JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Elevated serum levels of adiponectin and interlukin-28B after IFN/RIB therapy in hepatitis C virus-infected patients

Kaveh Sadeghi[1], Abbas Ahmadi Vasmehjani[1,2], Rasoul Baharlou[3,4], Zamaneh Hajikhezri[1], Seyed Jalal Kiani[5]

[1] *Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[2] *Department of Immunology and Microbiology, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran*
[3] *Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran*
[4] *Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran*
[5] *Department of Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*

Abstract

Introduction: The *interleukin 28B* (*IL28B*) genotype is associated with changes of lipid metabolism in patients infected with hepatitis C virus (HCV). The association of steatosis with serum levels of adiponectin in chronic hepatitis C (CHC) patients has also been documented. This study aimed for the evaluation of serum levels of IL28B and adiponectin as well as the association of *IL28B* SNPs with different clinicopathological parameters in HCV-infected patients.

Methodology: All 142 HCV-infected patients received peg-interferon plus ribavirin. Detection of rs8099917 and rs12979860 IL-28B genotypes was done with specific primers. Serum IL28 and adiponectin levels were measured using commercial ELISA kits.

Results: Higher levels of both IL28 and adiponectin were found in patients. In Genotype 3a (G3a) -infected patients, IL28 and adiponectin serum levels were significantly higher than those infected with G1a. A correlation was found between increasing levels of AST and ALT in G3a-infected patients and the decrease in IL28 and adiponectin serum levels, respectively, in contrast to G1a-infected patients. Higher levels of both IL28 and adiponectin were associated with both CT allele of rs12979860 and TT allele of rs8099917 in patients in comparison with corresponding alleles in controls.

Conclusions: In contrast to other studies, this study showed higher serum adiponectin levels in HCV-infected patients compared to that in healthy controls. This finding is possibly due to adiponectin resistance caused by down-regulation of adiponectin receptors or tumorigenic effects of adiponectin. Our genotype-based analyses revealed, at least in part, the involvement of the viral factors in the outcome of HCV infection.

Key words: Hepatitis C virus; adiponectin; interleukin 28B; Single Nucleotide Polymorphism; interferon therapy.

J Infect Dev Ctries 2019; 13(5):434-444. doi:10.3855/jidc.11190

(Received 29 December 2018 – Accepted 28 March 2019)

Introduction

Hepatitis C virus (HCV) infection is not cytopathic for hepatocytes. However, the host immune system, in an attempt to clear the infection, destroys virus-infected cells and causes liver damage. The extension of immune responses determines the outcome; a strong and consistent CD4 and CD8 T cell response with extensive destruction of HCV-infected hepatocytes results in an acute stage accompanied with self-clearance of infection in about 15-30% of individuals. On the other hand, virus strategies to evade host innate (IFN and NK cells) and adaptive (helper and cytotoxic T cells) immune responses result in a chronic inflammation, mostly associated with subsequent liver fibrosis, cirrhosis, and occasionally hepatocellular carcinoma [1, 2].

Several studies have shown that the success in interferon-based therapies is associated not only with viral factors, but also with multiple host factors, including age, sex, obesity, steatosis, and genotype [3, 4]. Single nucleotide polymorphisms (SNPs) in *IL28B* gene have been shown to be an important predictor of treatment response [5]. The *IL28B* gene encodes for the interferon-λ3. Upon binding to its receptor, this cytokine activates a common downstream signaling system with type I interferons (IFN-α and INF-β). It has been suggested that elevated serum levels of interferon-stimulated genes (ISGs) in *IL28B* T allele carriers may

be responsible for poor treatment response in comparison with CC homozygotes [6].

Moreover, the *IL28B* genotype has been shown to be associated with changes of lipid metabolism in HCV-infected patients. Patients with CC allele had significantly higher levels of total cholesterol, apolipoprotein B and low-density lipoprotein (LDL) cholesterol compared to T allele carriers [7]. In addition, disordered lipid metabolism and liver steatosis are common in chronic hepatitis C (CHC) and may be associated with an increased risk of disease progression and impaired response to antiviral therapy [8]. The association of steatosis with serum levels of adiponectin in CHC patients has also been documented [9].

Adiponectin is one of adipokines secreted by adipose tissue which are involved in inflammation, tumorigenesis, metabolism and immune response [10]. By binding to its specific receptors, AdipoR1 and AdipoR2, adiponectin activates signaling pathways that result in upregulation of β-oxidation, down regulation of lipid synthesis, and prevention of excess lipid storage by promoting lipolysis [11, 12]. Moreover, it has been shown that adiponectin can downregulate C-reactive protein (CRP), suppress TNF-α production, and induce IL-10 production from kupffer cells, in some mouse models [13]. Fas-mediated hepatocyte apoptosis and inflammation has also been blocked by adiponectin, which indicates a potential hepatoprotecive effect [14]. It can also inhibit the generation of reactive oxygen species (ROS) as an anti-oxidative effect [15]. Most clinical studies have demonstrated lower adiponectin levels in CHC patients and an increase in its level following IFN therapy [16, 17].

The aim of the current study was the evaluation of serum levels of IL28B and adiponectin and their correlation with the outcome of IFN-based therapy. Moreover, the association of IL28B SNPs with different clinicopathological parameters was investigated in HCV-infected patients.

Methodology

Patients

All patients were registered at Honary Medical Clinical Centre in Jahrom, a town in the southern part of Iran, between September 2015 and February 2016. This study included 142 subjects with HCV infection in acute or chronic phases of the disease. We also included 70 healthy blood donors as control. Informed consent was obtained from all participants. Inclusion criteria were as follows: (a) adults aged more than 18 years old with the presence of anti-HCV antibodies and detectable serum HCV RNA and (b) serum alanine aminotransferase (ATL) higher than the normal range. Patients were excluded if they had the following concurrent diseases or conditions: (1) co-infection with HBV (hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb)), (2) co-infection with human immunodeficiency virus type 1 (HIV-1) (gp120 and gp41 HIV type 1 antibody and p24 antigen). Recent HCV infections such as acute or early chronic infections were defined by an initial positive anti-HCV antibody test within 6 months of enrolment. Acute clinical infection was defined by symptomatic seroconversion illness or abnormal ALT levels ranging from 2–20 fold higher than the upper limit of normal level at the time of HCV detection [18]. Chronic HCV infection was defined as detectable HCV RNA at 6 months after the time of infection.

