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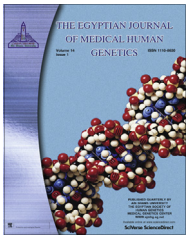

ORIGINAL ARTICLE

Serum level of interleukin-10 with its gene polymorphism can be predictors of response to treatment in Egyptian patients with chronic hepatitis C virus

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KEYWORDS

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Abstract *Purpose:* The aim of this study was to demonstrate the role of interleukin-10 (IL-10) gene polymorphism and its serum level in predicting response to treatment in patients with chronic hepatitis C virus.

Patients and methods: This study was carried out on 35 Egyptian patients with chronic HCV (Hepatitis C Virus) and 15 age- and sex-matched healthy subjects as control. They were divided as follows: Group I: 35 chronic HCV patients. They were subdivided according to their response to combination therapy of pegylated interferon alpha 2b and ribavirin into: Group I (a): 21 responder patients. Group I (b): 14 non responder patients. Group II: 15 healthy subjects as a control group. IL-10 serum level was assessed by ELISA (Enzyme Linked Immunosorbent Assay) before, during and after treatment. IL-10 gene polymorphism and genotype were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: A significant higher level of serum IL-10 was detected in HCV patients compared to the control group. A significant reduction was detected during treatment and a persistent decrease was found in patients with SVR. Low serum level of IL-10 pretreatment was associated with high treatment response. High pretreatment of the serum level of IL-10 was associated with the severity of chronic necroinflammation and non response to treatment. A positive correlation was found between IL-10 and serum ALT. The frequency of IL-10 592 genotype polymorphism was higher

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in HCV patients compared to control. A significant higher frequency of the IL-10 592 C/C polymorphism was found in the responder group compared to non responder. No correlation was observed between IL-10 polymorphism and liver histopathology.

Conclusion: Serum IL-10 level pretreatment is useful for predicting treatment response in HCV patients. IL-10 may be a useful marker to assess necroinflammation and to monitor the evolution of liver damage. IL-10 gene polymorphism has no relation to liver histopathology. IL-10 592 C/C genotype was more frequent in responder patients.

1. Introduction

Hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease worldwide. More than 80% of patients with acute HCV infection become chronic, and a significant proportion of chronic HCV patients develop cirrhosis and even hepatocellular carcinoma in their lifetimes [1]. The mechanism underlying the persistence and pathogenesis is not fully understood, but probably includes virological and immunologic factors [2]. Generally, two distinct patterns of cytokine production occur. Type I responses are characterized by production of interleukin-2 (IL-2), tumor necrosis factor alpha (TNF alpha) and interferon gamma, which prime and maintain antigen specific cellular immunity and are important in defense against viruses [3,4]. Type 2 responses are characterized by interleukin-4 (IL-4) and interleukin-10 (IL-10) production which promote humoral immune responses [5,6]. An imbalance in helper T-cell type 1 (Th1) and type 2 (Th2) cytokine is suggested to play an important role in the pathogenesis of chronic hepatitis C [7]. IL-10 is a Th2 cytokine produced by monocytes, macrophages and T cells. It has potent immunoregulatory and anti-inflammatory properties [8]. The human IL-10 gene is located on chromosome 1 and encodes 5 exons. The capacity of cytokine production in an individual might depend on a major genetic contribution. The role of IL-10 polymorphism in HCV clearance has been investigated [9,10].

The aim of this study was to demonstrate the role of interleukin-10 gene polymorphism and its serum level in predicting response to treatment in patients with chronic hepatitis C virus.

1.1. Patients and methods

This study was carried out on 35 Egyptian patients with chronic HCV and 15 age- and sex-matched healthy subjects as a control group. Patients were followed up at outpatient clinics of Tropical medicine department, Tanta University Hospital, Tanta, Egypt during the period between May 2011 and November 2012 to assess treatment responders & non-responders. Diagnosis of chronic HCV was based on persistence of HCV antibodies and HCVRNA in the serum of the patients for more than 6 months. Chronic hepatitis was confirmed by liver biopsy.

1.2. Inclusion criteria

Patients with positive HCV antibodies by ELISA and HCVRNA by reverse transcriptase polymerase chain reaction (RT-PCR) and histological evidence of chronic hepatitis were included in the study.

