Serum IL-10 level and response to combined pegylated interferon and ribavirin therapy in Egyptian patients with chronic hepatitis C virus infection

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Abstract: Introduction: Hepatitis C virus (HCV) infections is a major health problem. Egypt has the highest prevalence worldwide. Currently, combined pegylated interferon and ribavirin therapy is the standard treatment but the outcome is not satisfactory. It has been reported that patients with chronic HCV infection show enhanced serum IL-10 concentration and it was found to be correlated to the histopathological alterations of the liver. Objectives**:** To assess the possible association of serum IL-10 level and response to combined interferon α-2a and ribavirin therapy for chronic HCV infection. Patients and methods This study was conducted on 50 consecutive patients with chronic HCV infection and 20 healthy controls. All the patients were subjected to clinical and laboratory assessment, abdominal ultrasound, and liver biopsy. All the patients were treated with combined therapy and followed up for end of treatment and sustained virologic response (SVR). Determination of IL-10 serum level using ELISA test were done at the baseline and at the end of treatment. Results: Pre-treatment serum IL-10 was significantly positively correlated with BMI and grade of positivity of HCV RNA PCR. Pre-treatment serum IL-10 levels were significantly lower in responders at the end of treatment and SVR in comparison to non responders ($P<0.001$). There was significant reduction of serum IL-10 level after therapy in comparison to baseline in responders with no significant change in non responders. Conclusions: increased serum levels of IL-10 are a poor prognostic marker of response to combined treatment in patients with chronic HCV infection.
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Key words: hepatitis C, IL10, pegylated interferon, SVR.

Introduction:

Chronic hepatitis C virus (HCV) infection is a significant health problem (1) affecting almost 170 million individuals worldwide. It is the most frequent cause of chronic liver disease (2,3). Egypt has the highest prevalence worldwide (4). Interferon-α monotherapy and, more recently, combination IFN- α and ribavirin therapy are the only treatments for chronic hepatitis C shown to be effective (5). As a result of adverse events, a moderate rate of virologic response and high costs associated with HCV therapy, finding early markers of sustained treatment response is a clinical priority (6).

IL-10 is an anti-inflammatory cytokine involved in T helper2 (Th2) immunity (7). The Th2 profile inhibits the development of effectors mechanisms being involved in the pathogenesis of the chronic C hepatitis, as well as in the severity of the chronic liver disease (8,9). It has been reported that patients with chronic HCV infection show enhanced serum IL-10 concentration (10,11).

IL-10 was found to be correlated to the histopathological alterations of the liver in patients with chronic HCV infection (12). It was found to be correlated with disease progression of chronic liver disease and hepatocellular carcinoma (13). Furthermore, long term IL-10 therapy to treat chronically HCV-infected patients leads to a significant improvement of inflammation and fibrosis (14).

This study was designed to assess the possible association of serum IL-10 level and response to combined interferon α-2a and ribavirin therapy for chronic HCV infection.

Materials and methods

Population samples: This study was conducted on 50 consecutive patients with chronic HCV infection and 20 healthy control subjects. All the included patients were diagnosed as chronic HCV infection with

positive HCV antibody and PCR for HCV RNA and they were candidate for treatment with pegylated interferon α and ribavirin. All the patients were treatment-naïve.

Exclusion criteria: Patients who are younger than 18 years, older than 60 years, have co-infection with hepatitis B virus, alcohol intake, clinically evident liver cirrhosis, esophageal varices, hepatic encephalopathy, hepatocellular carcinoma, any end organ failure, hematological diseases, major psychiatric disorder, pregnant and breast feeding women were excluded from the study. Informed consent was obtained from all participants before enrollment in the study.

Methods:

All the patients were subjected to clinical assessment. Height and weight were determined at baseline and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Before starting therapy, all patients and controls were subjected to the following: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma Glutamyle transferase, albumin, total bilirubin, and kidney function (synchron CX4- clinical system), international normalized ratio (INR), complete blood count, alfa-fetoprotein, HCV antibody (Axyam machine-Ireland) and abdominal ultrasound. Quantitative HCV PCR using Real Time PCR (Stratagene) and liver biopsy were done for all the enrolled patients. Determination of IL-10 serum level was done using ELISA test (Diasorine Catalog Number D1000B). Blood samples were obtained in the morning after 12 hours of fasting. They were centrifuged and serum was separated, and then stored at -20ºC prior to use.

Percutaneous liver biopsy was performed under ultrasound guidance using 16 gauge needles. Specimens of at least 2.5 cm in length, including a minimum of 12 portal tracts were considered reliable for adequate grading and staging using modified Knodell's score (15). Reading of liver biopsies was done by a single pathologist who was blind to the clinical data.

