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Assessment of Hepatitis C Virus RNA in Peripheral Blood Mononuclear Cells as a Predictor of Response to Pegylated-Interferon and Ribavirin: A Cohort Study

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Abstract

Background: Sustained virologic response (SVR) to pegylated-interferon (PegIFN) and ribavirin (RBV) in hepatitis C virus (HCV)-infected patients could be predicted by detection of serum HCV RNA whereas detection of HCV RNA in other reservoirs such as peripheral blood mononuclear cells (PBMCs) for prediction of treatment response is still a mystery.

Objectives: This study aimed at assessing the prediction of SVR by detection of HCV RNA in PBMCs or serum in patients during treatment.

Methods: In a cohort study (2011 to 2014), 100 chronic HCV patients at Tehran Hepatitis Center were treated with PegIFN and RBV. Serum HCV RNA level was measured at baseline, 4, 12, and 24 weeks during treatment and at 24 weeks after termination of treatment. Meanwhile, HCV RNA was evaluated in PBMCs at weeks 4, 12, and 24 during the treatment.

Results: Out of 100 patients treated in this study, 91 completed the course of treatment. Most patients were young males infected with HCV genotype 1. Cirrhosis and previous history of treatment was found in 16.5% and 26.5% of patients. Sustained virologic response was achieved in 65 (71.4%) patients. Among baseline parameters, only female gender was significantly associated with SVR. Undetectable serum HCV RNA at week 4 (OR = 4.74) and week 12 (OR = 11.63) of treatment predicted SVR rate while the same was not true for detection of serum HCV RNA at week 24 of treatment. Moreover, detection of HCV RNA in PBMCs at weeks 4 and 12 of treatment was not associated with the rate of SVR, while absence of HCV RNA in PBMCs at week 24 of treatment was associated with SVR (OR = 4.55).

Conclusions: Detection of HCV RNA in PBMCs, especially at week 24 of treatment with PegIFN and RBV, could be considered as an additional marker for prediction of treatment response. It is recommended to assess HCV on-treatment kinetic in PBMCs of patients treated with direct-acting antiviral agents for prediction of treatment response.

Keywords: Hepatitis C Virus, Peripheral Blood Mononuclear Cells, Pegylated-Interferon, Ribavirin

1. Background

Hepatitis C virus (HCV) infection with a prevalence of approximately 130 to 150 million individuals results in 700000 deaths, annually (1, 2). The treatment of chronic HCV infection can prevent long-term complications of disease, such as cirrhosis and Hepatocellular Carcinoma (HCC) (3). Pegylated-interferon (PegIFN) and ribavirin (RBV) were recognized as standard of care for treatment of hepatitis C for more than a decade (4, 5). Introduction of direct-acting antiviral agents (DAAs) caused a revolution in treatment of HCV infection during the recent years, however, the PegIFN and RBV combination therapy is still used in countries where the DAAs are not available or affordable (6, 7).

Sustained virologic response (SVR) to PegIFN and RBV treatment was 40% to 80% in different studies and can be predicted by a set of baseline parameters, such as HCV genotype, polymorphisms near the *IFNL3* gene, gender, age, HCV RNA level and etc. (8-11). Moreover, on-treatment response and viral kinetic are stronger predictors of SVR to PegIFN and RBV treatment compared to baseline parameters (12, 13). Among on-treatment responses, rapid virologic response (RVR) and early virologic response (EVR) were used as predictors of SVR and for response-guided

therapy (4, 5).

While HCV can be detected in serum as a marker for monitoring treatment and evaluation of treatment success, other reservoirs, such as peripheral blood mononuclear cells (PBMCs) or liver can be assessed for detection of HCV RNA (14). The kinetic and detection of HCV RNA in PBMCs through the course of treatment attracted attention as a potential marker for prediction of SVR (14, 15).

2. Objectives

This study aimed at assessing the prediction of SVR by detection of HCV RNA in PBMCs specimens at weeks 4, 12, and 24 of treatment with PegIFN and RBV in patients with chronic HCV infection.

