

RESEARCH ARTICLE

Lack of Any Relationship Between Circulating Autoantibodies and Interleukin–6 Levels in Egyptian Patients Infected with the Hepatitis C Virus

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Abstract

Introduction: Elevated serum interleukin (IL) 6 has been reported in patients infected with the hepatitis C virus (HCV), but it remains debatable whether this influences the production of autoantibodies and the biochemical profile of HCV disease. Therefore, this current study was conducted to evaluate the relationship between IL-6 and circulating autoantibody levels in HCV positive patients. **Methods:** Levels of IL-6 in serum samples from 102 patients with HCV and 103 normal controls were determined by enzyme linked immunosorbent assay (ELISA). Autoantibodies were detected by immunofluorescence. **Results:** Levels of IL-6 were significantly higher ($p=0.028$) in patients infected with (HCV) compared with normal group. Autoantibodies were noted in in 43.1% of the patients; of these, 23.5% featured anti-nuclear antibodies (ANA+), 16.7% anti-smooth muscle antibodies (ASMA+), 7.8% anti-mitochondrial antibodies (AMA+), 17.6% anti-parietal cell antibodies (APCA+), 7.8% anti canalicular antibodies, and 2.9% anti reticulin antibodies (ARA+). No patients were found to be positive for anti-brush border antibodies (ABBA) or anti-ribosomal antibodies. (ARiA). No links with IL-6 levels were apparent. **Conclusions:** IL-6 levels are increased in patients infected with HCV disease and could influence the production of autoantibodies. However, this study did not provide evidence of a specific relationship between IL6 and circulating autoantibodies in such cases.

Keywords: Autoantibody- IL-6- hepatitis c virus

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Introduction

Chronic infection with hepatitis C virus (HCV) is a life-threatening disease that causes progressive liver damage and different autoimmune manifestations (Bonkovsky et al., 2001; Kim et al., 2012). Autoantibodies are characterized by the loss of tolerance against self-antigen and activation of auto reactive lymphocyte and pathological damage of single or multiple organs (Dammacco et al., 2000). As a Secondary event Autoantibodies can be measured in different liver diseases associated with etiological factors as drugs and chemical induced autoimmunity, viral and microbial infection induced. (Christopher et al., 2012)

In particular, IL-6 is a multifunctional potent, pleiotropic inflammatory cytokine that promotes the survival of plasma cells that secrete immunoglobulin or pathological autoantibodies. It is involved in the regulation of different cellular processes, including proliferation and differentiation and plays a functional essential role in acute phase response and in the control of the equation between pro-inflammatory and anti-inflammatory pathways (Shihara et al., 2000; Chihara et al., 2011).

High circulating levels of IL-6 have been reported in

many clinical studies (Inflammatory, neoplastic diseases) and especially in several liver diseases (Martinez et al., 1993; Soresi M et al., 2006; Giannitrapani et al., 2011, 2013).

Many specific and systemic autoantibodies are usually found in the serum of an infected patient with viral chronic hepatitis. Antinuclear Antibodies (ANA) are seen mostly in patients with Chronic systemic autoimmune disease as systemic lupus erythematosus (SLE), rheumatoid arthritis, and Sjogren's syndrome, also it may be detected in the serum of HCV infected patients, The propagation of (ANA) in HCV patients ranges between 6% and 22%, and they are usually found in the patient's serum at a low titer (Eva et al., 2005).

Similarly, Anti mitochondria antibody (AMA), and Anti smooth muscle antibody (ASMA) are repeatedly found in patients with primary biliary cirrhosis, and in HCV infected patients, the prevalence of ASMA in HCV infected patients ranges between 10-66% of cases (Greorio et al., 1998; Luo et al., 1998; Lenzi et al., 1999; Kammer et al., 1999; Drygiannakis et al., 2001; Eva et al., 2005).

Anti reticulin antibody (ARA) is seen in Crohn's disease, dermatitis herpetiformis, celiac disease and in low prevalence in chronic hepatitis C viruses (Eva et al., 2005).