All the patients were treated with pegylated interferon α-2a, 180 µg/week subcutaneously, plus ribavirin (1000-1200 mg orally /day based on body weight) (16).

Quantitative PCR for HCV-RNA after 12 weeks was done to assess early virological response. Those who showed response manifested by negative PCR or decrease of viral load by 2 logs, continued the therapy for 48 weeks. Qualitative PCR for HCV-RNA was repeated at 48 weeks for assessment of end of treatment response (ETR), and 72 weeks for the sustained virologic response (SVR), defined as negative HCV-RNA 6 months after the end of treatment (17).

All the patients were followed up regarding clinical and biochemical parameters every week for 1 month, every 2 weeks during therapy, then every month up to 6 months after end of therapy. Serum IL-10, liver function tests, kidney function test and complete blood count were done at the end of treatment.

Data analysis

Data were expressed as Mean ± SD for quantitative measures and analyzed using SPSS 11 for Windows. Comparison between groups was made by using Student's t-test. Spearman correlation coefficient test was used to study the association between each two variables among each group. A probability of error value of $P < 0.05$ was considered statistically significant while P-value < 0.01 is highly significant.

Results

Fifty patients with chronic HCV infection and 20 healthy control subjects were enrolled in this study. The patients were 34 males (68 %) and 16 females (32 %), their ages ranged from 20 to 52 (mean 40.1±7.9 years). The controls were 9 males (45 %) and 11 females (55%), their ages ranged from 18 to 47 (mean 38.9 ± 6.4 years) with no statistically significant difference between cases and controls as regards age and gender. Regarding BMI, there was no statistically significant difference between cases (24.4 ± 7.7) and controls (24.8 ± 8.0) ($P>0.05$).

Laboratory findings in patients before the start of therapy and control subjects are shown in table (1). There were significantly higher mean ALT, AST, total bilirubin and AFP among cases compared to controls ($P<0.01$). Also, pretreatment serum IL-10 levels were significantly higher among cases in comparison to control subjects ($P<0.01$).

All the patients were treated with pegylated interferon α-2a subcutaneously every week plus ribavirin orally. None of our patients required discontinuations of treatment due to adverse effects or laboratory abnormalities. But, four patients required temporary dose reduction due to hematological adverse effects. All the patients were adherent to treatment. Adherence to treatment is defined as taking 80 % of each drug for at least 80 % of the duration of therapy (18).

Regarding the response to therapy, 32 (64%) patients showed negative PCR at the end of therapy (end of treatment response); 3 of them relapsed and 29 (58%) showed SVR; while 18 (36%) patients didn't achieve ETR and were considered non responders.

BMI was significantly lower in responders (21±5.6) in comparison to non responders (30.4±7.2) (t 5, P<0.001)

After completion of therapy, there was a significant reduction of ALT (36.4±9.7), AST (46.3±22.8) and PT (0.9± 0.06) compared to pre-treatment level (P<0.01), while there was no significant difference regarding albumin (3.7±0.4), total bilirubin (0.9± 0.5) or AFP (9.5±3.5) (P>0.05). Also, serum level of IL-10 in cases showed a significant reduction (112.3±17.8) compared to pre treatment level (t 8.5, P<0.0001).

Pre-treatment serum IL-10 levels were significantly lower in responders at the end of treatment and patients who showed SVR in comparison to non responders (P<0.001) (Table 2).

Serum IL-10 levels before and after treatment in responders and non responders to therapy are shown in table 3. In responders, there is significant reduction in serum IL-10 level after therapy in comparison to pretreatment level. While, there was no significant change in serum IL-10 levels before and after treatment in non responders.

Using Spearman correlation test, pre-treatment serum IL-10 was significantly positively correlated with BMI and grade of positivity of HCV RNA by PCR (table 4).

Discussion:

Many cytokines secreted by Th1 and Th2 cells are involved in the immune response to HCV infection and progression of HCV-related liver disease. Th1 cells release TNF-α, INF-γ and IL-2, causing inflammation and necrosis, and Th2 release IL-4 and IL-10, which modulate hepatic injury by suppressing the Th1 response and counteracting the fibrogenic effects of TNF-α, INF-γ and IL-2 (19).

Interferon is still an optimal agent for the treatment of hepatitis C, but the outcomes are not satisfactory in some patients. Various factors were found to contribute to the outcomes (20).

This study was designed to assess the possible association of IL-10 level and response to combined pegylated interferonα-2a and ribavirin therapy in patients with chronic HCV infection.