3. Methods

3.1. Study Design

In a cohort study from 2011 to 2014, 100 patients with chronic HCV and a history of positive results for HCVAb and HCV RNA (> 50 IU/mL) in their sera for longer than 6 months were selected to be included in this study at Tehran Hepatitis Center (Tehran, Iran). Exclusion criteria were co-infection with hepatitis B or human immunodeficiency viruses, acute hepatitis C, HCC, liver transplantation, low (< 50 mL/min) creatinine clearance, poorly controlled psychiatric disease, poorly controlled diabetes, malignant or neoplastic disease, severe cardiac or chronic pulmonary disease, active substance abuse, immunologically mediated disease, retinopathy, and clinical or laboratory evidence of liver decompensation. The ethics committee of Baqiyatallah Research Center for Gastroenterology and Liver Diseases approved the study design. All study participants provided an informed consent. The authors asserted that all procedures contributing to this work complied with the ethical standards of the relevant national and institutional committees on human experimentation and with the 1975 Helsinki Declaration, as revised in 2008.

3.2. Treatment Protocol

Patients were treated with a regimen of once-weekly injections of 180 μg of PegIFN-α-2a (Pegasys®, Roche, Basel, Switzerland) or 1.5 μg/kg of PegIFN-α-2b (PegIntron®, Schering-Plough, Las Piedras, Puerto Rico, USA), and oral RBV (Copegus®, Roche or Rebetol®, Schering-Plough), administered at a weight-adjusted dose of 1000 to 1200 mg/day. The patients with HCV genotype 1 or 4 infection were treated for 48 weeks while the treatment duration was 24 weeks for patients with HCV genotype 2 or 3 infection.

Serum HCV RNA level was measured at baseline, 4, 12, and 24 weeks during treatment and at 24 weeks after termination of treatment. Meanwhile, HCV RNA was detected in PBMCs at weeks 4, 12, and 24 during treatment. Undetectable serum HCV RNA (< 10 IU/mL) at the end of a 4-week treatment course was considered to be a rapid virologic response (RVR). Early virologic response (EVR) was defined as undetectable serum HCV RNA at the end of week 12. Undetectable serum HCV RNA 6 months after treatment cessation was considered to be SVR, which indicated treatment success. Treatment non-response was defined as a < 2-log decrease in the serum HCV RNA level at week 12 compared to the baseline, or detectable HCV RNA at week 24. If an undetectable level of serum HCV RNA was achieved at the end of treatment and the serum HCV RNA became detectable again 6 months later, the case was considered to be a relapse. Non-Virologic Response (NVR) was considered in patients with relapse, breakthrough or non-response.

3.3. Laboratory and Liver Fibrosis Assessments

Serum HCV RNA levels were quantitated using COBAS® TaqMan® HCV Test v2.0 (Roche Diagnostics), according to the manufacturer's instructions. Hepatitis C virus genotyping was performed using restriction fragment length polymorphism (RFLP) based on the method developed by Pohjanpelto et al. (16). The detailed protocol of the PCR-RFLP method for genotyping the rs12979860 SNP has been described previously (17). The protocol for detection of HCV RNA in PBMCs was described previously by Bokharaei-Salim et al. (18). Briefly, blood samples with ethylenediaminetetraacetic acid anticoagulant were collected from patients before treatment. The PBMCs were separated from anti-coagulated blood samples using Ficoll density gradient centrifugation (Lympholyte H, Cedarlane, Hornby, Canada). Following 3 washes with phosphate buffered saline, PBMCs were resuspended in 200 μL RNA later solution (Ambion, Inc, Austin, Texas, USA), and stored at -80°C. For extraction of RNA from PBMCs, the RNeasy® mini kit (Qiagen, Hilden, Germany) was used, according to the manufacturer's instructions. The extracted RNA was subjected to cDNA synthesis using RevertAid First Strand cDNA Synthesis Kit (Fermentas of Thermo Fisher Scientific, Waltham, MA, United States). Hepatitis C virus 5'-untranslated region (5'-UTR) was detected in the product of cDNA synthesis by PCR, using the following primer set for the first PCR amplification round: fap, 5'-GCAGAAAGCGTCTAGCCATGG-3' and zap, 5'-CTCGCAAGCACCCTATCAGGC-3'. The amplified products were subjected to a nested-PCR amplification, using the following primer set: fip, 5'-TCTAGCCATGGCGTTAGTA-3' and zip, 5'-CAGTACCACAAGGCCTTTC-3'. The nested-PCR ampli-

fication resulted in a 214 bp DNA fragment separated on agarose gel for detection.