1.3. Exclusion criteria

Previous IFN-a therapy; other liver diseases such as hepatitis A, hepatitis B, schistosomiasis, autoimmune hepatitis, alcoholic liver disease, drug induced hepatitis, or decompensated liver disease; coinfection with human immunodeficiency virus; neutropenia ($<1500/mm^3$); thrombocytopenia ($<100{,}000/mm^3$); anemia (<12 gm/dl for females and <13 gm/dl for males), creatinine concentration >1.5 times the upper limit of normal; serum alpha fetoprotein concentration >25 ng/ml; organ transplant; neoplastic disease; severe cardiac or pulmonary disease; unstable thyroid dysfunction; psychiatric disorder; current pregnancy or breast feeding; or therapy with immunomodulatory agents within the last six months were exclusion criteria.

15 healthy individuals with normal transaminase levels and negative serologic results for hepatitis B and hepatitis C were considered as a control group. All patients and control were subjected to the following:

1.4. Blood sampling and laboratory tests

6 ml of blood samples was collected from each case, 3 ml was centrifuged at a rate of 3000r/m and the serum was kept at −60 °C for serological assay. The other 3 ml was used for RT-PCR.

1.5. ELISA assay of serum IL-10 level

Serum IL- 10 level was measured by a commercially available ELISA kit with the detection limitation of 20 pg/mL (Human IL-10 DuoSet®, R&D Systems, MN, USA) according to the manufacturer's instructions (10).

1.6. Quantitative assessment of HCNRNA by real time-PCR for HCV patients

RNA extraction: RNA was extracted using QIAamp viral RNA Kit (50) Cat. #52904 (QIAGEN).

QuantiTect™ Probe RT-PCR Kit (QIAGEN) was used for quantitative, real-time, one-step RT-PCR using sequence-specific probes.

Primers used in this research: [sense primer], 5′-TGCGGAA CCGGTGAGTACA-3′ and [antisense primer],5′-CTTAAGGT TTAGGATTCGTGCTCAT-3′ -Fluorogenic probe used 5′-(FAM) CACCCTATCAGGCAGTACCACAAGGCC(TAMRA)-3′]. Primers are designed according to program Primer Express (Perkin–Elmer) to design the primers and probes, following the guidelines for the best performance of the PCR. Real-time cycler conditions using TaqMan probes: -1-Reverse transcription: 50 °C f 30 min 2-PCR: Initial activation step: 95 °C for

15 min (HotStarTaq DNA polymerase is activated by this heating step; Omniscript RT and Sensiscript RT are inactivated), 2-step cycling: a-Denaturation 94 °C for 15 s then b-Annealing/Extension 60 °C for 60 s. Cycle number35–45 (11).

1.7. Single nucleotide polymorphism of IL-10 promoter and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp blood kit (Qiagen, Hilden, Germany), and quantity and purity were assessed on the basis of spectroscopic absorbance at 260 nm. Samples were stored at −20 °C until further use. IL-10 single nucleotide polymorphisms (SNP) at promoter 592 site were detected by PCR using primer that amplify a short fragment of DNA containing the polymorphism. The primers for −592 SNP was sense CCTAGGTCACAGTGACGTGG, anti-sense GGTGAGCACTACCT GACTAGC. Amplification of the specific DNA fragments was performed using a PTC-150 MiniCycler (MJ Research Inc., Watertown, MA, USA) according to the following thermocycler conditions: denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 45 s, and extension at 72 °C for 1 min. This was followed by a final extension at 72 °C for 10 min. IL-10-592A/C single nucleotide polymorphism was genotyped by restriction fragment length polymorphism (RFLP). RFLP assays were performed in a 20 uL reaction volume containing PCR products and specific restriction enzyme (RsaI). In the presence of the IL-10-592A allele, RasI cut the 412 bp PCR product into two bands of 236 and 176 bp. The digestion products were stained with ethidium bromide and visualized on a 3% agarose gel [11].

