is safe and effective in autoantibodies-positive chronic HCV patients with no need for further follow-up or worry during the treatment in absence of systemic autoimmune disorders.

It is estimated that approximately 130–210 million individuals, i.e. 3% of the world population, are chronically infected with HCV (1). Hepatitis C follows a variable course ranging from minimal or no significant liver disease to progressive liver fibrosis, cirrhosis up to hepatocellular carcinoma. Disease progression was associated with viral factors and host factors as genetic polymorphism, age at infection, gender, immune status and alcohol consumption (2).

HCV infection has been associated with several immune-mediated phenomena, presented as extrahepatic HCV manifestations including cryoglobulinaemia, lichen planus, porphyria cutanea tarda, lymphocytic sialoadenitis and membranous glomerulonephritis (3). There is an association between non-Hodgkin lymphoma and hepatitis C infection (4). The mechanism of development of the extrahepatic syndromes may also be attributed to chronic stimulation of B cells by HCV (5).

HCV infection tends to induce non-specific autoimmune reactions, as demonstrated by the high prevalence of various non-organ-specific autoantibodies, usually in low titres (6). Serum autoantibodies as ANA and smooth muscles antibodies (SMA) are detected among the chronic HCV patients (7, 8). It is recorded that the prevalence of serum autoantibodies is higher in HCV positive patients than in healthy population (9).

Autoimmunity in HCV infection is not limited to autoantibody seropositivity, but embraces the full

spectrum of textbook autoimmune disorders (10). Distribution of the autoantibodies shows no differences between patients infected with different HCV genotypes (10–12). The mechanism of production of these antibodies in HCV infection remains obscure; it may relate to disturbances in self-tolerance as a result of the molecular mimicry between viral proteins and autoantigens (13).

The current guidelines recommend that the autoimmune profile namely ANA should be assessed in chronic HCV patients before the treatment decision with interferon and ribavirin and consider the presence of active autoimmune disorders as a contraindication for treatment (14). The effect on treatment response and the disease progression outcome is not properly studied with controversy results (15).

Objectives

The objectives of this study are to assess the prevalence of autoantibodies (ANA) among patients with chronic HCV and the effect of its presence on the histopathology and response to standard of care treatment with pegylated interferon and ribavirin.

Material and methods

Selection of patients

Retrospective, cross-sectional study including 3673 chronic naïve HCV patients were candidate for treatment with pegylated interferon and ribavirn according to the body weight for 48 weeks in the period from 2007 to 2010. Patients were recruited from Cairo Fatemyia Hospital a referral centre for treatment of chronic HCV in Egypt under the supervision of Ministry of health as part of the Egyptian national project for combating chronic HCV.

Patients were subjected to thorough history taking with special emphasis on history of autoimmune disorders, clinical examination and routine pre treatment workup including: complete blood picture (CBC), serum transaminases aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and conjugated bilirubin, albumin, alkaline phosphatase, GGT, urea, creatinine, prothrombin time and concentration and HBsAg, HBcAb, anti-shistosomal antibodies, alpha foetoprotein (AFP), thyroid stimulating hormone (TSH), blood sugar, serum creatinine and HCV quantitative PCR.

Antibodies detection

Serum ANA was assessed by enzyme-linked immunosorbent assay (ELISA). If the ELISA method results in a positive or equivocal finding, the sample is titred using indirect immunofluorescence (IFA) assays on Hep-2 cells. Positive reactions were titred by double dilution to the end point. Any value less than or equal to 1:40 dilution (or <1/40 IU) is negative.

Histopathological examination

Histopathological examination of ultrasound guided percutaneous liver biopsy using 16 gauge semi-automated biopsy needles. Sections were stained with haematoxylin and eosin for histological assessment, and Masson trichrome stains for detection of fibrosis according to Metavir score (16) with focus directed to the presence of autoimmune element in the liver biopsy e.g. interfaces hepatitis on histological examination, which is needed for diagnosis of autoimmune hepatitis and/or lobular hepatitis.

Patients' classification

Patients were classified according to the presence of serum ANA in to two groups; group I with ANA-positive (titre ≥1:40) and group II with ANA-negative.

Patients consent

Patients' informed written consent from each patient and local ethical committee approval were available before starting data collection. With respect to patients' confidentiality, patients were represented in the study by code numbers and not by their names with all personal data concealed. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Statistical analysis

The quantitative data of the studied patients were described with mean, standard deviation (SD) or inter quartile range (IQR) and compared by *t*-student test in univariate analysis. Stepwise logistic regression analysis was used to assess the virological response associated with serum autoantibodies. Chi-square test was used for analysing the qualitative variables. *P*-value was considered significant if <0.05.