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Anti-brush border antibodies (ABBA) is detectable in thyroiditis, scleroderma, and also were detected in chronic hepatitis c virus (Ezaki et al., 1992). HCV infection may lead also to the production of anti-parietal cell antibody (APCA) (Cassani et al., 1997).

Anti canalicular and Anti ribosomal antibodies are quite rare, but have been found in low titer in HCV infected patients. (McMurry et al., 1997; Gregorio et al. 1998; Dammacco et al., 2000; Obermayer et al., 2001).

Every antibody is directed against a specific intracellular antigen emitted during (Apoptosis) cell death and presented to the immune system. Their pathogenic function and clinical significance still unclear (Eva et al., 2005; Campisi et al., 2016).

There are only a limited number of studies had examined the relationship between circulating autoantibody and IL-6 levels in HCV patients. In this study, we aimed to evaluate the relationship of IL-6 and different circulating autoantibodies (ANA, AMA, ASMA, ARA, ABBA, Anticanalicular and Anti-ribosomal) in untreated Hepatitis C virus patients.

Material and Methods

One hundred and two consecutive Egyptian individuals; 74 males and 28 females aged from 19-69 years; with clinically and laboratory confirmed chronic HCV was included in the present study, other causes of chronic liver disease were ruled out. Patients were from Oncology Hospital, Shebein El-kom, Minufiya Governorate, Minufiya University, Egypt. One hundred and three unrelated healthy blood donors served as normal controls (donors are living in the same geographical area). Patients' medical history, complete blood count, liver and renal function tests include Serum (albumin, AST, ALT, bilirubin, creatinine, Thyroid-Stimulating Hormone (TSH), and Alpha-fetoprotein (AFP)). The study was previously approved by the Ethical Committee of The Institute of Genetic Engineering and Biotechnology Research, Written informed consent was obtained from all patients.

Detection and differentiation of Circulating Autoantibodies by Indirect Fluorescence

ANA detection was performed by Indirect Fluorescence (IIF) using HEP-2 cells (ANAFLUOR DiaSorin Kits Immunofluorescence assay; DiaSorin, Stillwater, Minnesota, USA). The cells were fixed on a microscope slide. IIF was performed according to the protocol suggested by the manufacturer. In brief, serum samples diluted 1:80 were incubated with the HEP-2 cell substrate for 30 minutes at room temperature. After washing with PBS-Tween, the slides were incubated for another 30 minutes with goat anti-human IgG conjugated with fluorescein isothiocyanate and propidium iodide for counterstaining (ANAFLURO) to label precisely bound antibodies. After a second washing step and embedding, the slides were examined under a fluorescence microscope (Leica DM3000). The result of ANA test was considered positive when an apple-green fluorescence stain in the nuclei of the Hep-2 cells was observed.

Circulated autoantibodies were detected using Fluro kits Immunofluorescence assay (DiaSorin, Stillwater, Minnesota, USA). Serum samples were diluted in 1:20 in phosphate buffered saline, and then applied to the tissue section: rat kidney, rat stomach, and a rat liver cryostat section which fixed on the microscope slides. The test was performed according to the protocol suggested by the manufacturer as described previously in ANA. The result was considered positive when an apple-green fluorescence stains was observed in specific tissue organelles as a following at AMA, when present stain the cytoplasm of the kidney distal tubules with a coarse granular fluorescence. ASMA will stain the muscularis mucosa and the muscularis externa of the stomach tissue as well as the muscle layer of the arterioles that may be present in any of the tissue sections. ARA will stain peritubular fibers, Bowman's capsule, vascular endothelium and perivascular fibers. ABBA will stain the internal feather edge of kidney proximal tubules. APCA stains only the stomach's gastric parietal cells and the parietal cell cytoplasm of the stomach tissue. Antiribosomal antibody, if present, stains the gastric chief cell cytoplasm. Anticanalicular antibody, stains the bile canaliculi which exist as minute channels between cells of the hepatic laminae branching laterally between cells.

A Measurement of Serum IL-6 by Enzyme-linked Immunosorbent Assay (ELISA)

Total concentrations of IL-6 in the serum samples were measured using a commercial ELISA kit (R and D System, Inc., Minneapolis, MN), according to the manufacturer's instructions. The intensity of the developed color was measured by reading optical absorbance at 450 nm using a microplate reader (Sunrise™, Tecan Group Ltd. Ma'nendorf, Switzerland) Results were expressed as pictogram of cytokine per milliliter plasma (pg/ml). Talaat et al., 2015.