1.8. Liver biopsy for HCV patients

METAVIR scoring system [11] was used to assess necroinflammation and fibrosis in liver biopsy. Necroinflammation activity (A) was graded as A0 (absent), A1 (mild), A2 (moderate), and A3 (severe). Fibrosis stage (F) was scored as F0 (absent), F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis) and F4 (cirrhosis) [12].

1.9. Therapeutic regimen

Pegylated interferon alpha 2b subcutaneous injection at a dose of 1.5 μg/kg body weight per week was given in combination with a weight adjusted dose of oral ribavirin (1000 mg/day for <75 kg and 1200 mg/day for >75 kg) for 48 weeks. Patients were followed up at 12, 48 and 24 weeks after completion of treatment.

1.10. Treatment outcome

Patients who had undetectable HCV RNA at treatment week 12 were considered to have a complete early virological response(cEVR), while those who had $\geqslant 2$ log reduction in HCVRNA level compared to baseline HCVRNA level were considered to have a partial early virological response(pEVR). Patients who failed to clear HCVRNA from serum after 24 weeks of therapy were considered as nonresponders and did not receive further treatment. Those patients who had cEVR or pEVR continued treatment until 48 weeks. Patients who had undetectable HCV RNA at the end of 48 weeks were considered to have end treatment response (ETR) and were followed up for 24 weeks after finishing therapy. Those patients with an undetectable HCV RNA at 24 weeks after completion of treatment were determined to have sustained virological response (SVR). Patients with ETR in whom there was reappearance of HCV RNA in serum during the follow-up for 24 weeks were having relapse.

Subjects were divided into

- Group I: 35 chronic HCV patients.
- Group Ia: 21 responder patients.
- Group Ib: 14 non responder patients.
- Group II: 15 healthy persons as control.

All patients and control gave their informed consent and the study was approved by Ethical, and Research Committee, Tanta Faculty of Medicine, Tanta, Egypt.

1.11. Statistical analysis

The statistical data are reported as the mean ± SD, frequencies (number) and percentages when appropriate. A comparison of numerical variables between the study groups was performed using Student's *t*-test to compare independent samples from two groups when the samples were normally distributed and the Mann–Whitney *U*-test to compare independent samples when the samples were not normally distributed. A comparison of the numerical variables between more than two groups was performed using a one-way analysis of variance test to compare normal data, and the Kruskal–Wallis test was used when the data were not normal. To compare categorical data, a χ^2 test was performed. The accuracy is represented using the terms sensitivity and specificity. Spearman rank correlation was used to quantify the association between continuous or ordered categorical variables. A receiver operator characteristic (ROC) analysis was used to determine the optimum cut-off value for the studied prognostic markers. *P*-values less than 0.05 were considered statistically significant. All statistical calculations were performed using the computer program SPSS (Statistical Package for the Social Science; SPSS, Chicago, IL, USA) version 15 for Microsoft Windows.

2. Results

The results of this study showed that there was no statistical difference between HCV patients and control as regards age and gender ($P > 0.05$), significant higher level of the serum ALT and AST in patients with HCV compared to the control group ($P < 0.05$), and the frequency of the IL-10 592 genotype and IL-10 serum level was significantly higher in HCV patients compared to control ($P < 0.05$) (Table 1).

Also, the results of the present study showed a significant higher frequency of the IL-10 592 C/C genotype in responder patients compared to nonresponders. IL-10 base line serum level was significantly lower in responders than nonresponder patients. Liver histopathology revealed a significant higher grading of necroinflammation in non responder than responder patients. No significant difference was detected as regards fibrosis between responders and nonresponders (Table 2).

Table 1 Baseline parameters in both HCV patients and control groups.

