Association of Interleukin–18 with Sustained Virological Response (SVR) To Interferon Therapy in Patients with Chronic Liver Diseases

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Abstract: Background: Pegylated interferon plus ribavirin is the standard therapy for treatment of Chronic hepatitis C (CHC) patients, this therapy is only effective in 50–60% of infected individuals. Pretreatment predictors associated with sustained virological response (SVR) to interferon treatment have not yet been fully investigated. Several factors have been shown to influence response to antiviral treatment, one of them is interleukin – 18 (IL-18). There are few data on IL-18 in relation to infectious diseases, but it plays a prominent role in chronic HCV infection. Thus, the current study is designated to assess serum Interleukin – 18 levels in CHC genotype 4 patients and its association with treatment response. **Patients and Methods**: This study was performed on fifty Egyptian chronic hepatitis C patients, who received combined pegylated interferon alpha and ribavirin therapy and twenty healthy blood donors as a control group. Quantitative hepatitis C virus RNA was done by real time RT-PCR technique. Serum IL-18 level was assayed using quantitative ELISA plate method. **Results**: The mean level of IL-18 was significantly higher in CHC patients (212.15 Pg/ml) compared to the controls (48.95 Pg/ml). IL-18 level in responders was significantly reduced after 6 months from the end of treatment compared to those at baseline level (P=0.001), but such decrease was lower than that in non responders (P=0.058). IL-18 level was positively correlated with high and moderate viral load in non responders. **Conclusions:** Serum IL- 18 level has the potential to be used as a biological marker to predict outcome of antiviral therapy among CHC genotype 4 patients.

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1. Introduction

Hepatitis C virus (HCV) has been estimated by the World Health Organization to infect 170-200 million patients worldwide, with the highest prevalence rate about 22% among Egyptian population[(1)]. Characteristic features of HCV infection include its persistence in the host and progression to chronic hepatitis, leading to cirrhosis, which is a strong risk factor for the development of hepatocellular carcinoma (HCC) [(2)]. Interferon based therapy can reduce HCV to undetectable levels and improve prognosis. The primary aim of antiviral therapy in HCV patients is a sustained virological response (SVR), which is defined as undetectable serum HCV RNA 24 weeks after completion of therapy. Combined pegylated interferon-α and ribavirin therapy has sustained virological response rates reaching in different clinical trials from 54% to 61% [(3,4)].

Cytokines play an important role in the pathogenesis, progression, and treatment outcome of HCV infection. Because the control of cytokine production is highly complex and the effects of cytokines are widespread throughout multiple regulatory networks, it would seem that screening for multiple biomarkers could clarify the immunopathogenesis of the disease and predict antiviral therapy response[(5)]. IL-18 previously has been known as interferon-gamma-inducing factor, is a pleiotropic proinflammatory cytokine that is expressed mainly by peripheral blood mononuclear cells and macrophages. In the liver, besides its expression in Kupffer cells, IL-18 can also be synthesized by injured hepatocytes [(6,7)]. The fundamental function of IL-18 is an enhancement of T helper type 1 (T_H1) cytokine production [(8)]. The dynamics of TH1/TH2 response determine the outcome of antiviral therapy to chronic hepatitis C (CHC) and that IL- 18 is an important mediator of the TH1/TH2 balance [(4)]. The balance of TH1 and TH2 CD4+ responses has proven relevant to HCV infection. Accumulating evidence has shown that an early TH1 immune response leads to viral clearance, whereas a TH2 response favors chronic evolution [(23)]. IFN-α treatment has a negative influence on IL- 18 concentration, independently of viral effects [(21)]. Therefore, TH1 decreased, TH2 increased and incidence defect in dynamics of TH1 / TH2 balance occurred. Studies in murine models and cell lines

indicate that IL-18 has potent antitumor effects that are mediated by enhancement of cytotoxic T lymphocyte and NK activity, induction of apoptosis and reduction of tumor development. Hence, it has been hypothesized its role in prevention of HCC [9].Moreover, there is a dearth of data on the nature of the involvement of IL-18 in HCV pathogenesis and host defense [9]. However, the effect of IL-18 on antiviral therapy for chronic hepatitis C is still unclear [4]. There are few data on IL-18 in relation to infectious diseases, but it plays a prominent role in chronic HCV infection In this context, the aim of the present study is to investigate role of IL-18 and its association with outcome of PEG-IFN and ribavirin therapy hoping to find a non invasive marker can be used to predict response to antiviral therapy in chronic hepatitis C genotype 4.

2. Patients and Methods

Patients

This prospective study was conducted with 70 participants divided into two groups. The first group comprised 50 patients with CHC-4 patients diagnosed and treated at Ain Shams University Specialized Hospital, Cairo, between October 2010 and January 2012. The second group included 20 apparently healthy participants who had donated blood at the National Cancer Institute, Cairo University. The Ethical Committee of Ain Shams University Specialized Hospital approved the study protocol, which was prepared in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and later revisions.