Blood samples were collected at baseline (from all healthy controls and the HCV patients) and at weeks 4, 12, 24, and 48 of treatment (from HCV-infected patients).

Treatment schedule

All the participants subcutaneously received peg-interferon α-2a (180 μg/week) plus weight-based ribavirin (800-1200 mg/day). A 24-week regimen was administered to patients with rapid virologic response (RVR), which was defined as negative HCV RNA after 4 weeks of treatment. For those who failed to achieve RVR, the treatment course was extended to 48 weeks. Sustained virologic response (SVR) was defined as clearance of the serum HCV RNA at the end of the therapy and lasting for more than 24 weeks after the cessation of therapy [19]. Early virological response (EVR), defined as an undetectable viral RNA or ≥ 2 log reduction of HCV RNA at week 12 of treatment to the baseline viral level.

Clinical and laboratory assessment

ALT and AST activity was determined by the colorimetric method using Olympus AU400 auto-analyser machine (Mishima Olympus Co. Ltd., Shizuoka-ken, Japan) in the plasma samples. Reference values for ALT and AST were set at 56 and 41 U/L, respectively, and data were reported as international units (IU)/L.

Fibroscaning

Level of liver fibrosis was measured based on METAVIR score according to Eslam *et al.* [20]. Level of Fibrosis was described as follows: F0 as no fibrosis; F1 as portal fibrosis; F2 as periportal fibrosis; F3 as

fibrous septa with architectural distortion, no obvious cirrhosis; and F4 as definite cirrhosis.

HCV viral load and genotyping

Ten milliliters of whole blood sample were collected in 0.5 M EDTA-containing tubes. Plasma was isolated by centrifugation at 3000 rpm for 15 minutes and then preserved at -80° C. According to the manufacturer's instructions, RNA was extracted from 100 µL of plasma, using the AccuPrep Viral RNA Extraction Kit (Bioneer, Daejeon, South Korea). HCV genotyping was performed using type-specific primers according to the protocol described previously [21]. We used different samples with specific HCV genotypes as positive controls. Moreover, genotyping was confirmed by sequencing as described elsewhere [22].

Hepatitis C RNA levels were assessed by quantitative reverse transcription-polymerase chain reaction (qRT–PCR) according to Roche Cobas TaqMan HCV assay (Roche Molecular Systems, Pleasanton, CA), as described elsewhere [23].

IL28B SNPs genotyping

Detection of rs8099917 and rs12979860 IL-28B genotypes was done using PCR with specific primers as described previously [24]. Extraction of DNA was performed from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

Measurement of serum IL28 and adiponectin levels

Serum levels of adiponectin were measured in all patients and control group, using commercial ELISA kits (RayBiotech, Norcross, GA), according to the manufacturer's protocols. Serum IL28 levels were determined using commercial ELISA kit (Human IL28B ELISA kit, proteintech, USA), according to the manufacturer's instructions. Sensitivity of the detection for adiponectin and IL28 was 25 pg/mL and 15.6-1000 pg/mL, respectively.

Table 1. Clinical and laboratory parameters of HCV-infected patients and healthy controls.

Variables	Patients (n = 142)	Healthy control (n = 70)	*P*
Age	41.65 ± 11.39	43.07 ± 10.93	0.16
Gender			0.04
Male	101	51	
Female	41	19	
ALT (IU/l) [1]	49.51 ± 16.33	29.87 ± 5.06	0.001
AST (IU/l)[1]	36.36 ± 10.76	34.43 ± 9.25	0.34
Viral titer (IU/mL)[1]	1. $35\times10^6 \pm 1.69\times10^6$	-	
Phase of disease			
Acute	22	NA[2]	
Chronic	120	NA	
Genotypes			
1a	50	NA	
3a	84	NA	
3a/1a	6	NA	
2a/3a	2	NA	
Response to treatment			
SVR[3]	88	NA	
Non-SVR	36	NA	
Relapse	18	NA	
RVR[4]			
Yes	106	NA	
No	36	NA	
EVR[5]			
Yes	83	NA	
No	59	NA	
Fibrosis stage			
Portal fibrosis (F1)	114	NA	
Periportal fibrosis (F2)	22	NA	
Advanced fibrosis (F3)	6	NA	

[1]: Mean ± SD; [2]: not-applicable; [3]: Sustained virologic response; [4]: Rapid virologic response; [5]: Early virologic response.

Statistical Analysis

Continuous variable was expressed as mean ± standard deviation (SD). Quantitative data analyses were performed by t-test or nonparametric Mann Whitney U test. Analyses of qualitative data were performed by Chi-square or Fisher exact test. Relationship between two variables was tested by Spearman. p value ≥0.05 was considered significant for all results. All statistical analyses were implemented using SPSS software version 22 and GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) for Windows.