In the present study, the pre-treatment serum IL-10 levels were significantly higher in chronic HCV patients (124.7 ± 14) compared to control subjects (96.2 ± 21.6), in line with many studies (21, 22, 23,24). It has been reported that patients with chronic HCV infections show enhanced serum IL-10 concentration (25) and T-cells IL-10 production in response to stimulation with HCV Core protein (26).

In this study, a significant positive correlation was found between pretreatment IL-10 and body mass index, in agreement with Reuss et al. (27) who reported that decreasing body mass index appear to decrease IL-10 production.

A highly significant positive correlation was found between IL-10 and HCV viral load and this is constant with Flynn et al. (28) who stated that the magnitude of the HCV viral load correlated with IL-10 production. The direct mechanism linking high HCV replication to increased IL-10 production is not clear, but is may be via inhibition of the induction of HCV-specific effector T-cell responses (28).

In this study there was no correlation between IL-10 levels and the fibrosis stage this was agree with Imbert-Bismut et al. (29) who stated that IL-10 was not correlated with fibrosis stage

Also, we found that pre-treatment IL-10 serum levels were significantly lower in responders (116.7±5.6) compared to non responders (138.7±13.4), in line with Marín-Serrano et al. (24) who reported that baseline levels of IL-10 were significantly higher in patients without any response to treatment compared with those with sustained response. This might be explained by the finding that low IL-10 production may help to establish an effective immune response which helps HCV clearance (30), while the high level IL-10 could not only depress synthesis and secretion of inflammatory factors induced by HCV antigen but also refrain multiplication and differentiation of inflammatory cells such as cytotoxic T lymphocyte cells and NK cells, resulting in lowering of immunoreactive effect and failure of antiviral treatment (31). In contrary, Bozkaya et al. (32); in their study on 37 chronic HCV patients who were treated by IFN-alpha2b for 6 months; found no difference in baseline IL-10 levels in responders and non-responders.

As regarding serum level of IL10 before and after treatment, Bozkaya et al. (32) found that after treatment some patients lost their detectable IL-10 and some patients developed detectable IL-10 levels after treatment irrespective of the treatment response. Also, Marín-Serrano et al. (24) found that the concentration of IL-10 did not change with treatment. While in the present work, serum IL-10 levels showed significant decrease in responders compared to pre-treatment levels, with no significant difference between pre-treatment and post treatment levels in non responders, in accordance with Cacciarelli et al. (33) who reported that treatment with IFN-alpha for 12 weeks decreased the levels of IL- 10. This significant decrease of IL-10 after interferon administration might be explained by the fact that HCV was cleared by interferon through immunomodulation in addition to direct antiviral activity (31).

Table (1): Comparison between cases and controls as regards the laboratory findings before treatment

	Cases (N=50)	Control (N=20)	t	P
ALT (IU/ml)	56.7±15	30±6	7.6	0.000*
AST (IU/ml)	60.7±19.2	32.4±9	6.3	0.000*
Total bilirubin (mg/dl)	1±0.4	0.74±0.2	3.4	0.001*
Albumin(g/dl)	3.8±0.3	3.8±0.2	0.2	0.7
AFP (ng/ml)	9.4±5.2	5.8±2	4.0	0.000*
INR	0.93±0.06	0.93±0.05	0.1	0.8
pre-treatment IL-10 (pg/ml)	124.7±14	96.2±21.6	6.5	0.000*

* highly significant

Table (2) Pre-treatment serum IL-10 levels in responders and non responders

		IL-10 (pg/ml)	t	P
ETR	Responders	116.7±5.6	8.1	0.000*
	Non responders	138.7±13.4		
SVR	SVR	117.4±5.3	5.4	0.000*
	No SVR	134.7±10.1		

* highly significant

Table (3) Serum levels of IL-10 before and after treatment among responders and non responders (at end of therapy).

ETR	IL-10 (pg/ml)		t	P
	Pre-treatment	After treatment		
Responders (N=32)	116.7±5.6	100.5±4.8	14.1	0.000*
Non responders (N=18)	138.7±13.4	133.3±12.2	1.9	0.07

* highly significant

Table (4): Correlation coefficient between pre-treatment serum IL-10 levels and studied parameters

		ALT	AST	T.bilirubin	albumin	AFP	PT	BMI	HAI staging	HAI grading	PCR
IL-10	r	0.018	-0.274	0.042	-0.121	0.034	0.131	0.309	0.135	0.240	0.635
	P	0.09	0.05	0.7	0.4	0.8	0.3	0.02	0.3	0.09	0.000

Conclusion:

As pre-treatment serum IL-10 levels is significantly higher in non responders in comparison to responders with no significant reduction in post-treatment level in non responders, we concluded that increased serum levels of IL-10 are a poor prognostic marker of response to combined treatment in patients with chronic HCV infection.

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