Written consent was obtained from all participants prior to enrollment in the study and all were mentally and physically capable of answering a questionnaire. Inclusion criteria: adult patients of both sexes (18-46 years old), diagnosis within the previous 12 months, positive for HCV RNA in serum (by RT-PCR assay), with evidence of chronic hepatitis supported by liver biopsy. The control group was made from adults negative for HCV RNA and HBV DNA. Patients were not receiving hepatitis treatment at the time of sampling. The presence of HBV infection or co-infection was excluded by serum ELISA for anti-HBc and HBsAg. In addition to investigations needed to fulfill the selection criteria, all individuals included in this study were subjected to the following:

A. Medical History

Full history was taken with special reference to risk factors for liver diseases such as previous HCV exposure in surgical wards, blood transfusions, dental therapy, needlestick injury, history of HCV in the spouse and iv. injection.

B. Physical Examination

Complete medical examination with particular focus upon the manifestations of hepatitis such as jaundice, hepatomegaly, and tenderness in the right hypochondrium. BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m2). Abdominal ultrasonography was performed for all patients.

C. Histopathological investigations

Liver biopsy specimens were formalin-fixed and paraffin embedded then sectioned and stained (hematoxylin and eosin) for routine histopathological examination. Grading and staging of chronic hepatitis was performed according to Modified Knodell's Score [12].

D. Laboratory investigations

Venous blood samples were taken in the morning after 12-h overnight fast. Plasma glucose, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (Alb), total bilirubin levels (Bil) and cholesterol (Chol) were measured by using Synchron CX4 clinical system (standard clinical laboratory methods) at the Clinical Laboratory Department, Ain Shams Specialized Hospital. Serum alpha-fetoprotein levels were estimated by serological techniques (Axyam System, Abbott Laboratories). Prothrombin Time (PT) measurements were performed for all patients; normal PT was 13 seconds (100% concentration and International Normalization Ratio (INR) of 1). Interleukin (IL- 18) was calculated on Quantitative determination of IL- 18 by ELISA technique. ELISA was performed according to the manufacturer's instructions. The Human IL-18 ELISA Kit measures human IL-18 by using third generation kits (DiaSorin, Italy) according to the manufacturer's instructions. The concentration of IL18 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

E. Viral Markers

ELISA assays

Sera of all patients and controls were tested for HBsAg, anti-HBc and anti-HCV antibodies by ELISA, using third generation kits (DiaSorin, Italy) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) for detection of hepatitis C virus

RT-PCR was performed as previously described [13] and 10 µl samples of the amplicons were analyzed by electrophoresis (1.2% agarose gel, ethidium bromide staining).

HCV genotyping

HCV genotype was determined using INNO LiPAII and III versant Kit (Innogenetics, Ghent, Belgium) according to manufacturer's directions.

Quantitation of HCV-RNA in serum:

HCV-RNA was quantitated in all patients' serum samples using Real Time PCR (RT-PCR) [14] (primers and RT-PCR reagents from Stratagene, Qiagen, USA). Low viremia was defined as viral load lower than 100 x10^3 IU/L, moderate viremia as viral load 100-1000 × 10^3 IU/ L, and high viremia when viral load > 1000 x10^3 IU/ L [15].

Statistical analysis:

Mean ± standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) were used where appropriate. Comparison of quantitative variables between the study groups was done using paired-samples T test for dependent samples when comparing 2 groups. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. A probability value (p value) less than 0.05 was considered statistically significant. Pearson Correlation Coefficient - Benesty method was used to test correlation between numerical variables. Receiver-operator characteristic (ROC) curves were calculated the area under the curve (AUC) and 95% confidence interval (CI) were calculated for each plot. In order to select cutoff value that best combined sensitivity and specificity. All tests are two-tailed; a p-value<0.05 was considered significant. Statistical calculations were performed using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 17 for Microsoft Windows.

3. Results:

Detection and quantification of serum markers in CHC - 4 patients and their controls.

The mean baseline serum concentration of ALT (51.50 vs 35.30 IU/L; p = 0.000), AST (65.50 vs 48.35 IU/L; p = 0.000), and Hb (12.21 vs 10.35 g/dl; p = 0.000) were significantly higher in CHC patients than in healthy control (**Table 1**).

Of 50 patients receiving PEG-IFN and ribavirin therapy, 29(58%) were responders with accompanying normalization of ALT levels and 21(42%) were non responders. Three (6%) patients out of the 21 non responders had relapsed. For the patients at the baseline (before treatment), the mean AST level in responders group was significantly lower than that in non responder group (47.24 vs 74.81 IU/L; p = 0.000). But, mean ALT level in responder group was not significantly lower than that in non responder group (54.71 vs 59.29 IU/L; p = 0.381). Results are shown in **Table 2.**

As regards IL-18 level, the mean baseline level of IL-18 was significantly higher in patients with HCV-4 infection than in healthy controls (212.15 vs 48.95 pg/ml; p = 0.000), Table 1. However, mean baseline level of IL-18 was slightly higher though not significant in responders than in non responders (221.19 vs 217.33 pg/ml; p = 0.744), it showed a significant reduction after 6 months from end of treatment in responders group (194.57 vs 102.00 pg/ml; p = 0.001) compared with non responders (217.33 vs 194.57 pg/ml; p = 0. 058**).**

Clinico-pathological findings and serum IL-18 levels.