The study patients did not undergo liver biopsy because determining the histological severity is not required before starting HCV treatment. Liver elastography (Fibroscan) was performed on a proportion of patients and the results were defined as F0 - F4; patients with > F3 or > 12.5 Kpa were considered as severe fibrotic or cirrhotic. When Fibroscan was not accessible, evidence of cirrhosis included small liver size or irregular liver margins, dilated portal vein and/or splenomegaly on abdominal sonography, and endoscopic findings that included esophageal varices or hypertensive gastropathy.

3.4. Statistical Analysis

Categorical variables were expressed as frequencies and percentages. Continuous variables with normal distributions were expressed as mean ± standard deviation (SD); continuous variables that were deviated from normal distributions were expressed as the median (interquartile range). The Fisher's exact test was used to analyze the categorical variables. Odd ratios (OR) were calculated for the dichotomous variables and corresponding 95% confidence intervals were produced. P values of < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 20.0 (IBM SPSS, Chicago, IL, USA).

4. Results

In this study, 91 out of 100 patients with HCV were enrolled (Figure 1). Most of the patients were male (71 males versus 20 females) with age of 30.2 ± 9.47 years old. Overall, 61 patients (73.5%) were treatment naive and 26.5% had a history of treatment failure. The HCV infection progressed to cirrhosis in 15 (16.5%) patients. The dominant HCV genotype was 1a with 50.5% prevalence followed by HCV genotype 3a with 39.6% prevalence. The CC genotype of rs12979860 polymorphism was detected in 42.1% of patients, followed by CT and TT in 48.7% and 9.2%, respectively (Table 1).

Following treatment with PegIFN and RBV, 65 (71.4%) achieved SVR while 26 (28.6%) experienced treatment failure including 22 (24.2%) relapse, 3 (3.3%) virologic breakthrough, and 1 (1.1%) non-response. Comparison of the main variables between patients with SVR and those with failure showed that age, HCV genotype, rs12979860 polymorphism, and history of treatment (Naive or failure) did not impact treatment response rate. However, female gender was associated with SVR (P = 0.049) (Table 2).

On-treatment responses were assessed using detection of HCV RNA in serum and PBMCs during weeks 4, 12, and 24 of treatment. At week 4 of treatment, HCV RNA was detected in serum of 19 (29.7%) patients with subsequent SVR, and 17 (65.4%) with subsequent treatment failure (P = 0.002, OR = 4.74). At week 12 of treatment, serum HCV RNA was less frequently detected in patients with subsequent SVR than those with subsequent treatment failure (4.6% vs. 36.0%, P < 0.001, OR = 11.63). However, detection of serum HCV RNA at week 24 of treatment was not associated with achievement of SVR (Table 3). When analyzing the data of detection of HCV RNA in PBMCs, it was found that detection of HCV RNA in PBMCs at weeks 4 and 12 of treatment was not associated with achievement of SVR, while absence of HCV RNA in PBMCs at week 24 of treatment was signif-

Table 1. Baseline Characteristics of Patients[a,b]

	All Patients (n = 91)
Gender, n (%)	
Male	71 (78)
Female	20 (22)
Age (years), mean ± SD	30.20 ± 9.47
Serum ALT (IU/L), median (IQR)	40 (41)
Serum AST (IU/L), median (IQR)	38 (29)
Cirrhosis	
Yes	15 (16.5)
No	76 (83.5)
HCV RNA (Log IU/mL), median (IQR)	5.95 (6.09)
HCV genotype, n (%)	
1a	46 (50.5)
1b	5 (5.5)
2	1 (1.1)
3a	36 (39.6)
4	1 (1.1)
Mixed genotypes	2 (2.2)
rs12979860, n (%)	
CC	32 (42.1)
CT	37 (48.7)
TT	7 (9.2)
History of antiviral therapy, n (%)	
Naive	61 (73.5)
Relapse	21 (25.3)
Non-response	1 (1.2)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; IQR, interquartile range; n, number; SD, standard deviation.
[a] Data of rs12979860 was missed in 15 patients.
[b] Data for history of antiviral therapy was missed in 8 patients.