Results

Demographic and baseline data of the studied patients

Among the studied 3673 chronic HCV patients; ANA was positive in 1.6% (59 patients) of the studied population and 98.4% (3614 patients) were ANA-negative. Among the ANA-positive patients; male patients represent 71.2% and the age of 58.5% of the patients was above 40 years. There was no significant statistical association between the demographic data and the studied two groups of patients as shown in Table 1.

Concerning the baseline laboratory data of both groups, haemoglobin level was significantly higher in

Table 1. Demography of the studied patients in relation to ANA

	Group I (n = 59)	Group II (n = 3614)	P-value
Gender			
Male 2971 (80.8%)	42 (71.2%)	2929 (81%)	0.056
Female 702 (19.2%)	17 (28.8%)	685 (19%)	
Age (years)	41.87 ± 9.18	41.88 ± 9.7	0.991
BMI (body mass index)	28.08 ± 4.27	28.22 ± 4.31	0.804

ANA-negative patients than ANA-positive patients. However, no other significant relation was detected in the remaining laboratory data Table 2. Serum AST level was elevated in 58.5% of ANA-positive patients with 44% double-fold elevation.

The liver biopsy based on Metavir score, high histological activity (≥A2) represents 62% and 43.1% in groups I and II respectively. While, advanced fibrosis (≥F2) in group I represent 39% and 39.2% in group II with no statistical significance Table 3 and Fig. 1.

The exclusion of autoimmune hepatitis was based to the simplified international scoring system for the diagnosis of autoimmune hepatitis (17) which necessitates a score ≥7 for definite diagnosis or 6 for probable diagnosis. Although the level of immunoglobulin was not performed for the patients as routine lab, the presence of HCV and absence of histopathological features compatible for autoimmune hepatitis deprive the patient from needed score for diagnosis.

Follow-up data of the studied patients

During the treatment follow-up, no recordable autoimmune manifestations were detected among the ANA-positive patients. Regarding the follow-up labs, haematological treatment complications presented in significant anaemia (haemoglobin <10 g/dl), absolute neutrophilic count (ANC) falls (below 750/mm^3) and platelets count drop (below 50 000/mm^3) were comparable in both group.

While serum transaminases (AST and ALT) elevations 1.5 above the upper limit of normal (40 IU/ml) was statistically significant in group II with P-value <0.01 for AST and 0.03 for ALT. However, no flare-up of enzymes (above 10 folds) that necessitated stoppage of treatment or dose modifications was detected. Thyroid dysfunction recorded by TSH level changes every 12 weeks of therapy was not significant in both groups as in Table 4.

Treatment response of the studied patients

Concerning the response to pegylated interferon and ribavirin therapy, the overall response rate of the studied patients (n = 3673 patients) was 61.4%. In Fig. 2, the early virological response (EVR), end of treatment response (ETR) and sustained virological response (SVR) were comparable in both groups with no significant impact of the ANA positivity.

Table 2. Baseline laboratory data of the studied patients in relation to ANA

	Group I	Group II	P-value
Haemoglobin (g/dl)	13.62 ± 1.54	14.12 ± 1.52	0.011
White blood cells ×10^3 (/mm^3)	6.12 ± 1.52	6.47 ± 1.81	0.137
Platelets ×10^3 (/mm^3)	208.37 ± 51.54	213.50 ± 62.58	0.531
AST (40) IU/ml	59.33 ± 40.88	56.87 ± 43.10	0.9
ALT (40) IU/ml	60.92 ± 37.66	63.14 ± 42.85	0.66
Total bilirubin (mg/dl)	0.80 ± 0.31	0.80 ± 0.28	0.929
Albumin (g/dl)	4.08 ± 0.49	4.20 ± 0.47	0.057
Prothrombin conc. (%)	87.55 ± 11.02	86.56 ± 10.64	0.496
Creatinine (mg/dl)	0.87 ± 0.17	0.90 ± 0.20	0.214
Glucose (g/dl)	93.84 ± 27.68	99.23 ± 28.37	0.155
AFP (10) (ng/dl)*	2.6 (3.9)	3.5 (4.95)	0.35
TSH mIU/L	1.74 ± 1.23	1.57 ± 0.98	0.210
HCV RNA×10^6 (IU) *	0.36 (1.9)	0.91(4.2)	0.1

*Median (IQR) Mann–Whitney U-test.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha foetoprotein; IU, international unit, TSH, thyroid stimulating hormone.