	HCV $n = 35$	Control $n = 15$	P
Age	39.7 ± 1.8	41 ± 6.4	>0.05
Sex(M/F)	24/11	10/5	>0.05
ALT(U/L)	61.3 ± 12.3	26.2 ± 4.1	<0.05*
AST(U/L)	52.4 ± 11.4	24.1 ± 5.7	<0.05*
Bilirubin (mg/dl)	1.3 ± 0.5	0.8 ± 0.2	>0.05
Albumin (g/dl)	3.9 ± 0.7	4.3 ± 0.4	>0.05
PT (seconds)	12.1 ± 1.4	11 ± 0.5	>0.05
HCV ANA (10^6 copies/ml)	6.9 ± 6.5	–	–
Serum. IL-10 (pg/ml)	68.1 ± 24.5	46.9 ± 16.2	<0.05*
IL-10 592 genotype *n* (%)			
A/A	11 (31.4%)	7 (46.7%)	
A/C	10 (28.6%)	6 (40%)	<0.05*
C/C	14 (40%)	2 (13.3%)	

Table 2 IL-10 genotypes, IL-10 serum level and liver histopathology in responders and nonresponders of HCV patients.

	Responders $n = 21$	Non responders $n = 14$	P-value
IL-10 genotype			
A/A	4 (19.1%)	7 (50%)	0.033*
A/C	4 (19.1%)	6 (42.9%)	
C/C	13 (61.9%)	1 (7.1%)	
Serum. IL10 (pg/ml) (median)	56	79.5	0.013*
Histopathological activity			
METAVIR activity grade			
Mild (A1)	15 (71.4%)	1 (7.14%)	0.019*
Moderate (A2)	4 (19.1%)	5 (35.71%)	
Severe (A3)	2 (9.5%)	8 (57.14%)	
METAVIR fibrosis stage			
F1	14 (66.7%)	3 (21.4%)	>0.05
F2	5 (23.8%)	5 (35.7%)	
F3	2 (9.2%)	6 (42.9%)	

IL, interleukin; *$P < 0.05$ significant.

The results also showed significant reduction in the IL-10 serum level at 12, 48, and 72 weeks of treatment in responders (Table 3).

As regards different laboratory parameters and liver histopathology, a significant positive correlation was detected between severe grading of necroinflammation and each of IL-10, ALT, where as no significant correlation was found between IL-10 gene polymorphism and either necroinflammation or fibrosis (Table 4).

The present study revealed 95.5% sensitivity and 100% specificity of IL-10 as a prognostic marker for evaluation of response to interferon and ribavirin therapy (Table 5).

3. Discussion

IL-10 level differs widely between individuals probably due to polymorphism in the promoter region. This region includes two informative microsatellites and three frequent point mutations −1082(G/A, −819(C/T), and −592(C/A). The −1082(G), 819(G) and 592(C) alleles were associated with higher IL-10 production. The -1082(G/G genotype is known to be related to increase IL-10 production and is associated with a high risk of inefficient HCV clearance [13,14] and resistance to interferon therapy [13,14]. Recent study indicated that polymorphism of the promoter region of IL-10 gene (ATA haplotype) may determine spontaneous clearance of HCV infection [17].

This study revealed a significant higher serum level of IL-10 in HCV patients compared to control group. This was in accordance with Yoneda et al. [18], Shaker et al. [19] and several other studies [20,21], which reported a significant increase of the IL-10 level in the serum of HCV patients. In contrast, Marcello et al. [22] demonstrated no significant difference in the serum level of IL-10 between HCV patients and healthy control that is due to a different genotype of HCV included in this study. This study demonstrated a decrease in the serum level of IL-10 during treatment and remained low in patients with sustained virological response (SVR). These findings suggest that cytokines may in fact compromise host immune response to the virus .This was in agreement with Yoneda et al. [18], who reported decreased cytokine levels of IL-10, IL-12 and IL-18 during treatment with interferon and ribavirin.

This study demonstrated a significant low base line in the serum level of IL-10 in the responder group compared to non responder. Also, Shaker et al. [19] reported a significant

Table 3 IL-10 serum level changes before, during and after treatment.

Treatment outcome	Baseline	12 week	48 week	72 week	*P*-value
Responders	56 pg/ml	42	32	30	$< 0.001^*$
Non responders	79.5	77.5	78	76.5	> 0.05

Data are median values. IL, interleukin; $^*P < 0.05$ significant.

Table 4 Correlation between liver histopathology and serum parameters in HCV patients.

