Prevalence of Non Organ-Specific Auto Antibodies and its Effect on Response to Antiviral Therapy in Patients with Chronic Hepatitis C Virus Genotype 4

Mohamed Abd El-Maksoud[1], Hatem Elalfy[1], Maha Ragab Habeeb[2], Abd-Elmohsen E. El-desoky[2], Ziyad M. Tawhid[3], Basem S. Eldeek[4]

[1] *Tropical Medicine Unit, Mansoura University Hospital, Mansoura Faculty of Medicine, Egypt*

[2] *Internal Medicine, Mansoura University Hospital, Mansoura Faculty of Medicine*

[3] *Clinical Immunology Unit, Clinical Pathology Department, Mansoura Faculty of Medicine,Egypt*

[4] *Public health and community Medicine King Abdulaziz University faculty of medicine ,Jeddah, and Mansoura university, Egypt.*

Corresponding Author
Mohamed M. Abd El-Maksoud

Mobile:
+201224873738

E mail:
mohamedmaksoud2010@hotmail.com

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Background and Study Aim: Immunological disorders have been frequently described in the course of hepatitis C virus (HCV)–related chronic hepatitis. Our aim was to determine the prevalence of non-organ-specific autoantibodies (NOSAs) and evaluate its impact on the response to combined antiviral therapy in patients with chronic HCV genotype-4.

Patients and Methods: A total of 134 adult patients with chronic HCV genotype-4 were investigated for the presence of serum Antinuclear antibody (ANA), anti–smooth muscle antibody (SMA), and anti liver/kidney microsomal antibody type 1 (LKM1). 109 out of 134 HCV patients were treated naive and received combined antiviral therapy (pegylated interferon–ribavirin). The presence of these autoantibodies was studied in relation to the patient's characteristics and the outcome of antiviral therapy.

Results: Thirty-six (26.9%) patients were positive for at least one autoantibody. Various autoantibodies were presented as follows: ANA in 29 (21.6%) patients, SMA in 9 (6.7%) and anti-LKM-1 in 2 (1.5%). In two patients, both ANA and anti-SMA were positive, and in other two cases both ANA anti-LKM-1 were positive. Female patients had a higher prevalence of positive autoantibodies (P=0.005). Chronic hepatitis C (CHC) patients with positive autoantibodies had higher serum ALT, AST and GGT levels. The rate of sustained virological response to combined antiviral therapy was similar between autoantibody-positive and -negative groups (46.9% vs. 53.2%).

Conclusion: Autoantibodies can be induced in the course of CHC. Autoantibody-positive CHC patients are older and have higher disease activity and severity. However, the presence of these autoantibodies did not influence the response to combination antiviral therapy.

INTRODUCTION

Hepatitis C virus (HCV) is among the leading causes of chronic liver disease worldwide and affects approximately 170 million people [1]. Egypt has the highest prevalence of HCV infection of any country in the world, the situation is quite worse ,the overall prevalence (percentage of people) positive for antibody to HCV was 14.7% [2]. Immunological disorders have been frequently described in the course of HCV-related chronic hepatitis, and non–organ-specific autoantibodies (NOSAs) in particular are common examples of autoreactivity associated with HCV infection [3].

HCV has six major genotypes according to its viral genome, numbered one to six. These viral types and sub-types differ in their geographical distribution and anti-genicity [4]**.** Particular genotypes are associated with different courses and outcome of liver diseases, and also with different responsiveness to interferon therapy. 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. Genotype 4 is the predominant genotype of HCV in Egyptian patients (up to 91%) [5].