Table 3. Histopathological data of the studied patients in relation to ANA

	All patients (n = 3765)	Group I (n = 59)	Group II (n = 3614)	P-value
Fibrosis n (%)				
F0	26 (0.7)	1 (1.7)	25 (0.7)	0.55
F1	2207 (60.1)	35 (59.3)	2172 (60.1)	
F2	753 (20.5)	8 (13.6)	745 (20.6)	
F3	463 (12.6)	11 (18.6)	452 (12.5)	
F4	224 (6.1)	4 (6.8)	220 (6.1)	
Activity n (%)				
A0	4 (0.1)	0 (0)	4 (0.1)	0.06
A1	2075 (56.5)	23 (38)	2052 (56.8)	
A2	1264 (34.4)	28 (47.4)	1236 (8.8)	
A3	330 (9)	8 (14.6)	332 (8.8)	

Discussion

Chronic HCV is associated with a variety of immunological abnormalities previously described. The prevalence of autoantibodies in chronic HCV patients is higher than in normal population this may be attributed to chronic HCV infection proposed immunologic derangement. The available data on the effect of autoantibodies positivity in chronic HCV on the disease progression and the response to treatment remain

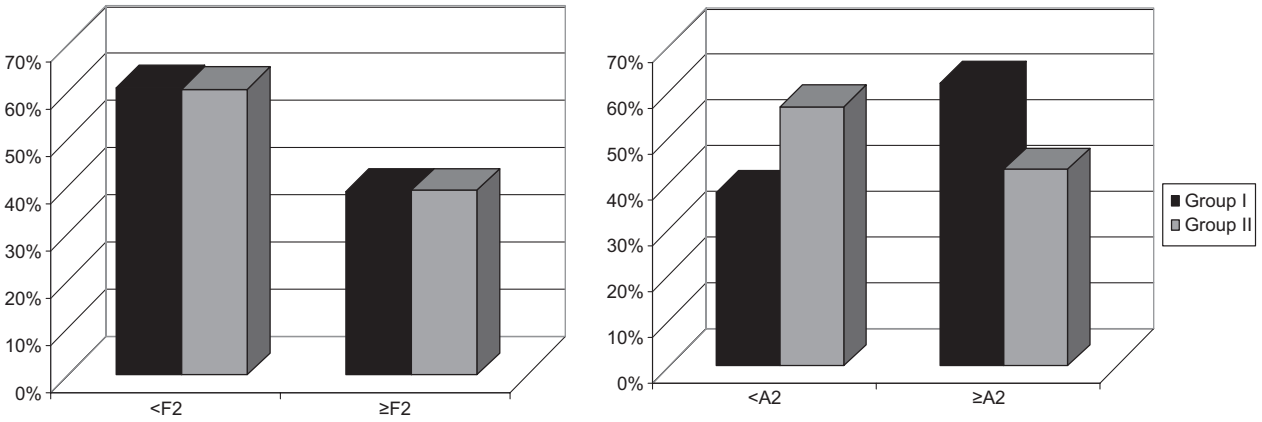

Fig. 1. Histological activity of the studied patients in relation to ANA Comparison between the degree of fibrosis; mild fibrosis (<F2) and moderate and marked fibrosis (≥F2) and the histological activity; no and mild activity (<A2) and high histological activity (≥A2).

Table 4. Follow-up laboratory data of the studied patients during treatment

	All patients (n = 3673) (%)	Group I (n = 59) (%)	Group II (n = 3614) (%)	P-value
Haemoglobin drop	1175 (32)	19 (32.2)	1156 (32)	0.97
Absolute neutrophilic count drop	819 (22.3)	16 (27.1)	803 (32)	0.37
Platelets drop	385 (10.5)	7 (11.9)	378 (10.5)	0.73
AST elevation	2622 (71.4)	33 (55.9)	2589 (71.6)	<0.01
ALT elevation	2582 (70.3)	34 (57.6)	2548 (70.5)	0.03
TSH abnormalities	416 (11.3)	8 (13.6)	408 (22.2)	0.59

AST, aspartate aminotransferase; ALT, alanine aminotransferase; TSH, thyroid stimulating hormone.

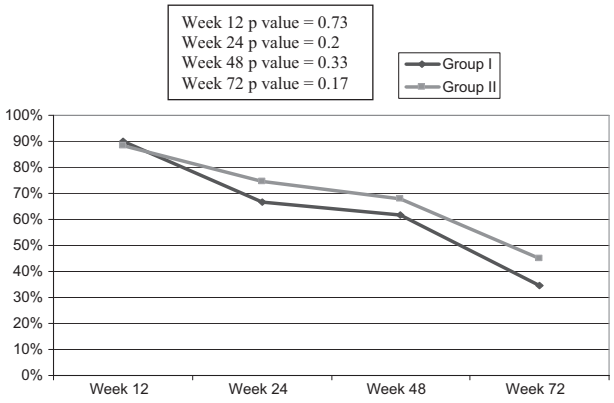

Fig. 2. Response rate of the studied patients in relation to ANA.

controversial. The aim of this study was to assess the prevalence of ANA-positive and impact on treatment response for proper choice of patients indicated for pegylated interferon and ribavirin therapy.