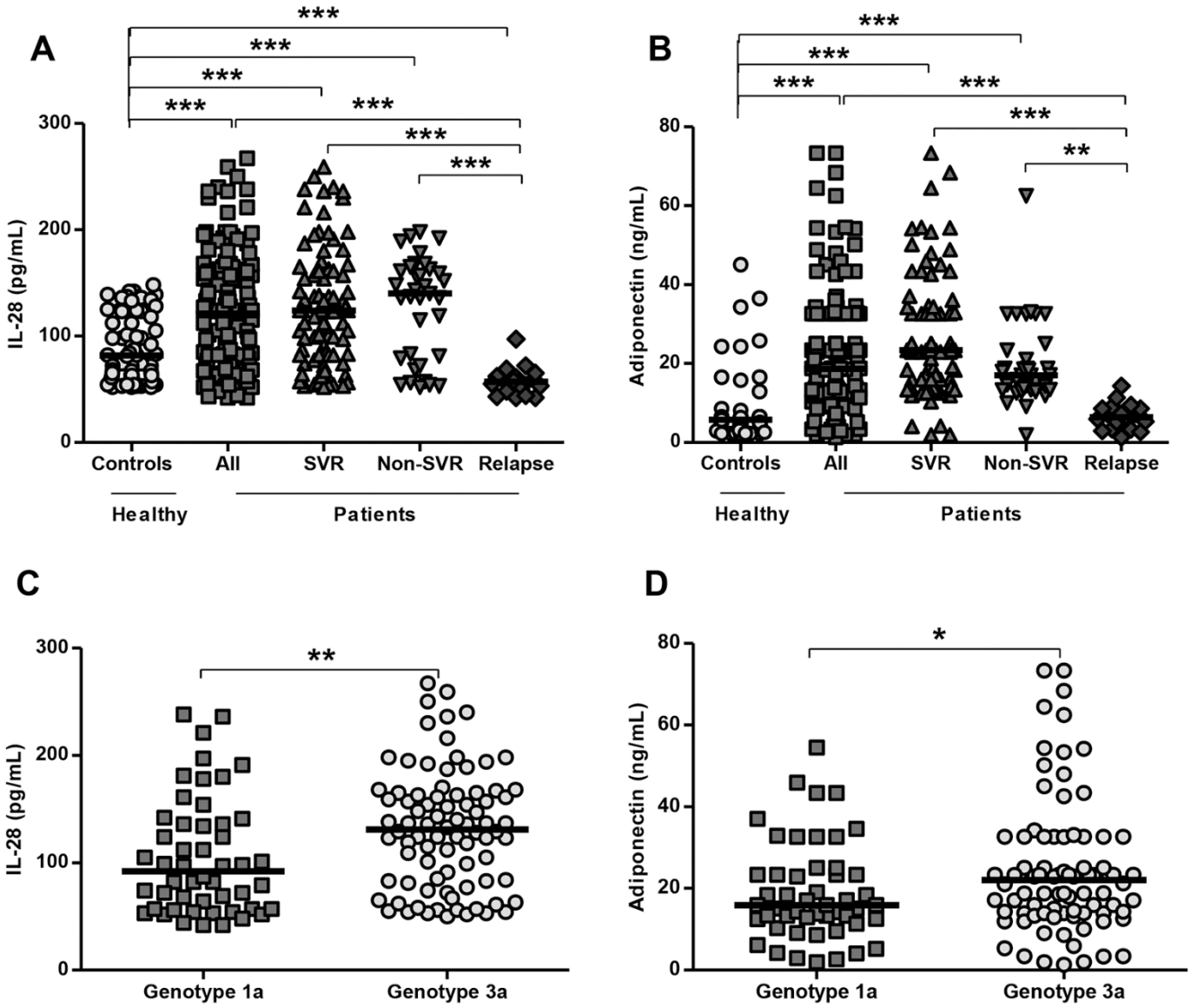
Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Figure 1. Comparison of IL28 and adiponectin serum levels between patients and healthy controls. A) IL28 serum level comparison between healthy controls and all patients as well as patients with SVR, non-SVR, and relapsed infection. B) adiponectin serum level comparison between healthy controls and all patients as well as patients with SVR, non-SVR, and relapsed infection. C) IL28 serum level comparison between patients infected with HCV genotypes 1a and 3a. D) adiponectin serum level comparison between patients infected with HCV genotypes 1a and 3a.



Results

Baseline characteristics of patients and controls

The baseline clinical characteristics of 142 cases and 70 controls were shown in Table 1. Of 142 patients, 101 (71%) were male and 41 (29%) were female, with a mean age of 41.65 ± 11.39 years. The healthy controls included 51 (73%) men and 19 (27%) women, with the mean age of 43.07 ± 10.93 years. The frequency of genotype 3a (G3a) and G1a in HCV patients was 59% (84/142) and 35% (50/142), respectively. After therapy, 88 (62%) patients had SVR, 36 (25%) were non-responders and 18 (13%) cases had relapsed infection. Advanced fibrosis was observed in 6 (4%) patients, while periportal and portal fibrosis were detected in 22 (16%) and 114 (80%) patients, respectively.

IL28 serum levels

There was a significant difference between HCV-infected patients and healthy controls according to IL28 levels (Figure 1). Higher levels of IL28 were found in HCV-infected patients (Figure 1A), however no significant difference was observed between patients in acute and chronic phases of disease (Table 2). Regarding response to IFN-based therapy, higher levels of IL28 were detected in both SVR and non-SVR groups in comparison with patients with relapsed infection (Figure 1A and Table 2). Although higher serum levels of IL28 were found in RVR cases, in the case of EVR, no significant difference was reported (Table 2). In addition, there was no difference in IL28 serum levels between different fibrosis stages.

In patients infected with G3a of the virus, IL28 serum level was significantly higher than those infected with G1a (Table 2 and Figure 1C). Moreover, a correlation was found between increasing levels of AST in G3a-infected patients and the decrease in IL28 serum level, while this was not observed in G1a-infected patients (Table 3). In addition, no correlation was found between viral loads in either genotypes and IL28 levels (Table 3).

Adiponectin serum levels

In comparison with IL28 levels, almost the same pattern was observed regarding to adiponectin serum level. There was a significant difference between case and control groups with regard to adiponectin levels (Figure 1). Higher levels of adiponectin were found in HCV-infected patients (Figure 1B), however no significant difference was observed between cases in acute and chronic phases of disease (Table 2). Regarding response to IFN-based therapy, higher levels of adiponectin were detected in both SVR and non-SVR

Table 2. Serum levels of IL-28 and adiponectin based on different clinicopathological parameters in HCV-infected patients.

