Antinuclear Antibody Positivity in Chronic Hepatitis C Patients: Effect on Histopathology and Impact on Early Response to Combined Antiviral Therapy

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Background and study aim: The prevalence of antinuclear antibody (ANA) has been documented in patients with hepatitis C virus (HCV) infection. Hepatitis C virus infection plays an important role in the pathogenesis of immunological derangement, but the mechanism remains unclear. The aim of this study is to detect the significance of ANA positivity and its impact on histopathology and early virological response (EVR) to combined antiviral therapy in chronic HCV patients.

Patients and methods: Two hundred Egyptian chronic HCV naïve patients were enrolled in this study. Antinuclear antibody (ANA) was detected by ELISA and it was considered positive with a titer > 1 : 14 by indirect immunofluorescence. Complete laboratory investigations and histological examination were done as a pretreatment work up for all patients. Patients were followed up during treatment and EVR was assessed in ANA positive and negative patients.

Results: There was a statistically significant difference between ANA positive and negative patients regarding viral load and histopathological criteria and no significant difference was detected regarding other demographic and laboratory criteria. EVR was close in ANA positive and ANA negative patients (77 for the former Vs. 80 for the later with P = 0.33). No autoimmune manifestations were detected during treatment among positive cases. Except for ALT & AST levels no statistically significant differences were detected between ANA positive and negative cases regarding haematological data, thyroid dysfunction. BMI, ALT levels, viral load and fibrosis stages were independent predictors of EVR.

Conclusion: ANA postivity in chronic HCV patients was associated with advanced fibrosis but didn't affect treatment response.

INTRODUCTION

Hepatitis C virus (HCV) infection is the most common cause of chronic liver disease in the world **[1].** It is estimated that 130-170 million people were chronically infected with HCV at the end of 20th century, and 2.4-4.7 million new infections per year **[2].**

Several immunologic abnormalities, such as production of auto antibodies, rheumatoid factor, and cryglobulin, has been associated with HCV infection. Antinuclear antibody (ANA) is one of the most frequently detected auto-antibody **[3].** Its prevalence in HCV infected individuals ranges from 21% to 34%. 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 auto antigens **[4].** Although ANA is the diagnostic hall-mark of systemic lupus erythrematosus (SLE) and type 1 autoimmune hepatitis, its role in chronic HCV infection is unclear **[5].**

The presence of serum ANA is associated with various factors including advancing age, genetic predisposition,

environmental agents, oestrogen-androgen balance, chronic infection and neoplasm **[6].** In some studies ANA positivity had no observed effect on HCV clinical outcome **[7].** The primary goal of HCV therapy is to cure the infection which results in eliminating detectable circulating HCV after cessation of treatment.

IFN-based treatment is frequently associated with significant side effects, some of them related to its immunomodulatory properties which can induce autoimmune phenomena **[8].**

It was recommended that the autoimmune profile namely ANA should be assessed in chronic HCV patients before treatment decision with interferon and ribavirin and consider the presence of active autoimmune disorders as a contraindication for treatment **[9].**

The objective of this study was to detect the significance of antinuclear antibodies (ANA) positivity in chronic HCV patients regarding the effect of its presence on histopathology and early virological response (EVR) to pegylated interferon and ribavirin.

PATIENTS AND METHODS

Selection of Patients:
This prospective study was carried out on 200 adult treatment naive patients with biopsy-proven chronic hepatitis C who were candidate for treatment with pegylated interferon and ribavirin, during the period from April 2012 to December 2012.

Patients were recruited from Hepatology Unit at Shebin El-Kom teaching hospital, a referal center of treatment of chronic HCV in Egypt under the supervision of Ministry of health as a part of the Egyptian national project for combating chronic HCV.

Patients with any other cause of liver disease (as HBV infection, AIH) ,decompensated liver disease, patients with hepatocellular carcinoma (HCC), ischemic cardiovascular diseases, pregnancy or breast feeding, poorly controlled diabetes, psychiatric, ophthalmological or cardiological disorders, substance abuse, patients with organ transplantation, previous treatment with interferon alpha, acute hepatitis, known history of hemolytic anemia were excluded from the study.