Statistical analysis

Data was fed into the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using numbers and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. A significance of the obtained results was judged at the 5% level.

Comparisons between both groups were performed by Chi-square test For categorical variables to compare between different groups, Student t-test For normally quantitative variables to compare between two studied groups, Mann Whitney test For abnormally quantitative variables to compare between two studied groups .

Results

Patients' characteristics

Table 1 demonstrates the number, age, and the results of different biochemical parameters carried out in this study, including the different liver function tests, TSH, AFP as well as different parameters of blood picture of individuals from all investigate the group. In the HCV

Table 1. Demographic, Biochemical Characteristics, Autoantibodies and IL-6 Level of All Subject

	Patients (n=102)	Control (n=103)	P
Gender			
Female	28 (27.5%)	48.0 (46.6%)	0.005*
Male	74 (72.5%)	55.0 (53.4%)	
Age	45.42 ± 10.01	28.9 ± 8.4	<0.001*
TLC	5.35 (2.20 – 33.0)	5.6 (4.2 – 10.1)	0.013*
Creatinin	0.90 ± 0.24	0.8 ± 0.2	<0.001*
Hemoglobin	13.06 ± 1.92	13.2 ± 0.9	0.64
AST	46.50 (12.0 – 209.0)	23.0 (10.0 – 40.0)	<0.001*
ALT	51.0 (12.0 – 318.0)	22.0 (10.0 – 37.0)	<0.001*
Alb	4.22 ± 0.61	4.45 ± 0.30	0.001*
Platelet	210.54 ± 61.03	265.22 ± 56.70	<0.001*
TSH	2.20 (0.03 – 36.49)	2.0 (0.40 – 4.20)	0.616
AFP	3.10 (1.20 – 2109.0)	2.0 (0.90 – 8.0)	<0.001*
ANA			
Less than 1:80	78 (76.5%)	98.0 (95.1%)	<0.001*
1:80	18 (17.6%)	5.0 (4.9%)	
1:160	6 (5.9%)	0.0 (0.0%)	
AMA			
Less than 1:20	94 (92.2%)	103.0 (100.0%)	0.004*
1:20	5 (4.9%)	0.0 (0.0%)	
1:40	3 (2.9%)	0.0 (0.0%)	
ASMA			
Less than 1:20	85 (83.3%)	103.0 (100.0%)	<0.001*
1:20	13 (12.7%)	0.0 (0.0%)	
1:40	3 (2.9%)	0.0 (0.0%)	
1:80	1 (1.0%)	0.0 (0.0%)	
ARA			
Less than 1:20	99 (97.1%)	103.0 (100.0%)	0.126
1:20	1 (1.0%)	0.0 (0.0%)	
1:40	2 (2.0%)	0.0 (0.0%)	
ABBA			
Less than Jan-20	102 (100.0%)	103.0 (100.0%)	-
1:20	0 (0.0%)	0.0 (0.0%)	
1:40	0 (0.0%)	0.0 (0.0%)	
APCA			
Less than 1:20	84 (82.4%)	103.0 (100.0%)	<0.001*
1:20	11 (10.8%)	0.0 (0.0%)	
1:40	6 (5.9%)	0.0 (0.0%)	
1:80	1 (1.0%)	0.0 (0.0%)	
Anti ribosomal ab			
Less than 1:20	102 (100.0%)	103.0 (100.0%)	-
1:20	0 (0.0%)	0.0 (0.0%)	
1:40	0 (0.0%)	0.0 (0.0%)	
Anticanalicular Antibody			
Less than 1:20	94 (92.2%)	103.0 (100.0%)	0.003*
1:20	6 (5.9%)	0.0 (0.0%)	
1:40	2 (2.0%)	0.0 (0.0%)	
IL-6	29 (4.0 – 1086.0)	29.3 (3.70 – 139.0)	0.028*