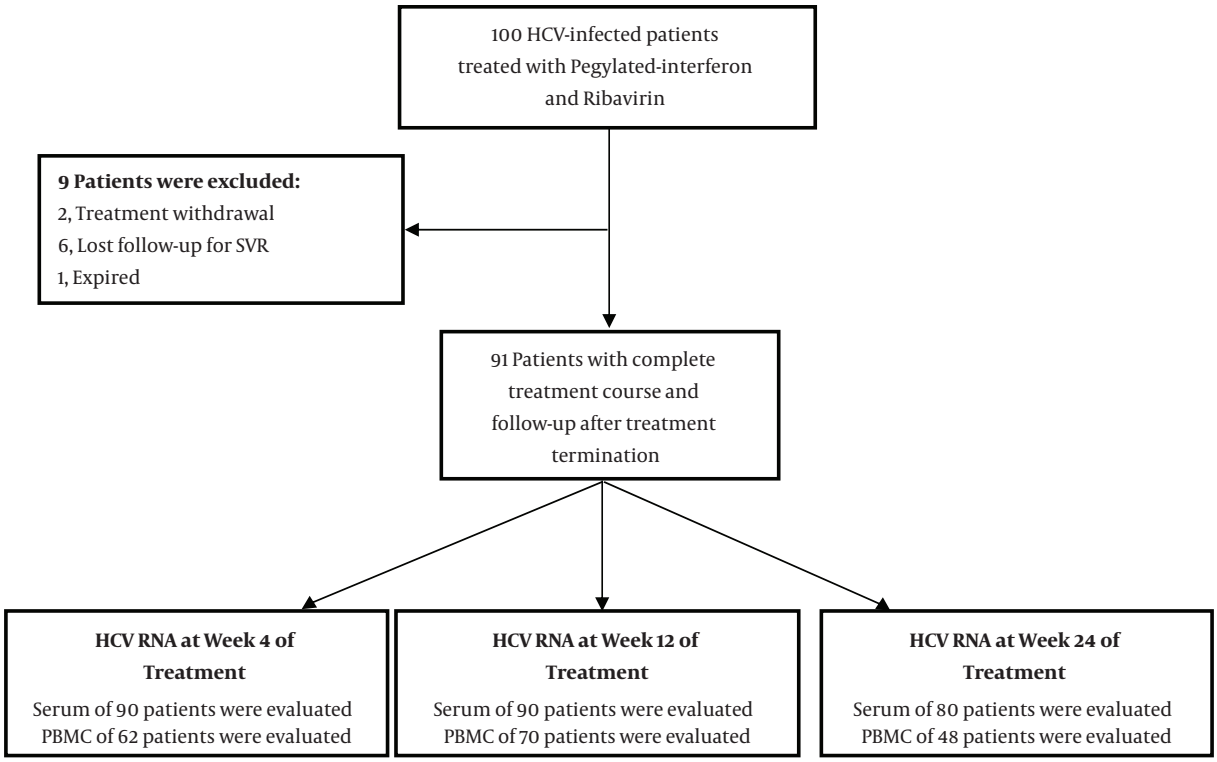

Figure 1. Follow Chart of Enrolled Patients

icantly associated with SVR, and at this time point, HCV RNA in PBMCs of patients with subsequent SVR was less frequently detected than those with further treatment failure (30.6% vs. 66.7%, P = 0.041, OR = 4.55).

