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A study of selected serological Autoimmunity markers in HCV patients before and after Sustained viral response by Direct-acting antiviral drugs

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Abstract: Background: Hepatitis C virus (HCV) infection is a significant cause of hepatic and extrahepatic manifestations. Direct-acting antiviral (DAAs) therapies proved highly effective in treating HCV. This study aimed to assess the autoimmune serological progression of asymptomatic Egyptian patients with chronic HCV after treatment with DAAs.

Methods: Twenty patients with chronic HCV with exclusion of any rheumatic clinical manifestations were treated with sofosbuvir 400 mg and daclatasvir 60 mg for three months. This study evaluated the autoimmune serological progression in these patients.

Results: All the 20-patients achieved sustained viral response (SVR), and none of them had clinical autoimmune manifestations. We found reduction in the number of positive **cryoglobulins** (15% before treatment and all became negative after treatment). There was no significant improvement in rheumatoid factor (**RF)** (25% before versus 20% after treatment) **or** Immunoglobulin-G **(IgG)** (25% before versus 20% after treatment), (P values <0.293 and <0.794 respectively). No significant improvement in Immunoglobulin-M (**IgM)** and Immunoglobulin**-A (IgA)** was observed (P values 0.097 and 0.538 respectively). We didn't find reduction in numbers of patients with positive antinuclear antibodies (**ANA).**

No marked changes in **Anti-Ro, Anti-La** antibodies, complement 3 (**C3)** and complement 4 (**C4)** after SVR.

Conclusion: HCV clearance by DAAs is accompanied with autoantibody changes. Nevertheless, most of the patients endure autoantibody-positivity; also some patients were negative before treatment and acquired autoantibodies following SVR. These findings prove that HCV is linked to autoimmunity and reveal that its autoimmune impact continues despite viral clearance by DAAs, implying that long-term monitoring is necessary

Keywords: Hepatitis C virus (HCV), Autoimmune serology, Direct-acting antiviral (DAAs) therapies

Introduction: Hepatitis C virus (HCV) infection is frequently associated with extrahepatic and autoimmune manifestations, which can have a major impact on morbidity, mortality, and medical costs (1). Some studies estimated that approximately 40 to 70% of patients with HCV will develop at least one extrahepatic manifestation at some point (2).

Serum cryoglobulins and hypocomplementemia are usually found in a large proportion of HCV-infected patients, and are considered markers or predictors for the development of extrahepatic manifestations (3).

The lymphotropic nature of HCV contributes to the proliferation of poly-oligoclonal B-lymphocytes, which is a common underlying abnormality observed in a significant number of HCV-infected individuals. This expansion of B-lymphocytes leads to the generation of various autoantibodies and immune complexes, including cryoglobulins which may lead to organ- and non-organ-specific immunological alterations. (4).

The presence of chronic hepatitis C infection has been linked to an increased risk of developing B-cell non-Hodgkin lymphoma, as well as primary hepatic lymphoma (5). Epidemiological studies conducted on individuals with chronic HCV infection have demonstrated a twofold higher risk of developing non-Hodgkin lymphoma in patients with symptomatic HCV-associated mixed cryoglobulinemia (6).

We aimed to study the effect of direct antiviral agents (DAAs) on the autoimmune serological profile in asymptomatic patients with chronic HCV infection and also to explore whether or not the autoimmune sequelae of HCV will continue despite viral clearance by DAAs, and the need for long-term monitoring of those patients.

Methods:

Study design:

We performed an observational prospective study. This study was performed in 2021 and 2022 and included 20 patients with HCV-RNA positive infection without any clinical autoimmune or other extrahepatic manifestations. They received treatment with DAAs according to the Egyptian Ministry of Health protocols which included Sofosbuvir (400 mg daily) plus Daclatasvir (60 mg daily), and were assessed clinically, serologically before and 6 months after finishing treatment.

Exclusion criteria:

The exclusion criteria included being already started on antiviral medication for HCV infection, co-infection with either HIV or HBV viruses. Patients with autoimmune or extrahepatic clinical manifestations, other autoimmune diseases. All patients on immunosuppressive drugs were excluded.

Data collection and follow up assessment time plan:

Patients were recruited from outpatient clinics.

They received treatment with oral DAAs and were assessed at baseline and after 6 months from the end of therapy.