To date, combination of pegylated interferon alpha (PEGIFN) and ribavirin is the treatment of choice for chronic HCV patients [6] with an (SVR) of 42%–52% in patients with genotype 1 [7, 8] and in 42-68 % in those with genotype 4 (9-12). The achievement of the SVR in patients with chronic hepatitis C (CHC) has been associated with improvements in liver histology as well as a reduced risk of hepatocellular carcinoma (HCC) and liver-related mortality[13-15] . However, several side-effects have been published in patients treated with IFN-α including the development or exacerbation of underlying autoimmune diseases and the development of a variety of organ and non-organ specific autoantibodies (NOSAs) .The association between these antibodies and either HCV per se or IFN-a related therapy is mainly based on epidemiological surveys [16-21] . Moreover, available data on the relationship between autoantibody seropositivity and the response to antiviral therapy in CHC patients are limited and controversial [22,23] .

In this study, we aimed to assess the prevalence of serum NOSAs in CHC patients. In addition, to evaluate its impact on the response to combined antiviral therapy (IFN or pegylated IFN plus ribavirin) in patients with HCV genotype 4-related chronic hepatitis and to identify clinical, biochemical, or immunological features predictive of response to antiviral treatment.

PATIENTS AND METHODS

The study was conducted into two stages:

Stage I: a comparative cross sectional study among patients with chronic hepatitis C virus Genotype 4

Stage II: a case control study between patients with chronic hepatitis C virus Genotype 4 and healthy cross matched control

Sample size and power of the study

The sample size was calculated by Medcalc program available at www. Medcalc.be. At a level of 95% confidence with alpha error 0.05. and the power of the study was settled at 80 and beta error .02. The prevalence of auto-antibodies was supposed to be ranged from 20% to 10%. The estimated sample is 86 patients. We try to increase the sample of patients to 134 patients to increase the power of the study. Limitation of our resources enforce us to have a control group of 60 subjects

A total of 134 consecutive CHC patients were admitted to this study during the period of July 2009 to January 2012 who visited clinics (inpatients and outpatients) of Mansoura University Hospital. They were 78 males and 56 females, with a mean age of 48.4 ± 3.2 years and 60 healthy controls with matched age and sex. All patients had positive HCV antibody with enzyme-linked immunosorbent assay (ELISA) (Murex anti-HCV (version 4.0) 7F51-06/-07, DiaSorin South Africa (Pty) Ltd, Republic of South Africa) and detectable HCV RNA (Appliedbiosystems, StepOne Real-time PCR system, USA) in the serum. Out of 134 HCV patients, 109 were treated with combined antiviral therapy (peg IFN plus ribavirin), while the remaining patients were missed during the treatment period.

The exclusion criteria included human immunodeficiency virus coinfection, hepatitis B virus infection, autoimmune hepatitis (using the simplified criteria for the diagnosis of AIH) [24], patients who showed evidence of alcohol, illicit drug, or potentially hepatotoxic medication use and major contraindications to IFN or ribavirin therapy. Informed consent was obtained from all patients, and the research protocols were approved by the Medical Ethics Committee of Mansoura University Hospital.

Detection of NOSA:

Serum ANA was detected by ELISA (ORG 538, ORGENTEC Diagnostika GmbH, Germany), ASMA was detected by ELISA (QUANTA LiteTM Actin IgG ELISA 708785, INOVA Diagnostics, Inc.USA) and anti-LKM-1 was also detected by ELISA (QUANTA LiteTM LKM-1 ELISA 708745, INOVA Diagnostics, Inc.USA).

Among the laboratory parameters measured at baseline serum levels of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), total and direct bilirubin, albumin, alkaline phosphatase, γ-glutamyl transpeptidase (GGT) and α-fetoprotein (AFP) were recorded and included in the analysis. Samples positive for HCV-RNA by real time PCR were subjected to genotyping of HCV,

by RT-PCR for the core domain using the primers modified by **Ohno et al. (1997)** [25] **.**

Histological assessment:

Liver biopsy was done for all patients before the initiation of therapy. The histological evaluation was assessed using the modified Knodell histology index and the Metavir scoring system reflecting the degree of hepatic inflammation and fibrosis [26,27]. Before treatment, informed consent was obtained from each patient.