Qualitative data were described using number and percent and was compared using Chi square test; while normally quantitative data was expressed in mean ± SD and was compared using student t-test; abnormally distributed data was expressed in median (Min. - Max.) and was compared using Mann Whitney test; *, Statistically significant at p ≤ 0.05

Table 2. Relation between Serum Level of IL-6 with Different Circulated Autoantibodies in Infected HCV Patients

	N	serum level of IL-6			N	Control		
		Median	Min. – Max	P		Median	Min. – Max	p
Gender	74	34.5		0.006*	55	31	5.2 – 139.0	0.204
Male	28	26.5	4.0 – 1086.0		48	26.8	3.7-100.4	
Female			5.0-561.0					
ANA								
Less than 1:80	78	29.0	4.0 – 1086.0	0.483	98	29.65	3.7 – 139.0	0.69
1:80	18	32.0	6.0 – 1073.0		5	19.6	9.3 – 43.6	
1:60	6	19.5	6.0 – 235.0		0	-		
AMA								
Less than 1:20	94	29.0	4.0 – 1086.0	0.515	103	29.3	3.7 – 139.0	-
1:20	5	27.0	6.0 – 987.0		0	-		
1:40	3	224.0	29.0 – 431.0		0	-		
ASM								
Less than 1:20	85	29.0	6.0 – 1086.0	0.543	103	29.3	3.7 – 139.0	-
1:20	13	29.0	4.0 – 431.0		0	-		
1:40	3	29.0	8.0 – 987.0		0	-		
1:80	1#	32.0			0	-		
ARA								
Less than 1:20	99	29.0	4.0 – 1086.0	0.031*	103	29.3	3.7 – 139.0	-
1:20	1#	29.0			0	-		
1:40	2	7.0	6.0 – 8.0		0	-		
APCA								
Less than 1:20	84	29.0	6.0 – 1086.0	0.453	103	29.3	3.7 – 139.0	-
1:20	11	35.0	4.0 – 987.0		0	-		
1:40	6	22.0	6.0 – 136.0		0	-		
1:80	1#	19.0			0	-		
Anticanalicular								
Less than 1:20	94	29.0	5.0 – 1086.0	0.443	103	29.3	3.7 – 139.0	-
1:20	6	30.5	4.0 – 224.0		0	-		
1:40	2	508	29.0 – 987.0		0	-		

Abnormally distributed data was expressed in median (Min. - Max.); and was compared using Mann Whitney test; *, Statistically significant at $p \leq 0.05$

patients group there were 74 (72.5%) male and 28 (27.5%) female patients and in the control group there was 55 (53.4%) male and female 48 (46.6%). The median age of HCV patients was 48.0 years (range: 23.0 – 69.0 years) and 26 (range: 19.0-54.0).HCV patients had significantly higher in Creatinine, AST, ALT, and AFP than in control cases ($P < 0.01$), No significant difference was found in the Hb and TSH.

Detection of circulating autoantibodies

ANA was the most frequent autoantibody detected in the HCV patients, it was found in 24 (23.5%), ANA in dilution 1:80 was detected in 18 (17.6%) patients and ANA in dilution 1:160 in 6 (5.9%) patients. ASMA was found in 17(16.7%), ASMA in dilution 1:20 was detected in 13 (12.7%), ASMA in dilution 1:40 in 3 (2.9%) patients, and ASMA in dilution 1:80 was positive in one patients (1.0%), the AMA was found in 8 (7.8%). AMA in dilution 1:20 was detected in 5 (4.9%) and AMA 1:40

in 3 (2.9%). APCA was positive in 18 (17.6%), APCA in dilution 1: 20 was detected in 11 (10.8%), in dilution 1:40 in 6 (5.9%) patients and in dilution 1:80 was detected in one patient (1.0%). ARA in 3 (2.9%), one patient (1.0%) in dilution 1:20 and two patients (2.0%) were detected in dilution 1:40. Anti canalicular Ab was found in 8 (7.8%), in dilution 1:20 was positive in 6 (5.9%) and two patients (2.0%) were positive in dilution 1:40. No patients were found to be positive for ABBA and anti-ribosomal antibodies in any of the 102 serum tested. In the control group only ANA was positive in 5 (4.9%) individuals in dilution 1:80, the rest of autoantibodies were negative.