	Inflammation		Fibrosis	
	R	*P*-value	*R*	*P*-value
S. IL-10 (pg/ml)	0.519	0.002^*	0.572	0.742
AST U/L	0.255	0.137	0.182	0.294
ALT U/L	0.528	0.001^*	0.263	0.126
IL-10 592 genotype	0.195	0.259	0.119	0.503

IL, interleukin, $^*P < 0.05$ significant.

low level of IL-10 in responders than non responders. In agreement with the present study Yoneda et al. [18] revealed a strong association between high base line serum IL-10 and a non response to interferon and ribavirin therapy. An association between low base line serum IL-10 and response to treatment was also reported by some previous studies [23–25]. Although humoral immunity is said to play a major role in recovery from HCV infection and B-cell is the strongest in those with persistent infection [26,27], a strong natural killer cell mediated and Th1 cell mediated immune response seems to be a key factor in protection from HCV infection [27].

This study demonstrated that the low IL-10 level before treatment was associated with SVR. This cytokine may offer the clinician another tool in predicting treatment outcome of HCV infection. It may be concluded that HCV patients who are genotyped as high IL-10 producers have a poor response to interferon therapy, these patients may therefore benefit from additional treatment strategies designated to enhance Th1 responses [28].

As regards liver histopathology, in this study serum IL-10 showed a significant positive correlation with METAVIR activity grade. This was in accordance with Marcello et al. [22] who found a significant higher level of IL-10 in patients with a grading score of 9–18 than in those with 1–8 grading score in HAI. In contrast, another study [29] reported no correlation between IL-10 and liver histology, ALT, age, sex and viral load. This can be explained by different genotypes of HCV. This study found no significant correlation between serum IL-10 value and the degree of fibrosis. This was in accordance with Lin et al. [30] and Marcello et al. [22], who observed absence of any relationship between IL-10 and staging score of fibrosis.

An experimental study conducted on animal models showed that IL-10 blockade strategy is capable of ameliorating the outcome of chronic viral infection [31,32]. IL-10 contributes to reduce the pathogenic effects of infection for the price of a weakening of the immune response aimed at the eradication of the virus and that timing as well as the relative amounts of IL-10 production is critical for safe resolution of infection [22]. It has also been reported that the heterozygous C/A variant in the IL-10 gene promoter region C592A is associated with a major risk of progression and chronic course of viral hepatitis [33]. Alteration of host immune system due to genetic predisposition determines the switch in the predominant activation from Th1 to Th2 cells with a consequent imbalance in the production of immunoregulatory cytokines. Such imbalances could determine a greater increase in IL-10 concentration, leading to impaired viral clearance and persistent necroinflammation [22].

This study demonstrated positive correlation between IL-10 values and serum ALT while no correlation was found with AST. Some other studies also reported the reduction in transaminase level was associated with a decrease in the IL-10 level in patients with chronic HCV treated with interferon and ribavirin [20,21]. In contrast Marcello et al. [22] reported a significant correlation between AST and IL-10 .

The association of the severity of liver disease and cytokine polymorphism was assessed in this study. IL-10 polymorphism did not show any significant association with either necroinflammation grading or fibrosis staging. This was in agreement with Chuang et al. [34] and Abott et al. [35], who found no correlation between IL-10 gene polymorphism and liver histopathology as regards necroinflammation and fibrosis. Genetic study of the IL-10 gene polymorphism in HCV has also been reported in other studies [36,15], suggesting that it has no role in the development of hepatic fibrosis. Dogra et al. [37] recorded the absence of correlation between IL-10 polymorphism and the severity of liver disease.

This study revealed that SVR at the end of treatment was obtained at 60% while non response was detected in 40%. This was in accordance with Slenzer et al. [38], Kurbanoy et al. [39] and Shaker et al. [19]. The frequency of the IL-10 592 genotype polymorphism was higher in HCV compared to control. This was in agreement with Shaker et al. [19], who reported a significant higher frequency of the IL-10 592 genotype polymorphism in HCV patients than control. In contrast Dogra et al. [37] and Chuang et al. [32] demonstrated no significant difference in

Table 5 Cutoff value, sensitivity, specificity, AUC, PPV and NPV of serum IL-10 level as prognostic marker in HCV patients.

Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
70.3 pg/ml	95.5	100	100	92.9	0.95

PPV: positive predictive value, NPV: negative predictive value, AUC.