Treatment regimens and outcomes.

A total of 32 (88.9%) of 36 autoantibodies positive patients and 77 (78.6%) of 98 autoantibodies negative patients had been treated with a combination therapy (either pegylated-IFN alfa-2a 180 µg subcutaneously once a week or pegylated-IFN alfa-2b 1.5 µg/kg subcutaneously once a week plus oral ribavirin 1000 or 1200 mg/day for subjects weighing <75 or ≥75 kg, respectively). According to HCV genotype, the predetermined duration of treatment was 48 weeks with a final efficacy evaluation at week 24 of follow-up.

Patients were regularly followed–up for physical examination, blood tests and virological assays. Treatment outcome was assessed as follows: sustained virological response (SVR) was defined as undetectable HCV RNA 24 weeks after treatment discontinuation; relapse was defined as HCV RNA clearance during treatment and reappearance during follow-up; and nonresponse was defined as a failure to clear HCV RNA at any time during treatment [28] .

Statistical Analysis

The data were collected and entered the computer. **The data were statistical analyzed by using Statistical Package of Social Science (SPSS).** The qualitative data were presented in the form of number and percentage. Chi-square with Yates correction was used as a test of significance for qualitative data when the expected cell less than 5. Chi-square test was used as test of significance for qualitative data when the expected cell more than 5. Significance was considered when p value less than 0.05.

The quantitative data were presented in the form of mean and standard deviation. Student t test was used as a test of significance for quantitative data of two groups. The fibrosis score was presented in the form of median and range. Mannwhitney u test was used as a test of significance for fibrosis score. Significance was considered when p value less than 0.05.

RESULTS

Prevalence of NOSA in patients with chronic hepatitis C:

Table (1) shows the prevalence of NOSAs in patients with chronic hepatitis C and control groups. Among total of 134 patients with chronic hepatitis C, thirty-six (26.9%) were positive for at least one autoantibody. ANA was present in twenty-nine (21.6%) patients, anti-SMA in nine (6.7%) patients , and anti-LKM-1 was found in two (1.5%) patients. In two of the patients, both ANA and anti–SMA were positive, and in other two cases both ANA anti-LKM-1 were positive. The prevalence of serum autoantibodies in patients with chronic hepatitis C was significantly higher than in healthy control (p< 0.05).

Clinical significance of NOSAs in patients with chronic hepatitis C:

Table (2) compares the clinical, laboratory and histological parameters between CHC patients with and without autoantibodies.

As regard demographic data, female patients had a higher frequency of positive autoantibodies (*P*=0.005) and age was significantly higher in the autoantibody-positive CHC patients (51.4 ±2.3) vs (45.4 ±4.1) (P <0.001).

The CHC patients with and without serum autoantibodies were analyzed with comprehensive clinical and biochemical examinations: autoantibodies-positive patients had significantly higher serum levels of ALT (102±20.3) vs (90±22.4) (*P*=0.013), AST (96±15.13) vs (72±16.7) (*P*=0.023), GGT (76.3±15.2) vs (50.9±12.7) (P<0.001) and AFP (18±4.5) vs (13±3.9) (P= 0.012). Autoantibodies-positive patients had also higher fibrosis scores and significantly lower platelet counts (144 ±30.2) vs (186 ±25.12) (P=0.004). No significant difference in HCV viral load between both groups.

Response to combined antiviral therapy:

Table (3) show the response of chronic HCV-infected patients to combined antiviral treatment (peg-IFN plus ribavirin). In autoantibodies positive patients, 15 (46.9%) of 32 HCV-infected patients had a sustained virological response (SVR), whereas 9 patients (28.1%) experienced

nonresponse and 8 (25%) experienced relapse. In their counterpart, autoantibodies negative HCV patients, the response rate was as follow: 53.2% SVR, 24.7% nonresponse and 22.1% relapse. The SVR rates were comparable between autoantiboies positive *vs.* autoantibodies negative patients (46.9% vs 53.2%).