A. Clinically by full history, systemic examination and assessment for extrahepatic or autoimmune disorders.

B. Laboratory investigations including CBC, AST, ALT, gamma-GT, alkaline phosphatase, serum albumin, serum creatinine, serum cryoglobulins, Rheumatoid Factor (RF), ANA by IF with titre and pattern, Anti Ro and Anti La, complement 3 (C3), complement 4 (C4) and serum protein electrophoresis, serum immunoglobulins (IgG, IgA, IgM), urine analysis as well as HCV viral load (quantitative PCR for HCV).

C. Abdominal ultrasonography.

Statistical analysis:

Data were statistically described in terms of mean ± standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Numerical data were tested for the normal assumption using Kolmogorov Smirnov test. Comparison of numerical variables between the study groups was done using Mann Whitney U test for independent samples. Within group comparison of numerical variables was done using Wilcoxon signed rank test for paired (matched) samples. For comparing categorical data, Chi-square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Paired categorical data were compared using McNemar test. Correlation between various variables was done using Spearman rank correlation equation. Two-sided p values less than 0.05 was considered statistically significant. IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows was used for all statistical analyses.

Results:

This study included 20 patients, 14 males (70.0%) and 6 females (30.0%). Their ages ranged from 19 to 60 years old (mean 37.75 ± 12.46 SD). None of them was diabetic or hypertensive. Five patients (20%) had parenchymatous liver disease; all of them were CHILD A. All patients received Sofosbuvir/daclatasvir combination for 12 weeks and achieved SVR. The duration of HCV diagnosis ranged from 2 to 8 years (mean 2.4± 1.35SD).

All patients were assessed for extra-hepatic and autoimmune manifestation and had none of them.

Autoimmune serology before antiviral treatment (table-1):

ANA was positive in 6 patients (30%) and negative in 14 patients (70%). **Anti-Ro** antibodies were borderline in one patient (5%) and negative in 19 patients (95%). **Anti-La** antibodies were positive in one patient (5%), borderline in one patient (5%), and negative in 18 patients (90%).

C3 was consumed in one patient (5%) and normal in 19 patients (95%). **C4** was consumed in two patients (10%) and normal in 18 patients (90%).

RF ranged from 6 to 47 IU/ml (mean 15.68±13.01 SD). RF was positive in 5 patients (25%) and negative in 15 patients (75%).

Serum **Cryoglobulins** were positive in 3 patients (15%) and negative in 17 patients (85%)

Serum **IgG** ranged from 775 to 5710 mg/dl (mean 1564.55±1029.55 SD). Serum IgG was high in 5 patients (25%) and normal in 15 patients (75%).

Serum **IgM** ranged from 53 to 247 mg/dl (mean141.1±55.7 SD).

Serum **IgA** ranged from 100 to 434 mg/dl (mean 232.3±100.32 SD).

SPEP was normal in 10 patients (50%), hypoalbuminemia with inverted A/G ratio in one patient (5%), and polyclonal gammopathy in 9 patients (45%) (Figure-1).

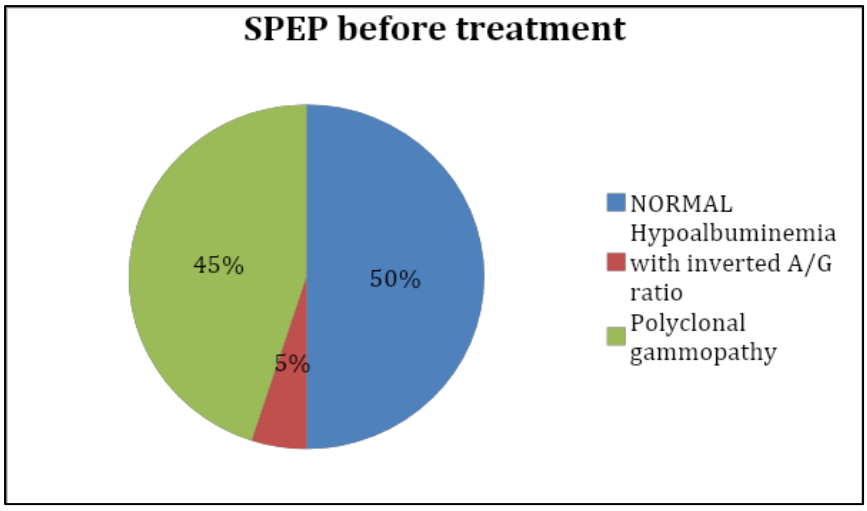

Figure-1: SPEP before anti-HCV treatment

Autoimmune serology after sustained viral response (Table-1):

ANA was positive in 6 patients (30%) and negative in 14 patients (70%).

ANA response after SVR showed 4/6 patients (66.7%) ANA became negative and was initially positive. In two patients (33.3%), the test remained positive and in 4 patients (28.6%), it became positive although it was initially negative.