	HCV genotype			***p-value*[1]**
	1a		**3a**	
IL28[2]	104.94 ± 7.65		131.14 ± 6.01	**0.008**
Adiponectin[3]	19.02 ± 1.70		24.42 ± 1.78	**0.04**
	Phase of disease			
	Acute		**Chronic**	
IL28	100.91 ± 8.324		123.07 ± 5.26	N.S[4]
Adiponectin	23.87 ± 4.58		22.30 ± 1.24	N.S
	Response to treatment			
	SVR[5]	**Non-SVR**	**Relapse**	
IL28	126.82 ± 5.77	131.42 ± 8.72	57.06 ± 3.35	**≤0.0001[6]**
Adiponectin	26.19 ± 1.57	21.19 ± 2.31	6.35 ± 0.85	**≤0.0001[c]**
	RVR[7]			
	Yes		**No**	
IL28	125.42 ± 5.29		23.84 ± 1.51	**0.03**
Adiponectin	102.61 ± 9.37		18.72 ± 2.11	N.S
	EVR[8]			
	Yes		**No**	
IL28	121.83 ± 6.29		23.80 ± 1.74	N.S
Adiponectin	116.54 ± 6.94		20.77 ± 1.78	N.S
	Fibrosis stage			
	Portal fibrosis (F1)	**Periportal fibrosis (F2)**	**Advanced fibrosis (F3)**	
IL28	119.26 ± 5.39	124.05 ± 9.94	110.50 ± 22.55	N.S
Adiponectin	22.90 ± 1.45	20.40 ± 2.82	23.61 ± 5.25	N.S

[1]: The presented data was analyzed with the t test and ANOVA; [2]: pg/mL; [3]: ng/mL; [4]: Non-significant; [5]: Sustained Virologic Response; [6]: $p \leq 0.0001$ vs. SVR and Non-SVR; [7]: Rapid virological response; [8]: Early virological response.

Table 3. Correlations[1] between serum parameters and viral load with IL-28 and adiponectin in HCV-infected patients.

Genotype		AST		ALT		Viral load	
		r	p	r	p	r	p
IL-28	**1a**	-0.164	N.S	0.071	N.S	0.153	N.S
	3a	-0.238	**0.02**	0.177	N.S	0.132	N.S
Adiponectin	**1a**	-0.164	N.S	0.071	N.S	0.153	N.S
	3a	-0.065	N.S	-0.234	**0.03**	-0.195	N.S

[1]: The correlation was evaluated using Pearson's rank correlation coefficient.

groups in comparison with patients with relapsed infection (Table 2). In addition, there was no difference in RVR cases, EVR cases, and between different fibrosis stages.

In patients infected with G3a of the virus, adiponectin serum level was significantly higher than those infected with G1a (Figure 1D and Table 2). Moreover, a correlation was found between increasing levels of ALT in G3a-infected patients and the decrease in adiponectin serum level, while this was not reported in G1a-infected patients (Table 3). In addition, no correlation was observed between viral loads in either genotypes and adiponectin levels (Table 3).

Frequency of IL28B genotypes

In HCV-infected patients, rs12979860 genotypes CC, CT, and TT were detected in 36.6%, 54.9%, and 8.5%, respectively and rs8099917 genotypes TT, GT, and GG were shown in 64.1%, 30.3%, and 5.6%, respectively. Meanwhile, the distribution of rs12979860 genotypes CC, CT, and TT in healthy controls were 50%, 38.6%, and 11.4%, respectively and the distribution of rs8099917 genotypes TT, GT, and GG were 51.4%, 40%, and 8.6%, respectively (Table 4).

The association of serum IL28 and adiponectin levels with IL28 genotypes

As shown in Table 4, there was not a significant difference between cases and controls according to single nucleotide polymorphisms of *IL28B* gene. Moreover, differences in distribution of SNPs regarding to response to IFN-based therapy in SVR, non-SVR, and relapsed infections as well as in RVR and EVR

Table 4. Genotype frequency of the IL-28 SNPs between study groups[1].

		rs12979860			rs8099917		
		CC (%)	CT (%)	TT (%)	TT (%)	GT (%)	GG (%)
Group	Patients	36.6	54.9	8.5	64.1	30.3	5.6
	Controls	50	38.6	11.4	51.4	40	8.6
	P		N.S[2]			N.S	
HCV genotype	1a	48	40	12	63.1	29.8	7.1
	3a	29.8	65.5	4.8	62	34	4
	P		**0.01**			N.S	
Phase of disease	Acute	68.2	31.8	0	65.8	29.2	5
	Chronic	30.8	59.2	10	54.5	36.4	9.1
	P		**0.003**			N.S	
Response to treatment	SVR[3]	36	55.1	9	58.4	38.2	3.4
	Non-SVR	36.1	58.3	5.6	75	16.7	8.3
	Relapse	41.2	47.1	11.8	70.6	17.6	11.8
	P		N.S			N.S	
RVR[4]	Yes	33	38.5	8.5	67	26.4	6.6
	No	47.2	44.4	8.3	55.6	41.7	2.8
	P		N.S			N.S	
EVR[5]	Yes	41	48.2	10.8	57.8	34.9	7.2
	No	30.5	67.4	5.1	72.9	23.7	3.4
	P		N.S			N.S	
Fibrosis stage	F1	36.8	55.3	7.9	61.4	35.1	3.5
	F2	40.9	54.5	4.5	77.3	9.1	13.6
	F 3	16.7	50	33.3	66.7	16.7	16.7
	P		N.S			**0.04**	

[1]: comparison using χ^2 test; [2]: non-significant; [3]: Sustained virological response; [4]: Rapid virological response; [5]: Early virological response.

cases were not significant. Interestingly, the distribution of rs8099917 TT allele was significantly higher in all fibrosis stages, while this was not observed in the case of rs12979860 in none of fibrosis stages.