Several demographic (age and sex) and clinical (AST, ALT, and HCV viral load), pathological (fibrosis and necro-inflammatory activity) finding were examined for their correlation with serum cytokine (IL-18) level in patients infected with CHC genotype 4. No correlation was observed between IL-18 levels and sex, ALT, AST, T. Bil, or AFP (**Table 3**).

Generally, number of CHC-4 patients who showed minimal scarring of fibrosis (F1) after 6 months of completion of antiviral therapy was higher than those before treatment (12% vs 4%). Also, number of patients who showed milder necro-inflammatory activity (A2) was higher after six months of end treatment than that before treatment (88% vs 34%). Such pathological observations were found to be correlated with HCV viral load, as number of patients with high viral load decreased from 26% before treatment to 2% after end of treatment. Data are shown in Table 1.

Regarding the correlation between clinic-pathological parameters and IL-18 level, mean level of IL-18 showed positive correlation though not significant with advanced stage of fibrosis and was dramatically decreased after 6 months from end of treatment compared to before treatment (**Fig. 1)**.

Similarly, mean level of IL-18 was found to be correlated with necro-inflammatory activity in CHC-4 responders and non responders patients. Patients with higher HAI index (A3) showed higher mean level of IL-18 than those with lower HAI (A1), **Fig. 2**.

IL-18 and viral load

Mean level of IL-18 was significantly correlated with HCV viral load after 6 months from the end of treatment. Mean IL-18 level in CHC-4 patients with high viral load was significantly higher than those negative for HCV-4 after end of treatment (r=-0.807, p =0.001). As regards response to treatment, mean level of IL-18 was significantly higher in non responders with moderate and high viral load than those with low viral load. But significant reduction in IL-18 level was observed in responders after end of treatment (p =0.001), **Fig. 3.**

Table 1: Demographic characteristics and laboratory parameters of the studied groups

Variable	Control N=20	CHC (Baseline) N=50	CHC (end of follow-up) N=50	*P*=value
Demographic characters				
Age (yr) mean ± SD	33.10 ± 8.169	41.30 ± 7.928	41.30 ± 7.928	0.005[a]
Sex				0.258[a]
Male	9	34	34	
Female	11	16	16	
M:F ratio	1:1.22	2.13:1	2.13:1	
BMI (kg/m2) mean±SD	24.85 ± 8.054	30 ± 10.407	30 ± 10.407	
Obese > 25 n (%)	7 (35 %)	22 (44 %)	23 (46 %)	0.111[a], 0.492[b], 0.000[c]
Non-obese n (%)	13 (65%)	28 (56 %)	27 (54 %)	
Biochemical tests (mean ± SD)				
IL- 18 (Pg/ml)	**48.95 ± 11.166**	**212.15 ± 40.977**	**153.70 ± 56.552**	**0.000[a], 0.000[b], 0.000[c]**
ALT (IU/L)	30 ± 6.00877	51.50 ± 13.930	35.30 ± 10.979	0.000[a], 0.036[b], 0.000[c]
AST (IU/L)	32.40 ± 9.064	65.50 ± 21.681	48.35 ± 23.006	0.000[a], 0.012[b], 0.000[c]
T.Bil (mg/dl)	0.745 ± 0.2012	1.130 ± 0.4378	0.955 ± 0.4850	0.002[a], 0.089[b], 0.265[c]
D.Bil (mg/dl)	0.15 ± 0.0607	0.29 ± 0.1917	0.22 ± 0.1824	0.004[a], 0.1[b], 0.53[c]
Albumin (g/dl)	3.85 ± 0.2164	3.790 ± 0.3243	3.585 ± 0.4826	0.466[a], 0.043[b], 0.079[c]
PT %	99.625 ± 0.2048	93.50 ± 6.902	91.25 ± 7.926	0.965[a], 0.634[b], 0.015[c]
Glucose(mg/dl)	101.85 ± 13.914	102.90 ± 18.148	98.25 ± 9.436	0.856[a], 0.405[b], 0.132[c]
HB (g/dl)	11.80 ± 1.735	12.21 ± 1.7177	10.35 ± 1.2258	0.472[a], 0.007[b], 0.000[c]
TSH (ng/dl)	2.78 ± 1.1813	3.435 ± 0.7235	4.05 ± 1.2976	0.074[a], 0.007[b], 0.028[c]
Serum AFP (ng/ml)	5.86 ± 2.0291	11.85 ± 4.44	11.35 ± 4.069	0.000[a], 0.000[b], 0.865[c]
Creatinine (mg/dl)	0.99 ± 0.181	1.385 ± 1.7989	1.13 ± 0.225	0.354[a], 0.043[b], 0.749[c]
HCV RNA (Viral load copies/IU/L) N (%)				0.000[c]
High		13(26%)	1(2%)	0.248
Moderate		23(46%)	9(18%)	
Low		14(28%)	11(22%)	
- ve			29(58%)	
Stage of fibrosis N(%)				0.250
F1		2(4%)	6(12%)	
F2		30(60%)	28(56%)	
F3		18(36%)	16(32%)	
F4		0	0	
Necroinflammatory activity index (HAI) N(%)				0.516
A1		5(10%)	24(48%)	
A2		44(88%)	17(34%)	
A3		0	0	
A4		1(2%)	9(18%)	