Serum levels of IL-6 in infected HCV patients

A statistically significant increase of IL-6 ($p = 0.028$) in infected HCV patients compared to healthy controls, 29.0 (160.9 ± 265.8) versus 29.3 (32.1 ± 24.2) was demonstrated (Table 1).

Relation between serum levels of IL-6 with different circulated autoantibodies:

As shown in Table 2, No correlation was observed between serum levels of IL-6 and different circulated autoantibodies (ANA, AMA, ASMA, ARA, APCA and Anticanalicular Ab). In spite of, serum level of IL-6 in infected male was higher than infected female.

Discussion

In this study, we aimed to evaluate the relationship between IL-6 and circulating autoantibodies in infected HCV patients, our study showed a highly increased in serological markers of autoimmunity among the patients infected with HCV. In other similar studies, these autoantibodies have been found to distinguish HCV-infected patients according to their clinical statuses. (Valentini et al., 1999; Zusinaite et al., 2005).

(HCV) has been related to many autoimmune diseases and can stimulate the production of non-organ specific autoantibodies (Sousa et al., 2011). These phenomena occur through molecular mimicry, induction of Toll-like receptor hypersensitivity or by creating immortal B and T cells (Barzilai et al., 2007).

Autoantibodies were detected in 43.1% of the patients; of these, 23.5% positive ANA, 7.8 % positive (AMA+), 16.7 % positive ASMA, 2.9% positive ARA, 17.6% positive APCA and 7.8 % positive Anti canalicular Ab which is similar to other studies (Clifford et al., 1995; Joanna et al., 2011; Rev et al., 2013; Deng et al., 2014). on the other hand no patients were found to be positive for ABBA and anti ribosomal antibodies in any of the 102 serum tested.

The study display a relation between the ages of HCV infected patients and the presence of different autoantibodies. This could be connected with the aggravation of the mechanisms protecting against autoimmune reactions and the longer duration of HCV infection. The contribution to worse outcomes of viral hepatitis in the elderly may be associated with several physiological changes (Carrion et al., 2012). Autoantibodies in HCV infected group were more prevalent in female and in older patients (Table1). The increased female tendency for autoimmune disease may relate to estrogenic effects that modulate the autoreactive response directly by affecting the pro- and anti-inflammatory cytokine pathways of lymphocyte differentiation (Markle et al, 2014).

Interleukin-6, a multifunctional cytokine produced by a variety of cells, plays a central role in regulating the immune system, acute phase and hematopoiesis (Zekri et al., 2005).

The results regarding the investigated Serum IL-6 levels showed that were higher in patients with chronic HCV infection in comparison to healthy adults, which is in accordance with that reported by Fallahi et al 2012; Comanescu et al., 2015. Serum IL-6 levels related with viral load and histological index (Malaguarnera et al., 1997). On the other hand, lower levels of IL-6 associated with sustained virologic response, at most, in men (Ueyama et al., 2011). In our group of patients, we found

significant differences between serum levels of IL-6 in male versus female ($p=0.006$) in contrast of Comanescu et al., 2015.

Although previous studies have suggested pathogenic roles for raising levels of IL6 and different autoantibodies in autoimmune disease (Ripley et al., 2005), but no correlation was found between IL6 and different autoantibodies, TSH or white blood cell (TLC) levels in HCV patients.

When levels of IL6 were compared between HCV patients and healthy group, levels of IL6 were clearly higher. Furthermore, high levels of different circulating autoantibodies in HCV patients were found. As a result, this study suggests for the first time a link between raised IL6 levels and different autoantibodies in HCV patients..

In conclusion, we investigated the relationship between IL6 and different circulated autoantibodies levels with infected HCV patients. We found no correlation between IL6 and autoantibodies in HCV. However, on further analysis, this apparent correlation was explained by the relationship between IL6 levels and autoantibodies levels in different autoimmune disease, which is not specific to HCV. Further studies are required, to understand more completely the mechanisms which IL6 might influence the development of autoantibodies production in HCV, both at the cellular and the molecular level.

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