HCV patients and control as regards IL-10 gene polymorphism. This can be explained by different virus genotypes.

Our study recorded a significant higher frequency of IL-10 592 C/C in responders compared to non responders (61.9% Vs 7.1%) respectively. This was similar to the results reported by Shaker et al. [19]. In contrast, Chang et al. [34] found a higher frequency of the IL-10 genotype AC and GG in responders compared to non responders. The patients enrolled in their study were with genotypes 1 and 2.

4. Conclusions

Serum IL-10 pretreatment level is considered a good predictive non invasive marker to predict response to interferon and ribavirin therapy in HCV patients. The IL-10 592 C/C genotype polymorphism is associated with response to treatment in HCV patients.

References

[1] Akkaya O, Kiyici M, Yilmaz Y, et al. Clinical significance fo activity of ALT enzyme in patients with hepatitis C virus. World J Gastroenterol 2007;13:5481.

[2] Constatntini PK, Wawrezynowicz M, Clare M, et al. Interleukin-1, Interleukin-10 and tumour necrosis factor alpha gene polymorphism in hepatitis C virus infection; An investigation of the relationship with spontaneous viral clearance and response to alpha interferon therapy. Liver 2002;22:404.

[3] Hohler T, Kruger A, Gerken G, et al. Tumour necrosis factor alpha promoter polymorphism at position -238 is associated with chronic active hepatitis C infection. J Med Virol 1998;54:173.

[4] Zein NN, Germer JJ, El-Zayadi AR, et al. Ethnic differences in polymorphism of tumour necrosis factor alpha, interleukin-10 and transforming growth factor beta gene in patients with chronic hepatitis C virus infection. Am J Trop Med Hyg 2004;70:434.

[5] Minton Elizabeth J, David S, Paula S, et al. Clearance of hepatitis C virus is not associated single nucleotide polymorphism in the IL-1 -6 or -10 genes. Hum Immunol 2005;66:127.

[6] Tzy-Yen C, Yih-Shou H, Wu Triang Tiau, et al. Impact of serum levels and gene polymorphism of cytokines on chronic hepatitis C infection. J Lab Clin Med 2007;150:116.

[7] Govan VA, Constant D, Hoffman M, et al. The allelic distribution of 308 tumour necrosis factor alpha gene polymorphism in south African woman with cervical cancer and control women. BMC Cancer 2006:24.

[8] Bidwell L, Keen I, Gallagher G, et al. Cytokine gene polymorphism in human disease: on line database. Genes Immune 1999;1:3.

[9] Nelson DR, Lauwer JY, Lau JYN, et al. Interlekin-10 treatment reduce fibrosis in patients with chronic hepatitis C: a pilot trial of interferon on responders. Gastroenterology 2000;118:655.

[10] Lio D, Caruso C, Distefano R, et al. IL-10 and TNF alpha polymorphism and the recovery from HCV infection. Hum immunol 2003;64:674.

[11] Brass V, Bieck E, Montserret R, Wolk B, Hellings JA, et al. An amino-terminal amphipathic alpha-helix mediates membrane association of the hepatitis C virus nonstructural protein 5A. J Biol Chem 2002;277:8130–9.

[12] Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. METAVIR Cooperat Study Group Hepatol 1996;24:289–93.

[13] Oleksyk TK, Thio CL, Truelove AL, et al. Single nucleotide polymorphism and haplotype in the IL-10 region associated with HCV clearance. Genes Immun 2005;6:347.

[14] Paladino N, Fainboim H, Theiler G, et al. Gender susceptibility to chronic gepatitis C virus infection associated with interleukin 10 promotor polymorphism. J Virol 2006;80:9144.

[15] Edwards-Smith CJ, Jonsson JR, Purdie DM, et al. Interleukin-10 promotor polymorphism predicts initial response of chronic hepatitis C to interferon alfa. Hepatology 1999;30:526.

[17] Mangia A, Santoro R, Piattelli M, et al. IL-10 haplotypes as possible predictors of spontaneous clearance of HCV infection. Cytokine 2004;25:103.

[18] Yoneda S, Umemura T, Katasoyama Y, et al. 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;14:1.