All patients included in the study were subjected to:Thorough history taking, complete clinical examination and laboratory investigations including: Complete blood count (CBC), Serum creatinine, Random blood glucose, Complete liver profile (pretreatment then weekly in the first month and monthly unless complications occurred) including :total and direct serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, serum prothrombin, serum alkaline phosphatase).

Patients Classification:
The studied populations were divided into 2 groups:

- **Group I**: included 100 patients with positive anti nuclear antibody titer.
- **Group II:** included 100 patients with negative anti nuclear antibody titer.

Detection of Anti nuclear Antibodies (ANA):
ANA was detected by using the enzyme-linked immunosorbent assay (ELISA) done at baseline and after 12 weeks of antiviral therapy.

If the ELISA method resulted in a positive or equivocal finding, the sample was titered using indirect immunofluorescence (IFA) assays on Hep-2 cells, and any value less than or equal to 1:14 dilution is negative, and any value more than 1:14 is considerd positive **[10].**

Histological assessment:
Liver biopsy was done for all patients before treatment using 16 gauge biopsy needle under complete aseptic conditions. Haematoxyline and eosin stains were used for sections staining for histological assessment and masson trichrome stains for fibrosis detections according to METAVIR scoring system on a scale of F0-4 **[11].** Special concern was directed towards the presence of autoimmune finding in liver biopsy to exclude autoimmune hepatitis. A single experienced pathologist who was unaware of the clinical data evaluated all liver biopsies.

Treatment protocol and definition of response:

All patients received 180 µg/week pegylated interferon alfa-2a (Pegasys) plus 1000-1200 mg/day weight-adjusted ribavirin (1000mg /d for patients < 75KG, and1200 mg /d for patients ≥ 75kg) **[12].** Treatment was discontinued if EVR was not achieved; these patients were defined as non responders. Early responders continued treatment for a total of 48 weeks. Complete early virological response (cEVR) was defined by undetectable HCV-RNA at 3 months after

initiation of therapy, while non-responders (NR)were defined by detectable HCV-RNA at 3 months after initiation of therapy.

Statistical Methods:
The collected data were tabulated and statistically analyzed using the suitable statistical methods"

For the quantitative data, range, mean and standard deviation were calculated. The difference between two means was statistically analyzed using student (t) test. P value of less than 0.05 was considered statistically significant. Continuous variables were compared using the student's *t*-test or the Mann-Whitney *U* test when appropriate. Categorical variables were compared using the Pearson's X^2 test or Fisher's exact test.

RESULTS

Patients characteristics and factors associated with ANA postivity:
This study was conducted on 200 patients with HCV infection (69.5% of them were males) who classiffied into 2 groups: 100 ANA positive and 100 ANA negative patients. Their mean age was 38.5 ± 6.3 years, their mean BMI was 22.3 ± 5.3 and their mean PCR was 1571636.5 ± 52391.21 IU/ml. There was no statistical significant difference between studied groups regarding demographic and laboratory criteria except for smoking and viral load which were significantly higher in ANA positive than ANA negative cases (p= 0.047 & p= 0.041 respectively) (Table 1).

Regarding liver biopsy, there was statistically significant difference between the two studied groups regarding the histological activity and fibrosis stages: 65% of ANA positive patients had high histological activity (≥ A2) versus 60% in ANA negative (p value 0.019). While 62% of group I had advanced fibrosis (F2 + F3) vs 54% of group II with statistically significant difference (p value = 0.021) (Table 2).

Follow up data and EVR of the studied cases:
No autoimmune manifestations were reported during treatment among ANA positive cases. TSH level was higher and Hb, WBCs and ANC were lower in ANA positive than ANA negative group but without statistically significant difference (P value 0.076, 0.085, 0.33, 0.096 respectively), EVR rate was close in ANA positive to that of ANA negative patients (77 vs. 80%) without significant difference (P = 0.3) Table (3).

As regard ANA level changes in ANA positive group at 12 weeks, there was increase in ANA level than the baseline in 93 patients (93%) vs 7 patients (70%) who had no changes or decreased ANA level during treatment. Seventy six patients (84%) out of those 93 patients achieved EVR vs 1 patient of the second group with highly significant difference (p value = 0.001) (Table 4).

ANA positivity had no significant impact on EVR by univariate analysis (Table 5). Only BMI, fibrosis stage (F2, F3), ALT and viral load were the predictors of EVR by multi variate analysis (Table 6).