Data are mean ± SD, frequency (%); obesity defined as BMI>25; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PT%, prothrombine concentration; T. Bil, total bilirubin; D. Bil, direct bilirubin; HB, hemoglobin; IL-18, Interlukin-18; AFP, alpha fetoprotein; p-value comparison between CHC patients at baseline and healthy control [a], CHC patients after 6 months from end treatment and healthy control [b], and CHC patients at baseline and CHC patients after 6 months from end treatment [c].

Table 2: Demographic characteristics and laboratory parameters of responders and non responders (at baseline and end of follow-up):

Variable	At baseline		At end follow-up		*P*=value
	Responders (n = 29; %58)	Non-responders (n = 21; %42)	Responders (n = 29; %58)	Non-responders (n = 21; %42)	
Demographic characters					
Age (yr) mean ± SD	40.14 ± 8.645	41.24 ± 7.622	40.14 ± 8.645	41.24 ± 7.622	0.651
Sex					0.772
Male	19	15	19	15	
Female	10	6	10	6	
M:F ratio	1.9 : 1	2.5 : 1	1.9 : 1	2.5 : 1	

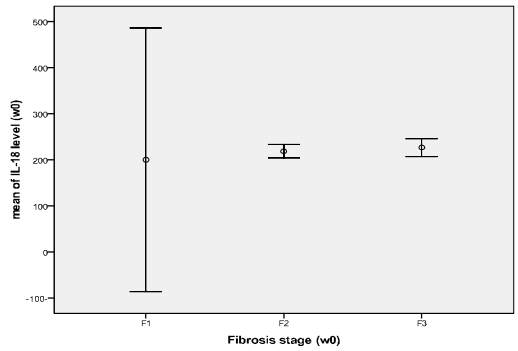
BMI (kg/m2) mean ± SD	20.24 ± 4.949	29.86 ± 7.512	20.24 ± 4.949	29.86 ± 7.512	0.001
Obese > 25 n (%)	5(17.2 %)	17(80.9 %)	5(17.2 %)	17(80.9 %)	
Non-obese n (%)	24(82.8 %)	4(19.1 %)	24(82.8 %)	4(19.1 %)	
Biochemical tests mean ± SD					
IL- 18 (Pg/ml)	**221.19 ± 51.493**	**217.33 ± 27.13**	**102 ± 4.416**	**194.57 ± 50.246**	**0.744[a], 0.000[b]**
ALT (IU/L)	54.71 ± 16.091	59.29 ± 13.517	33.14 ± 6.093	41.29 ± 11.637	0.381[a], 0.017[b]
AST (IU/L)	47.24 ± 12.429	74.81 ± 17.691	32.95 ± 7.131	64.24 ± 24.284	0.000[a], 0.000[b]
T.Bil (mg/dl)	0.981 ± 0.26	1.21 ± 0.53	0.781 ± 0.1504	1.252 ± 0.725	0.093[a], 0.009[b]
D.Bil (mg/dl)	0.219 ± 0.075	0.324 ± 0.2278	0.143 ± 0.0507	0.329 ± 0.2572	0.081[a], 0.004[b]
Albumin (g/dl)	3.924 ± 0.3714	3.757 ± 0.3091	3.919 ± 0.1965	3.376 ± 0.5088	0.126[a], 0.000[b]
PT %	90.95 ± 5.277	90.05 ± 7.940	90.95 ± 5.277	90.05 ± 7.940	0.637[a], 0.37[b]
Glucose(mg/dl)	99.33 ± 16.883	98.57 ± 17.829	95.81 ± 20.629	109.76 ± 18.566	0.885[a], 0.041[b]
HB (g/dl)	12.105 ± 1.6764	12.448 ± 1.7285	10.381 ± 1.244	10.19 ± 1.3274	0.557[a], 0.618[b]
TSH (ng/dl)	3.652 ± 0.6137	3.419 ± 0.6562	4.45 ± 1.459	4.43 ± 1.434	0.235[a], 0.966[b]
Serum AFP (ng/ml)	9.50 ± 4.323	9.90 ± 5.291	9.10 ± 1.998	10.90 ± 4.592	0.742[a], 0.084[b]
Creatinine (mg/dl)	1.333 ± 1.7625	0.995 ± 0.1532	0.938 ± 0.1533	1.229 ± 0.3036	0.393[a], 0.001[b]
HCV RNA (Viral load copies/IU/L) N (%)					
High	7(24.4%)	6(28.6%)	0	1(4.7%)	
Moderate	12(41.4%)	11(52.4%)	0	9(42.9%)	0.242[a], 0.000[b]
Low	10(34.5%)	4(19%)	0	11(52.4%)	
- ve	0	0	29(100%)	0	
Stage of fibrosis N(%)					
F1	2(6.9%)	0	5(17.2%)	1(4.8%)	
F2	20(69%)	10(47.6%)	24(82.8%)	4(19%)	0.620[a], 0.747[b]
F3	7(24.1%)	11(52.4%)	0	16(76.2%)	
F4	0	0	0	0	
Necro-inflammatory activity index (HAI) N(%)					
A1	4(13.8%)	1(4.8%)	22(75.9%)	2(9.5%)	
A2	25(86.2%)	19(90.4%)	6(20.7%)	11(52.4%)	0.099[a], 0.586[b]
A3	0	1(4.8%)	1(3.4%)	8(38.1%)	
A4	0	0	0	0	