[19] Shaker OG, Sadik NA. Polymorphism in interleukin-10 and interleukin-28 B genes in Egyptian patients with chronic hepatitis C virus genotype 4 and their effect on the response to pegylated interferon/ribavirin- therapy. J Gastroenterol Hepatol 2012;27:1842.

[20] Kakumu S, Okumura A, Ishikawa T, et al. Serum levels of IL-10, IL-15 and soluble tumour necrosis factor-alpha receptors in type C chronic liver disease. Clin Exp Immunol 1997;109:458.

[21] Inglot M, Gladysz A, Rymer W, et al. Cytokine assessment in untreated hepatitis C virus infected patients and during interferon alpha + ribavirin therapy. Wiad Lek 2008;61:13.

[22] Marcello CB, Valenti M, Bertino G, et al. Relationship between circulating interleukin-10 and histological features in patients with chronic C hepatitis. Ann Saudi Med 2011;31(4):360.

[23] Wan I, Kung YI, Lin YI, et al. Th1 and Th2 cytokines are elevated in HCV- infected SVR (-) patients treated with interferon alpha. Biochem Biophys Res Commun 2009;379:855.

[24] Kuzushita N, Hayashi N, Katayama K, et al. High levelas of serum interleukin-10 are associated with a poor response to interferon treatment in patients with chronic hepatitis C. Scand J Gastroenterol 1997;32:169.

[25] Marin-Serrano E, Rodriguez-Ramos C, Diaz F, et al. 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.

[26] Umemura T, Zen Y, Schechterly C, et al. Quantitative analysis of anti-hepatitis C virus antibody-secreting B cells in patients with chronic hepatitis C. Hepatology 2006;43:91.

[27] Takaki A, Wiese M, Maertens G, et al. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. Nat Med 2000;6:578.

[28] Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL-28 B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105.

[29] Manesis EK, Papaioannou C, Kaferi G, et al. Biochamical and virological outcome of patients with chronic hepatitis C with interferon alpha 2 b for 6 or 12 months: A 4 year follow up of 211 patients. Hepatology 1997;26(3):734.

[30] Lin CS, Chang CS, Yeh HZ, et al. Retrtspective evaluation of serum markersAPRI and AST/ALT for assissing liver fibrosis and cirrhosis in chronic hepatitis B and C patients with hepatocellular carcinoma. Intern Med 2008;47:569.

[31] Brook DG, Trifilo MJ, Edelmann KH, et al. Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 2006;12:1301.

[32] Ejrnaes M, Filippi CM, Martinic MM, et al. Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med 2006;203:2461.

[33] Naslednikoya IQ, Konencov VI, Ryazantseva NV, et al. Role of genetically determined production of immunoregulatory cytokines in immunopathogenesis of chronic viral hepatitis. Bull Exp Biol Med 2007;143:706.

[34] Chuang JY, Yang SS, Lu YT, et al. IL-10 promotor gene polymorphism and sustained response to combination therapy in Taiwanese chronic hepatitis C patients. Dig Liver Dis 2009;41:424.

[35] Abbott WC, Rigopoulou E, Haigh P, et al. Single nucleotide polymorphism in the interferon gamma and interleukin-10 genes do not influence chronic hepatitis C severity or T-cell reactivity to hepatitis C virus. Liver Int 2004;24:90.
[36] Knapp S, Branwen JW, Hennig AJ, et al. Interleukin-10 promotor polymorphism and the outcome of hepatitis C virus infection. Immunogenetics 2003;55(6):362.
[37] Dogra G, Chakravarti A, Kar P, et al. Polymorphism of tumor necrosis factor alpha and interleukin-10 gene promoter region in chronic hepatitis C virus patients and their effect on pegylated interferon alpha therapy response. Hum Immunol 2011;72:935.
[38] Selzner N, McGilvray I. Can genetic variations predict HCV treatment outcome ? J Hepatol 2008;49:494.
[39] Kurbanoy F, Abde-Hamid M, Latanich R, et al. Genetic polymorphism in IL-28B is associated with spontaneous clearance of hepatitis C virus genotype 4 infection in an Egyptian cohort. J Infect Dis 2011;204:1391.