Table (1): Demographic and Baseline Characteristics of the Studied Groups in Relation to ANA.

		ANA positive cases (>1/14) n=100	ANA negative cases (≤1/14) n=100	t. test	P value
Age		41.5±5.69	37.41±8.62	0.635	0.325
Sex	**M**	75(75%)	64(64%)	0.582	0.428
	F	25(25%)	36(36%)		
Smoking		(60)60%	(22)22%	2.635	**0.047**
BMI		28.10±3.24	25.60±2.10	0.568	0.442
DM		43(43%)	35(35%)	1.452	0.642
Hb(gm/dl)		12.5±0.19	12.7±0.13	1.625	0.635
WBC(c/mm³)		3963.5±5.63	4152.6±357.4	1.412	0.421
ANC(c/mm³)		1741.2±356.2	1836.4±742.2	1.520	0.152
Plt(c/mm³)		247.5±65.1	191.5±32.2	0.369	0.324
Blood glucose (gm/dl)		108.3±12.3	103.6±10.5	0.472	0.159
S.Creat(mg/dl)		0.89±0.16	0.81±0.16	0.530	0.752
TSH(mu/ml)		2.42±.0.96	1.73±0.25	1.635	0.085
AFP(ng/ml)		3.12±1.10	4.85±1.42	1.753	0.247
ALT(IU/L)		50.36±12.4	43.61±10.25	1.996	0.324
AST(IU/L)		36.24±10.56	32.15±11.52	0.369	0.427
S. albumin(g/dl)		4.5 ± 0.2	5.3 ± 0.4	1.542	0.231
Total BIL(mg/dl)		0.9±0.16	1.0±0.21	0.621	0.842
Viral load(IU/ml)		1341856.5±735220.4	1217538.5±25367.1	4.624	**0.041**

Table (2): Hisopathological Criteria of the Studied cases in Relation to ANA.

Activity grade N (%)	A1	35 (35%)	40 (40%)	3.336	**0.019**
	A2	47 (47%)	48 (48%)		
	A3	18 (18%)	12 (12%)		
Fibrosis stage N (%)	F1	38 (38%)	46 (46%)	2.996	**0.021**
	F2+3	62 (62%)	54 (54%)		

Table (3): Laboratory Data of Studied Groups at 12 Week

	ANA- positive Cases(>1/14)	ANA-negative cases(≤1/14)	t .test	P value
Hb(gm/dl)	10.35±2.82	11.74±2.27	1.332	0.085
WBC(cells/cm³)	2581.5±6.36	3159.2±274.1	1.259	0.332
ANC(c/mm³)	1654.3±241.6	1817.4±716.6	1.452	0.096
PLT (c/mm³)	236.5±60.1	170.5±32.2	0.635	0.325
Random blood Sugar(gm/dl)	107.3±9.31	101.6±8.63	0.447	0.442
S.Creat(mg/dl)	0.83±0.13	0.80±0.14	0.625	0.258
ALT(IU/I)	46.23±8.52	41.29±10.19	2.326	**0.049**
AST(IU/I)	35.36±11.52	30.14±5.22	2.639	**0.047**
TSH(IU/ml)	2.14±.0.12	1.15±0.22	1.241	0.076
AFP(ng/ml)	3.68±1.74	4.32±1.30	1.366	0.220
EVR	77	80	0.825	0.33

Table (4): ANA level changes in ANA-Positive Group (100 patients) at 12 Week

	No of patients with increase ANA level During treatment	No of patients with Decrease or no change in ANA level during treatment	X^2	p. value
At 12 weeks	93	7	10.336	0.001
EVR	84%(76)	16%(1)	8.336	0.001
Mean value of Baseline ANA level	14.23±2.43	15.42±1.32	2.532	0.053
Mean value of ANA level after12 weeks	16.75±5.36	14.52±4.85	2.336	0.076

Table (5): Univariate analysis of factors which predict achievement of early virological response (EVR)