Anti-Ro antibodies were negative in all patients (100%). **Anti-La** antibodies were positive in one patient (5%), and negative in 19 patients (95%).

C3 was normal in all patients (100%). **C4** was consumed in one patient (5%) and normal in in 19 patients (95%).

RF ranged from 10 to 19.2 IU/ml (mean 11.93 ± 3.28 SD). RF was positive in 4 patients (20%) and negative in 16 patients (80%).

RF response after SVR showed that 2/5 patients (40%) becoming negative and were initially positive and 3/5 patients (60%) were still positive and in 1/15 patient (6.7%) RF became positive while being initially negative. (Change before and after therapy: number (%) of patients who became negative and were initially positive).

Serum **Cryoglobulins** were negative in all patients (100%). Serum cryoglobulins after SVR showed complete response in all 3 patients (100%).

Serum **IgG** ranged from 870 to 5690 mg/dl (mean 1615.1±984.96 SD). Serum IgG was high in 5 patients (25%) and normal in 15 patients (70%).

Serum **IgM** ranged from 50 to 320 mg/dl (mean176.3±77.14 SD).

Serum **IgA** ranged from 100 to 622 mg/dl (mean 265.55±121.0 SD).

SPEP was normal in 11 patients (55%) and 9 patients (45%) had polyclonal gammopathy (Figure-2).

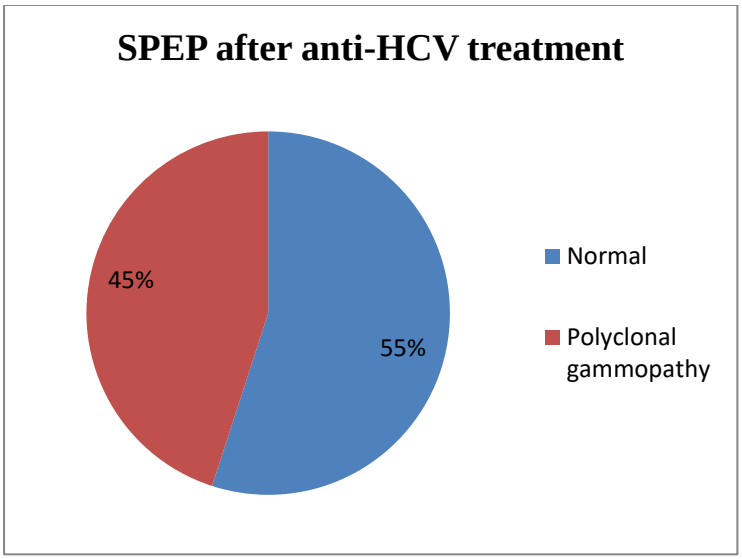

Figure-2: SPEP after anti-HCV treatment

Comparison between before and after SVR (table-1):

There was reduction in the number of positive **cryoglobulins** (15% before treatment to 0% after treatment).

There was no statistically significant improvement in **RF, IgG, IgM and IgA** after SVR when compared to those before starting anti-viral treatment (P values 0.293, 0.794, 0.097 and 0.538 respectively).

We didn't find marked reduction in numbers of positive patients of **ANA** (30% versus 30%)**.**

There was high statistically significant improvement in **ALT & AST** after SVR when compared to those before treatment (P value 0.001 in all).

There was statistically significant improvement in **albumin, GGT, direct bilirubin, PC and INR** after SVR when compared to those before treatment (P values 0.019, 0.004, 0.007, 0.007 and 0.24 respectively).

Table-1: Comparison of laboratory findings between before and after SVR:

	before treatment					After treatment					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	

RF titre	15.685	13.0067	10.500	6	47	11.93	3.278	10.00	10	19	0.293
IgG titre (700-1600)	1,564.55	1,029.558	1,405.00	775	5,710	1,615.10	984.959	1,395.00	870	5,690	0.794
IgM titre (40-230)	141.10	55.72	119.00	53.00	247.00	176.30	77.14	169.50	50.00	320.00	0.097
IgA titre (70-400)	232.30	100.32	215.50	100.00	434.00	265.55	121.00	246.50	100.00	622.00	0.538
ALT	40.25	14.12	42.50	17.00	60.00	22.55	8.12	20.00	13.00	40.00	< 0.001
AST	38.10	15.36	35.50	17.00	81.00	22.30	7.59	20.50	15.00	42.00	< 0.001
Albumin	4.30	0.40	4.30	3.20	4.90	4.07	0.15	4.00	3.90	4.50	0.019
GGT (11-60)	38.75	30.10	32.50	13.00	142.00	24.70	10.80	22.00	13.00	59.00	0.004
direct bilirubin	0.14	0.06	0.12	0.06	0.30	0.10	0.05	0.10	0.01	0.20	0.007
PC	98.30	2.30	100.00	93.00	100.00	99.95	0.22	100.00	99.00	100.00	0.007
INR	1.02	0.04	1.00	1.00	1.10	1.00	0.00	1.00	1.00	1.00	0.024