5. Discussion

In the current study, out of 91 patients, who completed the course of treatment, 71.5% achieved SVR. It was found that the predictor of SVR in weeks 4 and 12 was absence of HCV RNA in serum while at week 24 this was the absence of HCV RNA in PBMCs. Detection of HCV RNA in serum at week 24 of treatment and in PBMCs at weeks 4 and 12 of treatment was not useful.

In contrast to the current study, Chen et al. (19) and Taliani et al. (20) found that detection of HCV RNA in PBMCs specimens could not predict relapse after treatment. One of the weak points of Chen's study was the small number of patients with detectable HCV RNA in their PBMCs (19). Another possible reason could be the use of conventional IFN instead of PegIFN, which was used in this study. Besides, follow up time was different in the current study. In a study by Zaman et al. (14), 77 responders at the end of treatment were tested for HCV RNA in their PBMCs. The HCV RNA was detected in the PBMCs of 29 (37.7%) patients. Six months after the end of treatment, 15 (19.5%) of 77 patients with end of treatment response showed virologic relapse, while 62 (80.5%) patients attained SVR. Relapse appeared significantly more often in patients with HCV RNA in their PBMCs at the end of treatment when compared to the patients, who did not have the viral RNA at the same stage (34.5% versus 10.4%, respectively; P = 0.01; OR = 1.3) (14). Coppola et al. (21) declared that all 3 patients with treatment failure had HCV in their PBMCs in the first month of antiviral therapy while 1 patient among 15 with SVR had detectable HCV RNA in the PBMCs in the first month of treatment. Contrarily with the current study, Coppola et al. (21) concluded a good relationship between detection of HCV RNA in serum and PBMCs at 3 months during treatment, while the current study did not achieve the same results. Detecting HCV RNA in PBMCs of patients at weeks 4 and 12 of treatment did not conclude any significant relationship with response to treatment (SVR versus NVR). In a study performed by Xu et al. (22) on 16 HCV-infected patients with all achieving clearance of HCV RNA from serum at week 12 of treatment, it was shown that HCV RNA in PBMCs at the end of the 24-week IFN

Table 2. The Impact of Baseline Parameters on Achievement of Sustained Virologic Response in Patients with Hepatitis C Virus Infection[a,b]

	SVR (n = 65)	NVR (n = 26)	OR (95%CI)	P Value[c]
Gender, n (%)				
Male	47 (72.3)	24 (92.3)	Ref.	0.049
Female	18 (27.7)	2 (7.7)	4.55 (0.98 - 20.0)	
Age, n (%)				
> 24	48 (73.8)	24 (92.3)	Ref.	0.084
< 24	17 (26.2)	2 (7.7)	4.25 (0.91 - 19.92)	
Cirrhosis, n (%)				
Yes	10 (15.4)	5 (19.2)	Ref.	0.756
No	55 (84.6)	21 (80.8)	1.31 (0.40 - 4.28)	
HCV RNA (IU/mL), n (%)				
> 600000	41 (63.1)	18 (69.2)	Ref.	0.634
< 600000	24 (36.9)	8 (30.8)	1.32 (0.50 - 3.49)	
HCV genotype, n (%)				
1/4	36 (55.4)	18 (69.2)	Ref.	0.248
2/3	29 (44.6)	8 (30.8)	1.81 (0.69 - 4.76)	
rs12979860, n (%)				
CT + TT	29 (54.7)	15 (65.2)	Ref.	0.455
CC	24 (45.3)	8 (34.8)	1.55 (0.56 - 4.28)	
History of antiviral therapy, n (%)				
Treated	12 (21.1)	10 (38.5)	Ref.	0.113
Naive	45 (78.9)	16 (61.5)	2.34 (0.85 - 6.47)	

Abbreviations: n, number; NVR, non-virologic response; OR, odds ratio; SVR, sustained virologic response; Ref, reference.
[a] Data of rs12979860 was missed in 15 patients.
[b] Data for history of antiviral therapy was missed in 8 patients.
[c] Fisher-exact test.