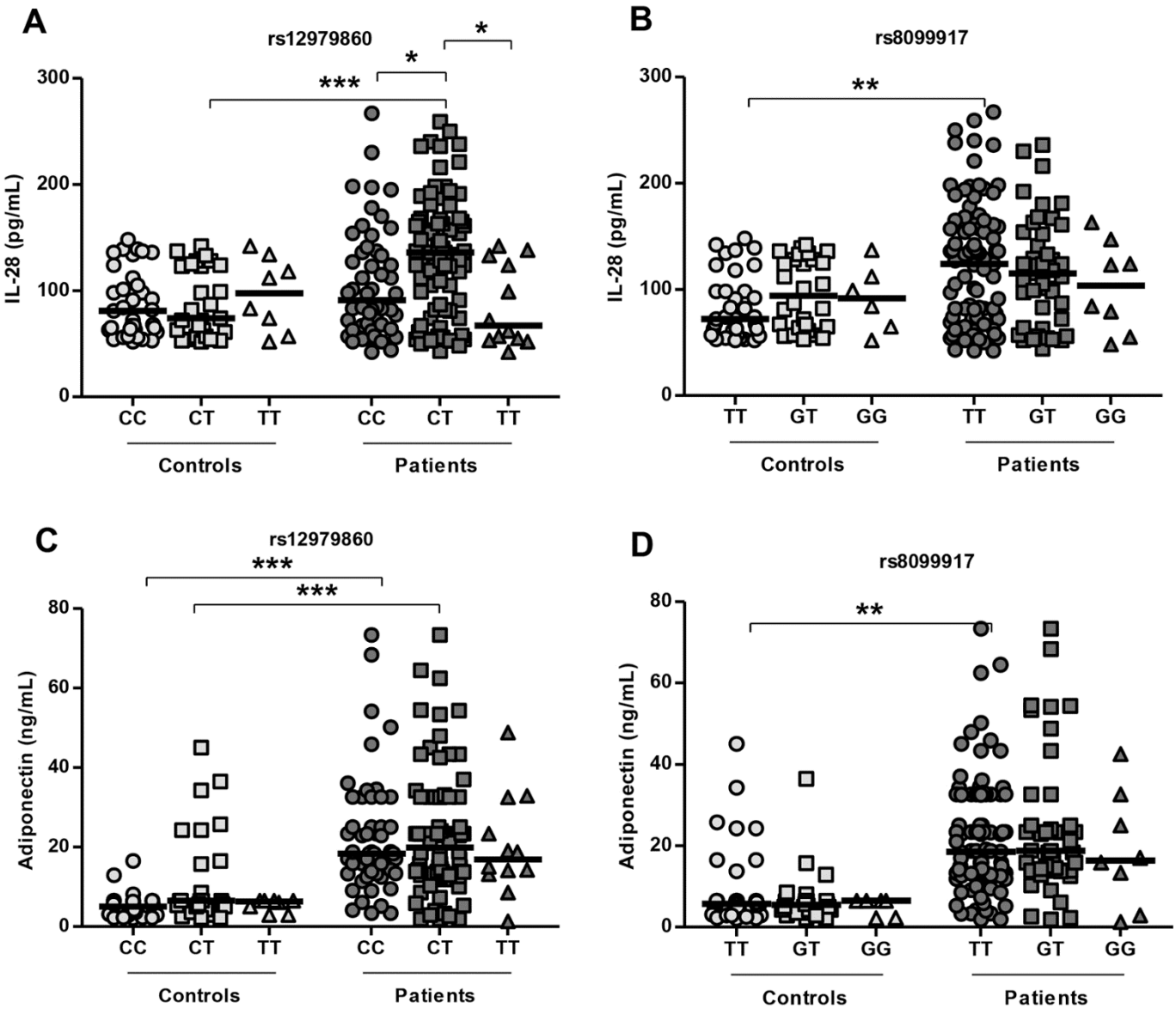
Higher levels of IL28 cytokine were associated with both CT allele of rs12979860 and TT allele of rs8099917 in patients in comparison with corresponding alleles in controls (Figure 2A and 2B). Moreover, patients' CT allele of rs12979860 was associated with significant higher levels of IL28 compared with CC and TT alleles. Higher levels of adiponectin were observed in patents with CT and CC alleles of rs12979860 and TT allele of rs8099917 in comparison with corresponding alleles in controls (Figure 2C and 2D).

Although higher levels of both IL28 and adiponectin were observed in patients infected with G3a of the virus, the differences were non-significant regarding the distribution of different alleles of rs12979860 and rs8099917 (Figure 2).

Discussion

Hepatitis C virus manipulates host lipid metabolism to take advantages for cell entry, assembly, replication and release [25, 26]. This is accompanied with lower pretreatment serum LDL cholesterol levels which is

Figure 2. Association of IL28 and adiponectin serum levels with *IL28* genotypes. IL28 serum levels differ significantly between patients and healthy controls with regard to *IL28B* genotypes rs12979860 (A) and rs8099917 (B). Adiponectin serum levels differ significantly between patients and healthy controls with regard to *IL28B* genotypes rs12979860 (C) and rs8099917 (D).



associated with poor response to combination pegIFN/RBV therapy in CHC patients [27]. Since this decrease in serum lipid and triglyceride levels is resolved after viral clearance, it seems that these effects are virus-dependent [28].

HCV infection results in the production of proinflammatory cytokines such as TNF-α, IL-1, IL-6, and IFN-γ. This is not only involved in the recruitment of inflammatory cells and subsequent cytokine production which leads to liver injury, but also ensues lipid peroxidation, ROS formation, NF-κB activation, and induction of TNF-α, IL-1, IL-6, and IFN-γ production, again. These cytokines are thought to be involved in repression of adiponectin expression, as well. In the absence of adiponectin, lipid accumulation in hepatocytes disturbs the microenvironment which may induce ROS formation and repetition of this destructive cycle.

Adiponectin, as an adipokine secreted from adipose tissue is considered to play some roles in lipid metabolism, tumorigenesis, inflammation, and immune response [10, 29]. The production of this adipokine is stimulated by insulin, angiotensin II, and by inflammatory cytokines such as TNF-α, IL-6, IL-1β, and IFN-γ and in certain pathological states [30]. After binding to its two main receptors, AdipoR1 (skeletal muscle, liver cells, and other tissues) and AdipoR2 (liver cells), adiponectin triggers some of intra-cellular pathways involved in down-regulation of lipid synthesis, and prevention of excess lipid storage by promoting lipolysis [11, 12].