Discussion:

Our results were similar to the results of **Yousif et al., 2021, Sise et al. 2016, Reyes-Avilés et al.'s and Benedetta Terziroli et al., 2019 who** studied the autoimmune serology in HCV patients and the effect of its successful treatment.

Yousif et al., 2021 (7), studied the clinical and serological course of rheumatic manifestations in 60 Egyptian patients with HCV infection before and after treatment with DAAs. The immunological features revealed positive RF in 56.7% (34/60) patients, positive serum cryoglobulins in 50% (30/60) of patients and ANA in 93.3% (56/60) of patients. They found marked reduction in both the number of positive patients and the mean levels of all immune markers after treatment, 38.3% were positive for RF, 61.6% for ANA, and 35% for cryoglobulin. This difference could be attributed to some of their patients had autoimmune features.

Sise et al. 2016 (8), also reported in their retrospective case study involving twelve HCV patients with mixed cryoglobulinemic syndrome; cryoglobulin levels dropped in the majority of patients and disappeared entirely in 44.4% (4/9) of the cases.

According to **Reyes-Avilés et al.'s study 2015 (9),** serum RF decreased and 30% of patients tested negative for RF after therapy. The authors also noted that following therapy, the fraction of mature activated memory B-cells in HCV RF+ patients decreased somewhat in comparison to their pretreatment values.

In Benedetta Terziroli et al., 2019 (10), ANA was positive in 96/235 (41%) of patients before DAAs and of the 96 patients, 34% (33/96) became ANA negative after HCV clearance, and among 139 patients who were ANA negative before DAA, 16% (22/139) became ANA positive. Hence it may be interpreted that HCV treatment doesn't alter ANA in one direction. A total of 235 patients were included. Unexpectedly, one or more autoantibodies appeared in 27% of pre-treatment negative subjects.

Also in Egypt, Tawfik et al., 2013 (11), studied 100 patients with chronic HCV infection, for whom SPEP was performed and revealed polyclonal gammopathy in a (40%) in the HCV-positive group and monoclonal bands in only two patients (2%).

Conclusion

HCV clearance by DAA is accompanied with autoantibody elimination in more than 40% of the individuals who were positive before therapy. Nevertheless, most of the patients remained autoantibody-positive and more than one third of those who were negative before treatment acquired autoantibodies following SVR.

These findings prove that HCV infection is linked to autoimmunity and reveal that the autoimmune sequelae continue despite viral clearance by DAAs, implying that long-term monitoring is necessary.

List of abbreviations:
ALT: Alanine transaminase
ANA: Anti-nuclear antibodies
AST: Aspartate aminotransferase
C3: Complement 3
C4: Complement 4
CBC: Complete blood count
DAAs: Direct antiviral Agents
HCV: Hepatitis C virus
Ig: Immunoglobulin
NHL: Non-Hodgkin lymphoma
PCR: Polymerase chain reaction
RF: Rheumatoid factor
RNA: Ribonucleic acid
SD: Standard deviation
SPEP: serum protein electrophoresis

SVR: Sustained viral response

All the procedures conducted in this study adhered to the ethical standards and received approval from the Research Ethics Committee of the Faculty of Medicine (REC code: D-28-19). Prior to their participation, all patients included in the study provided written informed consent. Prior to their participation, all patients included in the study provided written informed consent.

Consent for publication

A written informed consent for publication of their clinical details and/or clinical images was obtained from the patient. A copy of the consent form is available for review by the Editor of this journal.

Availability of data and material

All the data obtained or studied throughout this study have been incorporated into this published article.

Competing interests

The authors confirm that they have no conflicts of interest to disclose.

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Author contribution:

A.M was responsible for patient recruitment, conducting clinical examinations, sample collection, and management of clinical data. M.T.H assisted in patient recruitment. N.Z supervised and oversaw laboratory investigations. A.M, M.T.H., and G.R. contributed to manuscript writing. All authors participated in statistical analyses, data interpretation, and manuscript preparation. G.R. conceived the study, coordinated its implementation, and provided supervision.

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