therapy was a predictor of durable response to antiviral therapy in patients with chronic hepatitis C (22). In their study (22), at the end of treatment, all 9 out of 16 patients with negative results for detection of HCV RNA in serum, who showed HCV RNA positive results in PBMCs, relapsed 1 year after treatment, while only 1 out of 7 patients with undetectable HCV RNA in PBMCs relapsed within 1 year. These results were similar to that of the current study. Zayed et al. (15) aimed at investigating the predictive value of HCV RNA in PBMCs of 70 patients with chronic hepatitis C after 48 weeks of IFN treatment, which may act as the source of HCV reinfection of hepatic cells. They revealed the presence of detectable HCV RNA in the PBMCs of 27% of patients, despite clearance of serum HCV RNA. During the follow-up, 80% of the patients, who became serum HCV positive 6 months after the end of treatment had detectable level of HCV RNA in PBMCs at the end of treatment. Zayed et al. (15) concluded that the absence of HCV in the serum of patients by the end of treatment did not exclude future viremia. The patient might still be a source of infection to others. It is strongly encouraged to test for HCV in PBMCs to detect lack of response to treatment and persisting infection.

Due to the useful information afforded by HCV replication during antiviral therapy, seeking HCV RNA in both plasma and PBMCs specimens during treatment with PegIFN and RBV treatment has clinical importance (21). The PBMCs are actively infected by HCV and could be a site for extrahepatic viral replication. As a consequence, it is possible that impaired immune function of these cells may occur during the natural course of chronic infection and modify the efficacy of the treatment with interferon (20).

Sample size was one of the advantages of the current study compared to previous studies (14, 19-22). The study of Chen et al. (19) had 9 patients, Xu (22) had 16 patients, El-Awady (23) had 4 patients, while the current study had 91 patients. Zaman et al. studied 103 patients with HCV geno-

Table 3. The Impact of On-Treatment Responses in Serum and Peripheral Blood Mononuclear Cells on Achievement of Sustained Virologic Response in Patients with Hepatitis C Virus Infection

	SVR, n (%)	NVR, n (%)	OR (95%CI)	P value[a]
HCV RNA in serum				
Week 4 (n = 90)				
Detected	19 (29.7)	17 (65.4)	Ref.	0.002
Not detected	45 (70.3)	9 (34.6)	4.74 (1.70 - 11.80)	
Week 12 (n = 90)				
Detected	3 (4.6)	9 (36.0)	Ref.	< 0.001
Not detected	62 (95.4)	16 (64.0)	11.63 (2.82 - 47.98)	
Week 24 (n =80)				
Detected	1 (1.7)	1 (4.8)	Ref.	0.459
Not detected	58 (98.3)	20 (95.2)	2.9 (0.17 - 48.56)	
HCV RNA in PBMC				
Week 4 (n = 62)				
Detected	28 (63.6)	14 (77.8)	Ref.	0.375
Not detected	16 (36.4)	4 (22.2)	2.00 (0.56 - 7.12)	
Week 12 (n = 70)				
Detected	21 (42.9)	12 (57.1)	Ref.	0.306
Not detected	28 (57.1)	9 (42.9)	1.78 (0.63 - 4.99)	
Week 24 (n = 48)				
Detected	11 (30.6)	8 (66.7)	Ref.	0.041
Not detected	25 (69.4)	4 (33.3)	4.55 (1.13 - 18.32)	

Abbreviations: n, number; NVR, non-virologic response; OR, odds ratio; SVR, sustained virologic response; Ref, reference.
[a] Fisher's exact test.

type 3a at the end of treatment (14). Intervals of the follow up of patients in a study by Coppola (21) were the same as the current study.

5. Conclusion

This study showed the importance of on-treatment response in the course of treatment with PegIFN and RBV. While on-treatment detection and quantification of serum HCV RNA had a great role in prediction of response and decision of treatment strategy (response-guided therapy), detection of HCV RNA in PBMCs, especially at week 24 of treatment with PegIFN and RBV could be considered as an additional marker for prediction of treatment response. It is recommended to assess the HCV on-treatment kinetic in PBMCs of patients treated with DAAs for prediction of treatment response.

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