In this study, the prevalence of significant ANA-positive titre (≥1:40) was 1.6% of the studied patients. This prevalence is considered low when compared to previous similar studies where incidence ranges between 7 and 10% (15, 18, 19). The lower prevalence of ANA positivity in the studied population compared to other studies may be attributed to ethnic and racial considerations as these antibodies presence are not associated with any particular HCV genotype (10–12). Variations in the prevalence of ANA and SMA in Western countries have been reported (from 10 to 66%) (9, 20). In South Asia, the data reporting the prevalence of serum autoantibodies in chronic hepatitis are not uniform (19, 21).

A recent 2012 study of 4754 healthy American population over 12 years by Satoh *et al.* reported the presence of ANA in 13% of the studied patients (22). This higher prevalence may be contributed to the age difference between the patients studied in both studies. In Satoh study, ANA positivity was significantly higher ($P < 0.03$) in age groups of 50–59 years and >70 years, while in this study the patients included were below 60 years and the mean age of the ANA-positive patients is around 41 years.

Although some studies observed that women were more likely to have ANA positivity (20, 23) gender was not a significantly associated with the presence of autoantibodies in our patients. In the group of ANA-positive was 28.8% women and in the group without ANA women was 19%. This finding did not support the idea that ANA in HCV patients is part of autoimmune disorders as autoimmune diseases are common among the female gender and in the middle age population (24). Also, this may be explained by the fact that although HCV stimulates autoimmunity; autoimmuni-

ty itself does not amount to an autoimmune disease. This means that this autoantibodies positivity is epiphenomena in chronic HCV. HCV prevalence is higher in the males than females patients.

Regarding the pre-treatment workup, there was no statistical significance between the serum transaminases and the viral load between the two groups of patients. However, several reports related the presence of autoantibody with higher AST level (15, 18) and lower viral load (21). This discrepancy may be attributed to the different genotypes of the studied population, as around 90% of the Egyptian patients are genotype 4 and not genotype 1 or 3 as mentioned in the studies.

Concerning the histopathological features similar conflicting results where ANA positivity was accused to affect the progression of hepatic fibrosis and activity (18, 21) are reported. In this study, fibrosis stage and necroinflammatory grading were not influenced by ANA positivity.

The patients with positive ANA did not report any history of autoimmune diseases or present symptoms of immune-related disorders. Also, during treatment course no significant thyroid dysfunction or development of severe systemic autoimmune disease that required withdrawal of combination therapy was noticed in this group of patients. So, autoantibodies positivity in chronic HCV could be considered as an epiphenomena and not a part of immune-based disease (18).

Pegylated interferon alpha was reported to cause induction of autoimmunity as severe side effect (25, 26). There was no significant relation between the development of side effects to treatment, flare-up of the enzymes (>10 folds) and the autoimmune status of our studied patients. Surprisingly, ANA-negative patients and not those in ANA-positive group reported significant elevations of serum transaminases during treatment. Our data were in accordance with previous reports suggesting that autoantibodies in patients with chronic hepatitis C patients showed a favourable response to interferon (18, 27).

Response rates were comparable in both groups; either SVR or failure to respond. Similar results were recently recorded in 138 patients of HCV genotype 1 group, the SVR rate did not differ between patients with and without ANA titre ≥1:80 (28).

This study concludes that the positive ANA in patients with chronic HCV patients did not affect the disease progression or the treatment decision or development of significant side effects. Positive ANA is not a predictor of combined interferon and ribavirn treatment response. The presence of ANA could be considered as incidental finding in the patient workup as long as no further immune disorders manifestations are noted. The value of ANA assessment prior to treatment should be re-evaluated in guidelines to be restricted only to patients with history or manifest autoimmune disorders.

A limitation of this study is that the genotyping was not performed on our patients because of high cost for genotyping testing for the large number of patients included in the study (3673 patients). Also, it is known from previous epidemiological studies done in Egypt that genotype 4 represents over 90% of the infected patients with chronic HCV and the rest were of genotype 1 (29). Also, as long as the treatment with pegylated interferon and ribavirin is planned for 48 weeks, the response to treatment is not tailored according to the HCV genotyping so it is not cost effective to routine test for the genotype before starting therapy. Also, the pattern of ANA was not performed on all the patients included in the study as well as SMA. These limitations are attributed to the large number of the studied population and the related cost to perform these investigations.

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