An inverse correlation has been shown between both serum triglycerides and apolipoprotein B (ApoB), the main apolipoproteins of triglyceride rich VLDL and adiponectin levels [31]. A negative correlation has also been documented between adiponectin levels and steatosis [32]. A positive correlation with liver fibrosis, cirrhosis, and hepatocellular carcinoma has been reported, as well [33].

In contrast to other studies [16, 17] which reported lower adiponectin levels in CHC patients, the present study showed higher serum adiponectin levels in HCV-infected patients compared to that in healthy controls. This was also demonstrated in patients with SVR and non-SVR. This finding is possibly due to adiponectin resistance caused by down-regulation of adiponectin receptors or tumorigenic effects of adiponectin [33]. Moreover, the increase in adiponectin level may contribute in down-regulation of Fas-mediated hepatocyte apoptosis and inflammation in CHC patients [14]. Although some studies have shown a relationship between adiponectin levels and fibrosis [33, 34], in the present study no association was found between adiponectin and fibrosis stages in accordance with the results of Khedr *et al.* [35]. These discrepancies in results may be due to small sample size, and heterogeneity among the subject population.

On the other hand, *IL28B* gene polymorphism of rs12979860 has been shown to be associated with LDL cholesterol serum levels, as the only common genetic variant, in patients infected with G1 [7] and with severity of disease, steatosis and fibrosis in CHC patients [36]. It is confirmed that polymorphisms of *IL28B* gene have association with the rate of response to therapy in HCV-infected patients. Patients with rs12979860 CC genotype show higher SVR rates in comparison with patients with CT or TT genotype. In this study, in accordance with Fateh *et al.* [37] CT genotype of rs12979860 was the most prevalent genotype of *IL28B* gene among HCV-infected patients and it was associated with higher IL28 serum level. The C allele is more likely associated with higher expression rates of IL28B, IL28A, and IL29 which is followed by lower intrahepatic expression of interferon-stimulated genes (ISGs) and better response to IFN-based therapies [6]. These interleukins have antiviral activity similar to all interferons. They can induce the expression of MxA protein and 2',5'-oligo-adenylate synthase which is followed by better antiviral response. On the other hand, they can affect monocyte differentiation, maturation of dendritic cells, generation of antigen presenting cells, and development of regulatory T cells [38].

Another *IL28B* gene SNP affecting the treatment response rate is rs8099917 in which TT allele is associated with more self-clearance of infection and better response to IFN-based therapies. The exact mechanism has not been explained so far and further studies are needed to unveil this association [39].

Although some authors have selected one of these two SNPs as the most important predictor of treatment response [5, 40], some studies have reported significant association of both rs12979860 and rs8099917 with the treatment outcome [41, 42]. In the present study, regarding the absence of significant difference in the distribution of SNPs between patients and controls, there were no differences between patients with SVR, non-SVR, and relapsed infections as well as RVR and EVR. This was also reported regarding IL28 serum levels between patients with SVR and non-SVRs. These results are consistent with the results obtained by Alborzi *et al*. [43] but inconsistent with the investigation performed by Fateh *et al*. [44]. In

accordance with Shi *et al*. [45], in the present study, rs12979860 CT and rs8099917 TT genotypes of patients showed higher serum IL28 levels compared to CT and TT genotypes of healthy controls, respectively. In addition, there was a significant association between rs8099917 TT genotype and liver fibrosis stage while it was not shown for rs12979860.

In the present study, the predominant genotype among HCV-infected patients was G3a which is in contrast with other studies enrolled in Iran, introducing mostly G1a as the most prevalent genotype of HCV [46-48]. However, this finding is consistent with Taherkhani *et al*. [23] pointing out an increase in the frequency of infection with G3a in recent years and geographic predominance of this genotype in some parts of Iran. Our viral genotype-based analyses revealed that G3a-infected patients have higher serum levels of both IL28 and adiponectin in comparison with patients infected with G1a. This result is consistent with the results obtained by Meng *et al*. [49] and Liu *et al*. [50] indicating that serum IL28 and adiponectin are correlated with viral factors, including HCV genotype. Moreover, our results indicated a correlation between higher AST levels with lower IL28 as well as higher ALT levels with lower adiponectin in patients infected with G3a, but not in G1a-infected patients. However, there were no significant difference in IL28 and adiponectin levels with the distribution of *IL28B* gene SNPs, regarding different viral genotypes (data not shown). The pathological significance of these observations and the relationship between IL28, adiponectin, and aminotransferase levels in HCV-infected patients is unknown and more studies are necessary in order to elucidate these relationships.

With the introduction of new direct acting antivirals (DAAs) which are associated with lower treatment duration, lower side-effects, and activity against all genotypes, patient management will shift toward using these IFN-free regimens [51]. Albeit not available in all countries because of their cost, the immune profile involved in the clearance of HCV infection after DAA-therapy needs further investigations in future.

In conclusion, the results of this investigation showed elevated serum levels of both IL28B and adiponectin in HCV-infected patients after peg-IFN/Ribavirin therapy and further elucidated HCV genotype-based variations in host responses as G3a of the virus induced higher levels of IL28 and adiponectin in comparison with G1a.

Acknowledgements

The authors would like to thank all the participants and colleagues for cooperation in this investigation. This study was supported in part by Jahrom University of Medical Sciences (grant No: 16638).