As regard the systemic autoimmune manifestations, one patient with positive serum autoantibodies developed hypothyrodism while in autoantibodies negative group, one patient developed diabetes mellitus, and another one developed hypothyroidism. These complications were controlled on therapy and did not required withdrawal of combination therapy.

Predictors of response to antiviral therapy

In this study, we compared patients with and without SVR (Table 4) in order to predict the factors associated with a favorable response to combined antiviral therapy. Among the clinical, biochemical, and histological parameters studied, our results showed that younger age (P<0.001), lower body mass index (BMI) (P<0.001), higher serum ALT (P<0.001), lower GGT (P<0.001), lower HCV viral load (P<0.001) levels and lower fibrosis score were significantly associated with SVR. In comparison serum ANA, ASMA and LKM-1 were not significantly different between patients with and without SVR.

Table (1) Prevalence of NOSAs in 134 patients with chronic hepatitis C and the control group:

	Case (n)	**Autoantibodies** (n (%))	**ANA** (n (%))	**ASMA** (n (%))	**Anti-LKM-1** (n (%))
Patients with CHC	134	36 (26.9%)	29 (21.6%)	9 (6.7%)	2 (1.5%)
Control group	60	7 (11.7%)	7 (11.7%)	0 (0%)	0 (0%)
Test of significance		0.018*	0.098	.031*	.47

Table (2): Clinical, laboratory and histological parameters of patients with chronic hepatitis C who did or not test positive for non-organ specific autoantibodies.

Parameters	**Autoantibody positive N= 36**	**Autoantibody negative N= 98**	**P value**
Gender Male Female	 14 22	 64 34	P=0.005**
Age (year)	51.4 ±2.3	45.4 ±4.1	<0.001***
Body mass index	26.1±2.1	27.2±2.4	.059
Hb level (g/dl)	13.6 ±1.2	13.9 ± 1.5	.28
WBCs	5.9±2.1	6.2 ±2.3	.19
Platlet count (×10^9/l)	144 ±30.2	186 ±25.12	0.004**
Albumin (g/dl)	4.03 ±0.75	4.2±0.32	.49
Total Bilirubin (mg/dl)	1.04±0.5	0.9±.41	.101
ALT (IU/ml)	102.3 ±20.3	90.5 ±22.4	0.013*
AST (IU/ml)	96.4 ± 15.13	72.9 ±16.7	0.023*
ALP (U/l)	247.08±42.3	238.67±36.8	.37
GGT (IU/l)	76.3±15.2	50.9±12.7	<0.001***
HCV RNA(×10^6 IU/ml)	0.58±.2	0. 66 ± .3	0.141
AFP	18.2 ±4.5	13.3 ±3.9	0.012*
Fibrosis score (0-2/3,4)	21/15 1(1-4)	70/28 2 (1-4)	<0.001***

* SIGNIFICAT P LESS THAN 0.05

** HIGHLY SIGNIFICANCE LESS THAN .01

**** EXTREMLY SIGNIFICANCE LESS LESS THAN .001

Table (3): Response of chronic hepatitis C patients with and without autoantibodies to combined antiviral therapy

	Autoantibodies positive patients N= 32	Autoantibodies negative patients N=77	P value
SVR	15 (46.9%)	41 (53.2%)	**0.83**
Non responder	9 (28.1%)	19 (24.7%)	
Relapse	8 (25%)	17 (22.1%)	

Table (4): Comparison between patients with SVR and Non-SVR

	SVR n= 56	Non-SVR n= 53	P Value
Gender Male/ Female	31/25	33/20	0.46
Age (year)	44±5.21	52±4.2	<0.001***
Body mass index	25.3±2.31	28.5±1.41	<0.001***
ALT (IU/ml)	116±25.7	86±50.1	<0.001***
AST (IU/ml)	87.7±17.2	82.5±19.2	0.15
GGT (0-40IU/l)	47.7±5.7	82.3±6.2	<0.001***
ANA (+/-)	13/43	16/37	.41
ASMA (+/-)	4/52	5/48	.51
Anti-LKM-1	1/55	1/52	.96
HCV RNA ($\times 10^6$ IU/ml)	0.49±0.56	0.86±0.72	<0.001***
Fibrosis score	1 (1-3)	3 (2-4)	0.003**