Variables	No EVR(N =43) (21.5%)	EVR(N=157) (78.5%)	P value
Sex(females)	19	٤٢	0.526
Age	39.63±8.63	37.55±7.62	0.224
BMI	28.3±0.5	21.3±1.3	**0.046**
ANA positivity	23	77	0.213
Hb(gm/dl)	10.25+2.84	11.99+3.41	0.526
WBC(cells/cm³)	3869.2+258.6	4115.6+366.5	0.336
ANC	1785.7+174.6	1813.95+259.3	0.447
PLT (c/mm³)	251.6+33.6	196.3+44.5	0.529
Random blood Sugar (gm/dl)	110.4+20.4	99.4+15.60	0.357
S.Creat (mg/dl)	0.90+0.12	0.84+0.36	0.859
ALT (IU/I)	52.41+7.63	41.29+6.95	**0.027**
AST (IU/I)	37.63+7.19	34.19+6.37	0.147
Viral load (IU/ml)	1391722.5+63241.4	1296438.5+38367.9	**0.019**
TSH (mU/ml)	37.61+7.52	33.96+8.63	0.352
AFP (ng/ml)	3.68+1.34	4.63+0.96	0.639
Fibrosis stage(2,3)	40(93%)	76(48%)	**0.048**
Activity grade(2,3)	35 (81%)	90(57%)	0.053

Table (6): Multivariate Logistic Regression Analysis for predictors of EVR

	r.	P. value	Odd's ratio	95% confedence interval
BMI	0.491	0.045	0.591	0.34-1.57
Fibrosis stage(2,3)	0.457	0.048	0.718	0.36-1.42
ALT(IU/I)	0.436	0.036	0.638	0.35-1.25
Viral load(IU/I)	0.385	0.023	0.731	0.62-2.98

DISCUSSION

Hepatitis C virus infection plays an important role in the pathogenesis of the immunologic derangement, but the mechanism remains unclear. In patients with HCV infection, evidence of altered immune system homeostasis is indicated by a high prevalence of non organ specific autoantibodies (NOSAs) (50%).

Among NOSAs, anti-liver/kidney microsomal antibody type 1 has a direct influence over the enhanced severity of liver damage. However, for more frequently observed NOSAs, such as anti smooth-muscle antibody (ASMA) and anti-nuclear antibodies (ANA), there are insufficient data to provide a conclusive answer regarding their pathogenicity **[6].**

This study was conducted on 200 HCV infected patients (139 males &61females) to detect the significance of ANA positivity and its effect on histopathology and EVR to pegylated interferon and ribavirin in chronic HCV infected patients for proper selection of patients for combined antiviral therapy.

In the present study age was non significantly higher in ANA positive group than in ANA negative one [(41.5±5.69) vs. (37.41±8.62) respectively]. As regard sex, males were more likely to have ANA positivity than females (75% vs 25%) but without statistically significant difference (p= 0.4) (Table 1). These results were in consistance with Peng et al. **[5]** and Khairy et al. [**13]** who found that there was no statistical significant difference concerning the demographic data in ANA positive and negative patients. In contrary, Hsieh et al. **[6]** found that women had a significantly higher prevalence than men (41.2 vs 31.0%; p = 0.012). This difference may be due to large number of patients included in the previous study as it was carried out on 614 patients.

Regarding pretreatment evaluation of patients, BMI was higher in ANA positive group than ANA negative one (28.10±3.24 vs. 25.60± 2.10) but without statistical significant difference (p value =0.442) (Tabl 1). This was in agreement with Narciso–Schiavon et al. **[14]** who found no association between ANA positivity and BMI.

On the same hand viral load was higher in ANA positive than ANA negative group with significant difference (p= 0.041). This was in agreement with study done by Chen et al. **[15]** who found that PCR levels in ANA positive group were higher than ANA negative one but without statistical significant difference (p value 0.3). In contrast to the result of the present work, Hsieh et al. **[6]** concluded that antinuclear antibody positivity was associated with lower RNA levels in patients with chronic hepatitis C. This difference might be due to different HCV genotypes that were included in the previous study.

In the present study there was statistically significant difference between ANA positive and ANA negative groups as regards activity grades (p value =0.019)and fibrosis stages (p value= 0.021) (Table 2). This was in agreement with Chretien et al. **[16]** who reported that fibrosis, inflammation and hepatocellular necrosis were significantly more pronounced when NOSA positive patients were compared with NOSA negative patients.