p- value comparison between responders and non responders to IFN-treatments at baseline [a]; Responders and non responders after 6 months from end treatment [b].

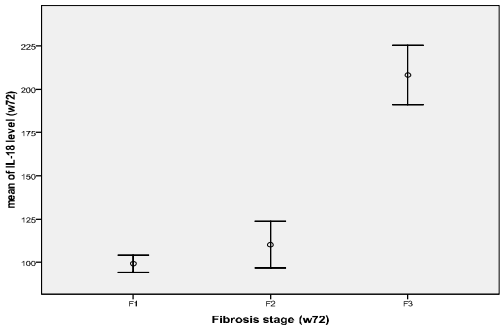
Table 3: Correlation between IL-18 and other parameters

Parameters	**IL – 18 level**							
	Control N=20		**CHC N=50**		**Responders (n = 29; %58)**		**Non-responders (n = 21; % 42)**	
	r	***P*= value**	**r**	***P*= value**	**r**	***P*= value**	**r**	***P* = value**
Demographic characters								
Age (yr)	-0.002	0.992	-0.292	0.040	-0.212	0.269	-0.498	0.022
Sex	-0.290	0.214	0.027	0.854	0.070	0.727	0.299	0.189
BMI (kg/m2)	-0.145	0.543	-0.053	0.712	0.077	0.692	-0.385	0.085
Biochemical tests								
ALT (IU/L)	0.061	0.798	0.109	0.449	0.037	0.849	0.362	0.107
AST (IU/L)	0.162	0.495	-0.016	0.914	0.115	0.551	-0.098	0.672
T.Bil (mg/dl)	-0.168	0.480	-0.033	0.820	-0.092	0.634	0.069	0.765
D.Bil (mg/dl)	-0.182	0.441	-0.069	0.640	-0.129	0.504	-0.011	0.962
Albumin (g/dl)	-0.008	0.975	0.044	0.759	-0.023	0.904	0.179	0.436
PT %	-0.292	0.212	0.118	0.413	-0.340	0.071	0.568	0.007
Glucose(mg/dl)	0.069	0.771	0.110	0.447	0.112	0.561	0.110	0.635
HB (g/dl)	0.171	0.472	-0.060	0.678	-0.026	0.894	-0.111	0.631
TSH (ng/dl)	-0.182	0.443	0.010	0.944	-0.026	0.894	0.035	0.879
Serum AFP (ng/ml)	0.128	0.592	-0.184	0.202	-0.250	0.191	-0.041	0.859
Creatinine (mg/dl)	-0.014	0.952	-0.034	0.815	0.37	0.850	-0.144	0.534

HCV RNA (Viral load copies/IU/L)	-	-	0.278	0.050	0.295	0.120	0.231	0.314
Stage of fibrosis	-	-	0.146	0.313	0.273	0.153	-0.049	0.832
Necroinflammatory activity index (HAI)	-	-	0.273	0.055	0.320	0.091	0.245	0.285

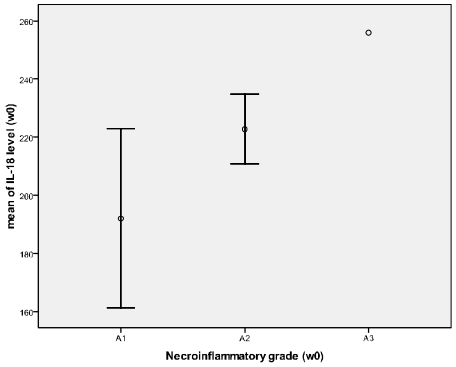


A- CHC-4 patients before starting treatment (r = 0.146, p = 0.313).



B- CHC-4 patients after 6 months from end treatment (r = 0.750, p = 0.000).