References

1. Sklan EH, Charuworn P, Pang PS, Glenn JS (2009) Mechanisms of HCV survival in the host. Nat Rev Gastro Hepat 6: 217-227.
2. Keyvani H, Fazlalipour M, Monavari SHR, Mollaie HR (2012) Hepatitis C virus-proteins, diagnosis, treatment and new approaches for vaccine development. Asian Pac J Cancer Prev 13: 5917-5935.
3. Asselah T, Estrabaud E, Bieche I, Lapalus M, De Muynck S, Vidaud M, Saadoun D, Soumelis V, Marcellin P (2010) Hepatitis C: viral and host factors associated with non-response to pegylated interferon plus ribavirin. Liver Int 30: 1259-1269.
4. Sadeghi F, Bokharaei-Salim F, Salehi-Vaziri M, Monavari SH, Alavian SM, Salimi S, Vahabpour R, Keyvani H (2014) Associations between human TRIM22 gene expression and the response to combination therapy with Peg-IFNa -2a and ribavirin in Iranian patients with chronic hepatitis C. J Med Virol 86: 1499-1506.
5. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461: 399-401.
6. Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, Hong L, McKenzie A, Patel K, Shianna KV (2010) IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. Hepatology 52: 1888-1896.
7. Li JH, Qian Lao X, Tillmann HL, Rowell J, Patel K, Thompson A, Suchindran S, Muir AJ, Guyton JR, Gardner SD (2010) Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. Hepatology 51: 1904-1911.
8. Valenti L, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, Dongiovanni P, Maggioni M, Fracanzani AL, Rametta R (2011) Patatin-Like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology 53: 791-799.
9. Saad Y, Ahmed A, Doa'a AS, Doss W (2013) Adipokines and insulin resistance, predictors of response to therapy in Egyptian patients with chronic hepatitis C virus genotype 4. Eur J Gastroenterol Hepatol 25: 920-925.
10. Matarese G, Mantzoros C, Cava AL (2007) Leptin and adipocytokines: bridging the gap between immunity and atherosclerosis. Curr Pharm Des 13: 3676-3680.
11. Awazawa M, Ueki K, Inabe K, Yamauchi T, Kaneko K, Okazaki Y, Bardeesy N, Ohnishi S, Nagai R, Kadowaki T (2009) Adiponectin suppresses hepatic SREBP1c expression in an AdipoR1/LKB1/AMPK dependent pathway. Biochem Biophys Res Commun 382: 51-56.
12. Buechler C, Wanninger J, Neumeier M (2011) Adiponectin, a key adipokine in obesity related liver diseases. World J Gastroenterol 17: 2801.
13. Park P-h, McMullen MR, Huang H, Thakur V, Nagy LE (2007) Short-term treatment of RAW264. 7 macrophages with adiponectin increases tumor necrosis factor-a (TNF-a)

expression via ERK1/2 activation and Egr-1 expression role OF TNF-a in adiponectin-stimulated interleukin-10 production. J Biol Chem 282: 21695-21703.
14. Wedemeyer I, Bechmann LP, Odenthal M, Jochum C, Marquitan G, Drebber U, Gerken G, Gieseler RK, Dienes HP, Canbay A (2009) Adiponectin inhibits steatotic CD95/Fas up-regulation by hepatocytes: therapeutic implications for hepatitis C. J Hepatol 50: 140-149.
15. Yuan F, Li Y-N, Liu Y-H, Yi B, Tian J-W, Liu F-Y (2012) Adiponectin inhibits the generation of reactive oxygen species induced by high glucose and promotes endothelial NO synthase formation in human mesangial cells. Mol Med Rep 6: 449-453.
16. Khattab MA, Eslam M, Mousa YI, Ela-adawy N, Fathy S, Shatat M, Abd-Aalhalim H, Kamal A, Sharawe MA (2012) Association between metabolic abnormalities and hepatitis C-related hepatocellular carcinoma. Ann Hepatol 11: 487-494.
17. Wójcik K, Jablonowska E, Omulecka A, Piekarska A (2014) Insulin resistance, adipokine profile and hepatic expression of SOCS-3 gene in chronic hepatitis C. World J Gastroenterol 20: 10449.
18. McGovern BH, Birch CE, Bowen MJ, Reyor LL, Nagami EH, Chung RT, Kim AY (2009) Improving the diagnosis of acute hepatitis C virus infection with expanded viral load criteria. Clin Infect Dis 49: 1051-1060.
19. Ghany MG, Strader DB, Thomas DL, Seeff LB (2009) Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 49: 1335-1374.
20. Eslam M, Kawaguchi T, Del Campo J, Sata M, Abo-Elneen Khattab M, Romero-Gomez M (2011) Use of HOMA-IR in hepatitis C. J Viral Hepat 18: 675-684.
21. Antonishyn NA, Ast VM, McDonald RR, Chaudhary RK, Lin L, Andonov AP, Horsman GB (2005) Rapid genotyping of hepatitis C virus by primer-specific extension analysis. J Clin Microbiol 43: 5158-5163.
22. Hafez AA, Baharlou R, Nasab SDM, Vasmehjani AA, Shayestehpour M, Joharinia N, Ahmadi NA (2014) Molecular epidemiology of different hepatitis C genotypes in serum and peripheral blood mononuclear cells in jahrom city of iran. Hepat Mon 14: e16391.
23. Taherkhani R, Farshadpour F (2015) Epidemiology of hepatitis C virus in Iran. World J Gastroenterol 21: 10790–10810.
24. Nasab SDM, Baharlou R, Piroozmand A, Toghyani H, Shadmand E, Fazel H, Sadeghi K, Hashemi SMA, Shokouh MR, Gheshlaghi A (2015) Distribution of IL-28B genotypes in patients with hepatitis C and healthy individuals in Jahrom city. Gastroenterol Hepatol Bed Bench 8: 278-287.
25. Gastaminza P, Cheng G, Wieland S, Zhong J, Liao W, Chisari FV (2008) Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. J Virol 82: 2120-2129.
26. Syed GH, Amako Y, Siddiqui A (2010) Hepatitis C virus hijacks host lipid metabolism. Trends Endocrinol Metab 21: 33-40.
27. Harrison SA, Rossaro L, Hu KQ, Patel K, Tillmann H, Dhaliwal S, Torres DM, Koury K, Goteti VS, Noviello S (2010) Serum cholesterol and statin use predict virological response to peginterferon and ribavirin therapy. Hepatology 52: 864-874.
28. Corey KE, Kane E, Munroe C, Barlow LL, Zheng H, Chung RT (2009) Hepatitis C virus infection and its clearance alter circulating lipids: Implications for long-term follow-up. Hepatology 50: 1030-1037.
29. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 116: 1784.
30. Chen X, Wang Y (2011) Adiponectin and breast cancer. Med Oncol 28: 1288-1295.
31. Zietz B, Herfarth H, Paul G, Ehling A, Müller-Ladner U, Schölmerich J, Schäffler A (2003) Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. FEBS Lett 545: 103-104.
32. Uraki S, Tameda M, Sugimoto K, Shiraki K, Takei Y, Nobori T, Ito M (2015) Substitution in amino acid 70 of hepatitis C virus core protein changes the adipokine profile via toll-like receptor 2/4 signaling. PLoS One 10: e0131346.
33. Liu D, Li S, Li Z (2017) Adiponectin: A biomarker for chronic hepatitis C? Cytokine 89: 27-33.
34. Sumie S, Kawaguchi T, Kuromatsu R, Takata A, Nakano M, Satani M, Yamada S, Niizeki T, Torimura T, Sata M (2011) Total and high molecular weight adiponectin and hepatocellular carcinoma with HCV infection. PLoS One 6: e26840.
35. Khedr MA, Sira AM, Saber MA, Raia GY (2015) Serum adiponectin, vitamin D, and alpha-fetoprotein in children with chronic hepatitis C: can they predict treatment response? Int J Hepatol 2015: 1-9.
36. Sato M, Kondo M, Tateishi R, Fujiwara N, Kato N, Yoshida H, Taguri M, Koike K (2014) Impact of IL28B genetic variation on HCV-induced liver fibrosis, inflammation, and steatosis: a meta-analysis. PLoS One 9: e91822.
37. Fateh A, Aghasadeghi MR, Keyvani H, Mollaie HR, Yari S, Hadizade Tasbiti A, Ghazanfari M, Monavari S (2015) High resolution melting curve assay for detecting rs12979860 IL28B polymorphisms involved in response of Iranian patients to chronic hepatitis C treatment. Asian Pac J Cancer Prev 16: 1873-1880.
38. Dembic Z (2015) The Cytokines of the Immune System: The Role of Cytokines in Disease Related to Immune Response. Academic Press.
39. Halfon P, Bourliere M, Ouzan D, Maor Y, Renou C, Wartelle C, Pénaranda G, Tran A, Botta D, Oules V (2011) A single IL28B genotype SNP rs12979860 determination predicts treatment response in patients with chronic hepatitis C Genotype 1 virus. Eur J Gastroenterol Hepatol 23: 931-935.
40. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S (2009) Genome-wide association of IL28B with response to pegylated interferon-a and ribavirin therapy for chronic hepatitis C. Nat Genet 41: 1105-1109.
41. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'hUigin C, Kidd J, Kidd K, Khakoo SI, Alexander G (2009) Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461: 798-801.
42. Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R (2010) Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 139: 120-129. e118.
43. Alborzi A, Hashempour T, Moayedi J, Musavi Z, Pouladfar G, Merat S (2017) Role of serum level and genetic variation of IL-28B in interferon responsiveness and advanced liver disease in chronic hepatitis C patients. Med Microbiol Immunol 206: 165-174.