Similarly, Hsieh et al. **[6],** Yee et al. **[7]**, Squadrito et al. **[17],** Takashi et al. **[18]** found significant association between autoantibody reactivity and severe hepatic fibrosis, inflammation, and cirrhosis. ANA positive patients had almost two fold higher chance of having quicker fibrosis. On the other hand Khairy et al. **[13]** concluded that fibrosis stage and necro inflammatory grading were not influenced by ANA positivity in their study. This discripasncy may be attributed to different number of ANA positive cases included in the previous study (59 patients).

In the present study, ALT and AST were significantly elevated in ANA positive than ANA negative group during IFN therapy (p= 0.049 & 0.047 respectively) (Table 3). This came in accordance with Sezaki et al. **[19]** who reported that ALT level may increase in patients with autoimmune features when received IFN- based therapies, and this may be due to IFN- induced immune mediated hepatocyte injury. On the other hand, Khairy et al. **[13]** concluded that ANA negative patients and not those ANA positive patients reported significant elevations of serum transaminases during treatment.

Also in this work, EVR was higher in ANA negative (80%) than ANA positive group (77%) but without significant difference (p value 0.85) (Table 3). This was in consistence with Wasmuth et al. **[20]** who found that absence of NOSA prior to &during combination therapy was associated with favorable treatment response. Similarly, Peng et al. **[5]** reported that patients with higher ANA titers before interferon therapy tend to be interferon-resistant.

Similarly, Gatselis et al. **[21]** found that ANA negativity was the predictor for achieving better treatment response to interferon therapy. On contradiction to the results of the present work, Wu et al. **[22]** demonstrated that EVR was significantly higher in ANA positive patients (77.8%) vs ANA negative (53%) (p value <0.05). This difference might be due to different sample size, patients characters and viral genotypes as the previous study was performed on 69 HCV patients, twenty of them were positive in auto-antibodies, not only ANA but also other auto-antibodies (anti-SMA, anti-AMA, and anti-LKM) and the studied cases had genotype 1 and 2.

In the present study there was statistically significant difference (Table 4) between ANA positive patients who had increased level of ANA during treatment and those who had decreased level of ANA during treatment in achieving EVR (84% vs 16% respectively), p value (0.001) and this was in contrast to what reported by Muratori et al. **[23]** who found that patients whom the NOSA titer developed and increased during treatment were non responders. The difference may be due to small sample size in the previous work (20 patients).

In the present work univariate and multi variate analysis of predictors of EVR revealed that BMI, ALT, viral load and fibrosis stage were an independent predictors of EVR (Table 5 & 6).

This was in consistence with Kim et al. **[24]** who found that rapid normalization of ALT by 4 weeks after treatment might be a useful response factor that is readily available in clinical practice.

This was in contrary to Rodrigues-Torres et al. **[25]** who stated that higher ALT , absence of cirrhosis, younger age and white non-Latino race/ethnicity were associated with successful achievement of RVR and EVR in patients infected with HCV. This difference may be explained by different genotype as the previous study was carried out on genotype 1only.

Regarding BMI, the result of the present study came in agreement with Bressler et al. **[26]** who stated that BMI greater than 30 kg/m², was an independent negative predictor of response to hepatitis C treatment. Also, Rodrigues-Torres et al. **[25]** concluded that lower BMI was associated with achieving EVR. On the other hand, Pattullo et al. **[27]** stated that neither body weight nor BMI influenced virological response.

Regarding viral load, the result of the present work was in consistant with Ridruejo et al. **[28]** who stated that lower virological responce associated with higher viral load. Also, Lee et al. **[29]** found that viral load < 2 million copies/mL was associated with responses 1.5- to two-folds better than cases with high viral load.

Regarding fibrosis stages, the result of the present work agreed with Rodrigues-Torres et al. **[25]**, Paqliaro et al. **[30]** Who stated that short-term and sustained response were independently predicted by lobular structure on pretreatment liver biopsy and by short disease duration.This was contradictory to Lee et al. **[29]** who stated that presence of significant fibrosis/cirrhosis was not important predictive response factor. This difference due to, may be, different sample size (360 patients) of the previous work.

This study concluded that ANA positivity in patients with chronic HCV was associated with advanced fibrosis but it was not a predictor for EVR.

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Ethical approval: Written informed consent was taken from all patients before participation in this study.The study protocol was approved by the ethical committee of Benha Faculty of Medicine and its University Hospitals.

Conflicts of interest: Non.

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