Figure1: Correlation between IL- 18 and Stage of fibrosis in CHC-4 patients



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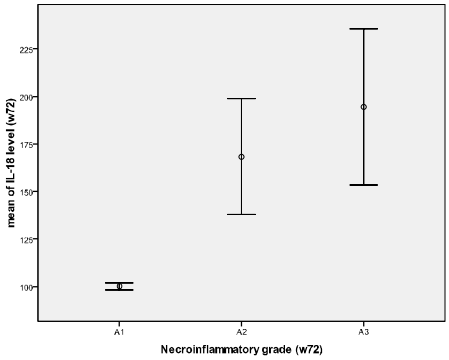
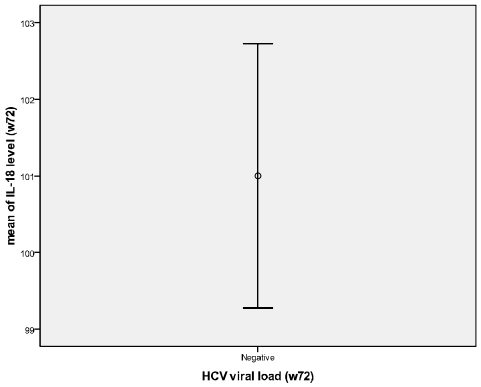
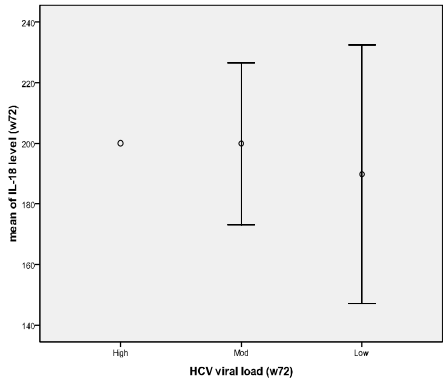


B- CHC-4 patients after 6 months from end treatment (r = 0.750, p = 0.000).

Figure2: Correlation between IL- 18 and Necro-inflammatory activity index in CHC-4 patients



A- In responders to IFN- treatment (r = -0.020, p = 0.916).



B- In non-responders to IFN- treatment(r = -0.438, p = 0.047).

Figure3: Correlation between IL- 18 and HCV viral load and response to antiviral therapy

4. Discussion

IL-18 has been reported by previous studies as a marker in HCC with others cytokines [16]. In the current study, it has been used as a predictor to the

outcome of PEG-IFN ad ribavirin therapy in chronic hepatitis C patients, where measuring of IL-18 level has been performed in those patients before treatment and after 6 months from end of treatment (week 72). We analyzed its association with outcome of PEG interferon and ribavirin therapy hoping to find biological marker that might be used in predicting antiviral therapy response.

Our results showed that IL-18 level was higher in patients with HCV infection than in control participants, in accordance with Sharma *et al.* [9], and Yoneda *et al.* [4]. In addition, IL-18 showed a marked reduction after 6 months from the end of treatment. Previous reports have demonstrated upregulated levels of IL-18 in CHC genotype 1 and 4 infected patients and its correlation with hepatic injury. McGunniess *et al.* [17] have demonstrated chemo-attractant properties of IL-18 (IL-1β, MIP-1β), mediating upregulation of TH1 associated IL-2 and IFNγ and down regulation of TH2 cytokine 10. This indicates its proinflammatory role in the course of hepatic impairment in CHC patients and its key role in disease pathogenesis. Ludwiczek *et al.* [18] have reported that plasma level of IL-18 was significantly higher in cirrhotic than non cirrhotic HCV patients.

In accordance with a study performed by Schvoerer *et al.*[19] who observed that IL-18 level was significantly correlated with hepatic histologic activity, our results showed such correlation but without significant difference. This may be due to small sample size which influenced statistical analysis.

As regards response to interferon treatment, it has been demonstrated that IL-18 levels were significantly higher at baseline in CHC genotype 1 infected patients who achieved a SVR than those who did not [4]. This was in agreement with our findings for CHC genotype 4 infected patients, indicating no relationship between HCV genotypes 1 or 4 and IL-18 production. Moreover, it has been reported that high levels of IL-18 is one of the independent factors related to SVR in patients treated with PEG-IFN and ribavirin [4]. This was also confirmed by Abiru and his colleagues [16] who stated that cirrhotic patients with low levels of IL-18 may progress to HCC, suggesting that IL-18 could be tested as a prognostic marker for cirrhotic patients who are likely to develop HCC. Such previous studies may support our results which have shown significant decrease in IL-18 level of responders compared with non-responders, suggesting positive correlation between IL-18 level and response to antiviral therapy.