DISCUSSION

Patients chronically infected by HCV present various immune-mediated phenomena mainly due to B lymphocyte dysfunction as mixed cryoglobulinemia and non-organ-specific autoantibodies (NOSAs) production [29]. Previous studies have shown that serum autoantibodies are commonly found in CHC patients [30]. In this study, the global prevalence of NOSAs among patients with chronic hepatitis C was 26.9%. ANA was the most commonly found autoantibodies being present in 21.6% of patients. The prevalence of ANA is higher than that reported by studies from some countries [31], while it is comparable to that reported from some other countries. Lenzi et al., demonstrated the occurrence of ANA in 16% of patients with chronic hepatitis C [21]. In Estonia, Zusinaite et al., reported 14.4% prevalence of ANA in patients with chronic hepatitis C [32] . As regard the prevalence of ASMA in patients with chronic hepatitis C, it was found to be 6.7%. This result is lower than that reported in some studies [16,21,32]. Anti-LKM-1 autoantibodies are detected worldwide in approximately 0-7% of patients with chronic hepatitis C [33,34] . Available data on the prevalence of anti-LKM-1 in Egyptian patients with CHC are relatively uncommon. Here we reported that the positive rate of anti-LKM-1 was 1.5%. These results confirm that AIH-related autoantibodies can exist in CHC patients.

Molecular mimicry between the HCV polyprotein and "self" proteins may account for the production of autoantibodies in chronic HCV infection. A sequence homology between the HCV polyprotein and cytochrome p450 2D6 (CYP 2D6), the antigenic target of anti-LKM1, was previously reported [35] . The reactivity against the viral protein would induce the production of anti-LKM1 in HCV-related CLD. Gregorio and colleagues documented molecular mimicry between HCV polyprotein and three nuclear host antigens including matrin, histone H2, and replication protein as a mechanism for the emergence of ANA [36]. Polyclonal B cell activation by persistent HCV infection has been proposed as another mechanism for the production of autoantibodies. In determining one

of the mechanisms for polyclonal B cell activation, Pileri and colleagues documented that HCV envelope protein (E2) represented a co-stimulatory signal to B cells by binding to CD 81 (tetraspanin) and thereby facilitated the production of autoantibodies [37] . B-lymphocyte activating factor (BAFF) appeared to play a crucial role in HCV-induced autoimmunity [38].

Variations in the prevalence of autoantibodies may be attributed to several factors. First, there may be differences in viral strains causing these differences [3]. Secondly, the differences in detection methods, ethnic background and geographic distribution of the study cohort [39] .

In our study, patients with positive autoantibodies were significantly older. This is in agreement with the findings of Squadrito et al., [16], who found that NOSAs positive HCV patients were older than those with negative autoantibodies. This phenomenon might result from functional defects in suppressor T cells in older patients [40,41]. However, other studies found no age difference between the two groups [42,43] . The positive rate of autoantibodies was higher in females, which is in accord with reports by other groups [31,44] . This may reflect the difference in autoimmune reactions between males and females after CHC infection, suggesting that hormones, such as estrogen, may play an important role in infection [45] .