44. Fateh A, Aghasadeghi M, Siadat SD, Vaziri F, Sadeghi F, Fateh R, Keyvani H, Tasbiti AH, Yari S, Ataei-Pirkooh A (2016) Comparison of three different methods for detection of il28 rs12979860 polymorphisms as a predictor of treatment outcome in patients with hepatitis C virus. Osong Public Health Res Perspect 7: 83-89.
45. Shi X, Pan Y, Wang M, Wang D, Li W, Jiang T, Zhang P, Chi X, Jiang Y, Gao Y (2012) IL28B genetic variation is associated with spontaneous clearance of hepatitis C virus, treatment response, serum IL-28B levels in Chinese population. PLoS One 7: e37054.
46. Bokharaei-Salim F, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Nasseri S (2013) Distribution of hepatitis C virus genotypes among azerbaijani patients in capital city of iran-tehran. Hepat Mon 13: e13699.
47. Bastani M-N, Bokharaei-Salim F, Keyvani H, Esghaei M, Monavari SH, Ebrahimi M, Garshasebi S, Fakhim S (2016) Prevalence of occult hepatitis C virus infection in Iranian patients with beta thalassemia major. Arch Virol 161: 1899-1906.
48. Samimi-Rad K, Rahimnia R, Sadeghi M, Malekpour SA, Marzban M, Keshvari M, Kiani SJ, Alavian S-M (2016) Epidemic history of hepatitis C virus among patients with inherited bleeding disorders in Iran. PLoS One 11: e0162492.
49. Meng Q-H, Duan Z-H, Lin Z-H, Yu H-W, Li J, Liu Y (2009) Serum level of adiponectin correlated with gender and genotype in patients with chronic hepatitis C. Dig Dis Sci 54: 1120-1127.
50. Liu C-J, Chen P-J, Jeng Y-M, Huang W-L, Yang W-S, Lai M-Y, Kao J-H, Chen D-S (2005) Serum adiponectin correlates with viral characteristics but not histologic features in patients with chronic hepatitis C. J Hepatol 43: 235-242.
51. Asselah T, Boyer N, Saadoun D, Martinot-Peignoux M, Marcellin P (2016) Direct-acting antivirals for the treatment of hepatitis C virus infection: optimizing current IFN-free treatment and future perspectives. Liver Int 36 Suppl 1: 47-57.

Corresponding author

Seyed Jalal Kiani
Department of Virology, School of Medicine, Iran University of Medical Sciences, Hemmat Highway, 14496-14535, Tehran, Iran
Tel: 00982186703007
Fax: 00982188602205
Email: Kiani.j@iums.ac.ir

Conflict of interests: No conflict of interests is declared.