In addition, Marin-Serrano and his colleagues [20] have reported that there was a significant decrease in IL-18 levels in both responders and non responders after 6 months from end of treatment, with no difference in the decrease of cytokine level between the two groups. In this context, several issues should be mentioned before explaining such contradictory results; first, there is a favorable effect of interferon treatment on reducing this cytokine and its detrimental effect on histologic damage such as necroinflammatory activity and fibrosis index irrespective of viral effect. Thus, IL-18 cytokine should be a target for therapeutic interventions to reduce liver damage in CHC patients. Second, decreased levels of IL-18 could be caused by negative feedback regulation through parallel increase in IL-18BP or receptors resulting in impairment of its bioactivity and then associated with progression of disease. Third, differential production of IL-18 during various stages of HCV infection could also be a result of polymorphism in the regulatory gene of IL-18. These polymorphisms may be associated with protection against or susceptibility to infections. Fourth, precise cellular and molecular mechanisms and mediators underlying the liver damage and also protective antiviral effect may differ. Thus the relationship among genotype, IL-18 production and HCV progression warrants further study. In addition to all previous factors other cytokines like IL-12 synergize the role of IL-18, in promoting Th1-mediated immune response and viral eradication.

Sharma *et al.* [9] stated that during early and asymptomatic stage of infection, decreased levels of IL-18 may stimulate and maintain high levels of HC virions, resulting in persistence. During later stage of infection, an increase in IL-18 might result in prevention of progression to end-stage of liver disease. This was explained by_ability of IL-18 to modulate the viability of the cells by increasing Fas-mediated apoptosis of infected hepatocytes, supporting HCV replication, favoring their elimination and dissemination of the viral particles. This mechanism is inhibited by core protein of HCV in persistent infection. Therefore, we suggest that our non responders with high and moderate viral load and high level of IL-18 are at higher risk for HCC development.

Regarding liver function tests, our results showed significant decrease in AST levels in responders than in non responders. This goes well with Yoneda *et al.* [4] who found that AST is one of the independent factors related to SVR. As regards correlation between AST and ALT levels with IL-18 level, our results was consistent with Sharma *et al.* [9], which didn't show any association between IL-18 level and ALT or AST values in responders and non responders. Also, in accordance with Mohran *et al.* [21] no correlation was observed between IL-18 level

and T.Bil, D.Bil., and albumin but significant correlation was observed between prothrombin activity and IL-18 in responders and non responders.

Our results showed also significant increase in AFP in CHC patients compared to controls and this was in agreement with previous reports done by Hamouda *et al.* [22], Tamura, *et al.* [23], and Mohran *et al.* [21]

Regarding TSH level, it has been demonstrated that the most common complications of IFNα therapy for chronic hepatitis C infection is interferon induced thyroiditis (IIT) by necrosis not by apoptosis mechanism. Also, HCV infection itself contributes to the initiation of thyroid autoimmunity [24]. Therefore, all patients were subjected to routine thyroid screening. Our results showed significant increase in TSH level after 6 months from end of treatment, but no correlation was observed between IL-18 and this variable in responders and non responders. Previous studies have shown a wide variation of thyroid dysfunction incidence in patients with CHC during and after IFN-α based therapy, at a rate of 2.5% to 35% in different countries [25, 26]. This variability can be attributed either to an underestimation of the true prevalence of thyroid dysfunction or to the diverse genetic predisposition of the subjects [27].

Conclusion:

IL-18 level was seen to be elevated in CHC genotype 4 patients than in apparently healthy subjects. IL-18 was correlated with histological stages of the liver injury, but small sample size affect significant correlation. IL-18 level in responders was significantly reduced after 6 months from the end of treatment compared with those at baseline level, but such decrease was not significant in non responders. IL-18 level was positively correlated with high moderate viral load in non responders, indicating its correlation with pathogenesis of liver disease in the course of HCV infection.

Authors' contributions.

AAM developed plan and performed the research; WSM, performed statistical analysis, tabulated data and shared in editing process, SAL evaluated and edited draft manuscript, responsible for publication process, MA gathered clinical data and contributed in practical part and editing process and MMH contributed to analytical tools and supervising work.

All authors have read and approved the final manuscript

Competing interests

The authors declare that they have no competing interests

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References

1. World Health Organization. Media centre of Hepatitis C. Fact sheet N* 164 June 2011.
2. Bataller R, Lemon SM.: Fueling fibrosis in chronic hepatitis C**.** Proc Natl Acad Sci. 2012, 109:14293-14294.
3. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY: Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010, 138:1338-1345.
4. Yoneda S, Umemura T, Katsuyama Y, Kamijo A, Joshita S, Komatsu M, Ichijo T, Matsumoto A, Yoshizawa K, Ota M, Tanaka E: Association of Serum Cytokine Levels with Treatment Response to Pegylated Interferon and Ribavirin Therapy in Genotype 1 Chronic Hepatitis C Patients. *J Infect Dis.* 2011, 203:1087-1095.
5. Wan L, Kung YJ, Lin YJ, Liao CC, Sheu JJ, Tsai Y, Lai HC, Peng CY, Tsai FJ: Th1 and Th2 cytokines are elevated in HCV-infected SVR(-) patients treated with interferon-alpha. *Biochem Biophys Res Commun.* 2009, 379:855-860.
6. Fantuzzi G, Dinarello CA.: Interleukin-18 and interleukin-1 beta: Two cytokine substrates for ICE (caspase-1). *J Clin Immunol*. 1999; 19:1–11.
7. Cales P, Oberti F, Michalak S.: A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology.* 2005; 42:1373-1381.
8. Lindsay KL. Introduction to therapy of hepatitis C. Hepatology. 2002, 36(5 Suppl1):114-120.
9. Sharma A, Chakraborti A, Das A, Dhiman RK, Chawla Y.: Elevation of interleukin-18 in chronic hepatitis C: implications for hepatitis C virus pathogenesis. *Immunology.* 2009, 128(1 Suppl):e514-522.
10. Belkaid Y.: Regulatory T cells and infection: a dangerous necessity. *Nat Rev Immunol*. 2007, 7:875-888.
11. Finotto S, Siebler J, Hausding M.: Severe hepatic injury in interleukin 18 (IL-18) transgenic mice: a key role for IL-18 in