As regard the biochemical finding, this study showed that autoantibody-positive CHC patients had significantly higher serum ALT and AST levels than those without autoantibodies. This is in agreement with previous reports by Lenzi, et al., who reported that NOSAs were significantly prevalent in patients with HCV-related chronic liver disease, and were especially so when the alanine aminotransferase activity was higher [21]. Moreover, Cassani, et al., showed in a prospective series of patients with HCV related chronic liver disease who were positive for autoantibodies, a biochemical and histological activity were higher than that of patients with no markers of autoimmunity [46]. In controversy, Stroffolini et al., showed no correlation between the positivity of autoantibodies and liver damage [43]. Muratori, et al., 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 [3]. This suggests that the presence of non-organ-specific autoantibodies is more likely associated with increased patient's age, 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.

Our finding also demonstrated that NOSA-positive CHC patients had low platelet count and more advanced fibrosis scores than seronegative CHC patients. These finding are in agreement with most published data, suggesting that HCV-infected autoantibody- positive patients have higher disease activity and severity than those who are autoantibody-negative [46,47] .

IFN-alfa is the treatment of choice for patients with chronic hepatitis C, but its immunomodulatory activity may also favor the appearance or amplification of autoimmune reactions [48]. The response to IFN-α in patients with HCV infection and autoimmune markers continue to be controversial [42]. In our study, we found that the presence of serum NOSA in CHC patients did not influence the response to combined antiviral therapy, which was similar in both serum NOSA-positive and -negative patients (46.9 % vs 53.2%). This results is in agreement with other studies who reported that the presence of autoantibodies such as ANA or anti-LKM1 in patients with CHC is less likely to affect the response to antiviral treatment [46,49] . In contrast, the favourable pridectors of SVR were younger age, lower body mass index (BMI), higher serum ALT, lower GGT, lower HCV viral load levels and lower fibrosis score. These results are in agreement with other reports as in all large prospective studies of (PEG) IFN and RBV combination therapy younger age correlated significantly with an SVR when assessed by univariate and multivariate analyses and patients younger than 40–45 years showed the best response rates [50] . GGT has been identified as a prognostic factor in other studies [51,52] . In this study, we found that low GGT level had a favorable prediction of SVR. This is in accordance with previous reports in which low pre-treatment serum GGT levels were significantly and independently associated with SVR in multivariate regression analysis[53,54] . The pathogenetic background of GGT elevation in chronic hepatitis C is not fully understood. However a close relationship between serum GGT levels and hepatic steatosis, advanced fibrosis, and insulin resistance has been described [55,56] . Moreover, GGT levels are

related with an increased expression of TNFα in the liver that seems to reduce the efficacy of antiviral therapy [57] . We also confirmed previous reports signaling that a low viral load is predictor of SVR. A low baseline viral load (<600,000–800,000 IU/ ml or less) was shown to be an independent predictor of SVR regardless of genotype in numerous studies[50,53,58,59].

In conclusion, serum $NOSA_S$ were frequently found in HCV-infected patients. Patients with positive serum autoantibodies were older, and have higher disease activity and advanced fibrosis scores than their negative counterparts. The positivity of autoantibodies did not influence the response to combination antiviral therapy. Combined antiviral treatment is safe and effective in autoantibodies-positive patients with CHC. Routine testing of autoantiantibodies may be needed to monitors the progress and severity of disease that might be areas for further research.

Limitation of the study:

Some limitations should be considered when interpreting our findings. First, detection of autoantibodies was based on ELIZA method, and there was no record of the distribution type of NOSAs. Whether the distribution type of autoantibodies has clinical relevance is worthy of future study. Second, the external validity of this study is questionable, since the sample of the patients may not be representative of all Egyptian population due to cost variable, long duration of follow up and the interferon therapy is not available for most of Egyptian patients; therefore, it is possible that our findings cannot be extrapolated to all CHC patients in Egypt.

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Peer reviewer: Abeer Nafee;Professor of Tropical Medicine, Faculty of Medicine, Zagazig Universiry, Egypt, Consultant Gastroenterologist and Hepatologist **.**

Editor : Tarik Zaher; Assistant Professor of Tropical Medicine, Faculty of Medicine, Zagazig Universiryb, Egypt, Consultant Gastroenterologist and Hepatologist