regulating hepatocyte apoptosis *in vivo*. *Gut.* 2004; 53:392-400.
12. Ishak K, Baptista A, Bianchi L, *et al.* (1995): Histopathological staging and grading of chronic hepatitis. J Hepatol. 22:696-699.
13. Hu Y, Shahidi A, Park S, Guilfoyle D, Hirshfield I.: Detection of extrahepatic hepatitis C virus replication by a novel, highly sensitive, single-tube nested polymerase chain reaction. *Am J Clin Pathol*. 2003, 119: 95-100.
14. Shaker O, Ahmed A, Doss W, Abdel-Hamid M.: MxA expression as marker for assessing the therapeutic response in HCV genotype 4 Egyptian patients. *J Viral Hepat.* 2010, 17:794-9.
15. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, O'Mahony L, Palomares O, Rhyner C, Ouaked N, Schaffartzik A, Van De Veen W, Zeller S, Zimmermann M, Akdis CA.: Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. *J Allergy Clin Immunol*. 2011, 127:701-21.e1-70.
16. Abiru S, Kato Y, Hamasaki K, Nakao K, Nakata K, Eguchi K. Spontaneous regression of hepatocellular carcinoma associated with elevated levels of interleukin 18. Am.J.Gastroenterol., 2002; 97:774-775
17. McGuinness PH, Painter D, Davies S, McCaughan GW.**:** Increases in intrahepatic CD68 positive cells, MAC387 positive cells, and proinflammatory cytokines (particularly interleukin 18) in chronic hepatitis C infection. *Gut*. 2000; 46:260–269.
18. Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Vogel W, Tilg H.: Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease. *J Clin Immunol*. 2002, 22:331-337.
19. Schvoerer E, Navas MC, Thumann C, Fuchs A, Meyer N, Habersetzer F, Stoll-Keller F.: Production of interleukin-18 and interleukin-12 in patients suffering from chronic hepatitis C virus infection before antiviral therapy. *J Med Virol*. 2003, 70:588-593.
20. Marín-Serrano E, Rodríguez-Ramos C, Díaz F, Martín-Herrera L, Girón-González JA.: Modulation of the anti-inflammatory interleukin 10 and of proapoptotic IL-18 in patients with chronic hepatitis C treated with interferon alpha and ribavirin. *J Viral Hepat.* 2006, 13:230-234.
21. Mohran ZY, Ali-Eldin FA, Abdel Aal HA.: Serum interleukin-18: does it have a role in the diagnosis of hepatitis C virus related hepatocellular carcinoma?. *Arab J Gastroenterol*. 2011, 12:29-33.
22. Hamouda SA., Deghady AA, Farid A, *et al.* (2006): Study of the serum level of interleukin-18 (IL-18) in patients with chronic hepatitis C virus infection. Bull. Alex. Fac. Med. 42: 1110-1834.
23. Tamura Y, Yamagiwa S, Aoki Y, *et al.,* 2009: Serum Alpha-Fetoprotein Levels During and After Interferon Therapy and the Development of Hepatocellular Carcinoma in Patients with Chronic Hepatitis C. Dig Dis Sci 54:2530–2537
24. Tomer Y: Hepatitis C and interferon induced thyroiditis. *J Autoimmun*. 2010, 34(3):J322-326.
25. Vasiliadis T, Anagnostis P, Nalmpantidis G, Soufleris K, Patsiaoura K, Grammatikos N, Orfanou-Koumerkeridou E, Kargiotis K, Slavakis A, Deliyiannidis A, Eugenidis N. Thyroid dysfunction and long-term outcome during and after interferon-alpha therapy in patients with chronic hepatitis C. Ann Acad Med Singapore. 2011 40:394-400.
26. Dabrowska MM, Panasiuk A, Flisiak R. Thyroid dysfunction in antiviral therapy of chronic hepatitis C. Hepatogastroenterology. 2010, 57: 826-831.
27. Carella C, Mazziotti G, Amato G, Braverman LE, Roti E. clinical review 169: Interferon-alpha-related thyroid disease: pathophysiological, epidemiological, and clinical aspects. J Clin Endocrinol Metab. 2004, 89: 3656-3661.

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