The Predictive Factors for Favorable Outcomes of Peginterferon and Ribavirin Combination Therapy in HCV-Infected Patients

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Abstract- We aimed to investigate the association of pretreatment host and/or viral related factors with sustained virological response (SVR) rate in chronic hepatitis C (CHC) infected patients. This cohort study was performed on 200 IFN-naïve Iranian CHC patients who were treated with pegylated interferon-α (PEG-IFN-α) plus ribavirin (RBV). Pretreatment levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), fasting blood sugar (FBS), HCV load and genotype were determined, and the pattern of changes was monitored throughout the course of treatment. The baseline FBS value in the non-responder group was significantly higher than that of the SVR group. The SVR group showed a rapid and continuous decline of ALT/AST activity from the beginning of the treatment, while the ALT level was fluctuating in non-responder and relapse groups. Persistent normalization of transaminases during combination antiviral therapy was significantly associated with SVR rate. Besides, age and FBS levels had the greatest impact on SVR. Minocycline seems to be a safe and effective adjuvant in the management of patients with schizophrenia.

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Keywords: Hepatitis C virus (HCV); Sustained virological response; Predictive marker; Transaminase

Introduction

Hepatitis C virus (HCV) is considered a major cause of liver diseases, cirrhosis, and hepatocellular carcinoma (1-4). Prevalence of HCV infection ranged from 0.2 to 40 percent in different countries, *i.e.*, 0.3 to 12% in Asian-Pacific regions (5) and less than 1% in Iran (6-8). The combination of peg-IFN-α and RBV, although largely replaced by the newer all-oral treatments, is still recommended in many countries, with the SVR rate of 20-56% (5,9-13). Some host-related factors (*i.e.* age, gender, history of diabetes, race, pretreatment ALT level, stage of fibrosis, and genetic factors), as well as some viral factors (i.e. HCV genotypes and viral load), may have an impact on antiviral treatment outcome (5,10,12,14-17).

Although the patterns of ALT changes have not been completely established (15,18,19), some reports suggest that a decrease in ALT and its normalization after week 12 could be considered as an indicator of response to treatment (18). On the other hand, SVR has not always been associated with ALT normalization (19); however, persistently elevated ALT during treatment was observed in 13% of patients who achieved SVR (18). Some researchers believe that the treatment course should be stopped in patients who have not been able to normalize ALT level within the first 12 weeks (19).

As the predictive markers could be useful tools to identify non-responders, these factors should be considered as early as possible. Most studies focused on single factors for prediction of HCV treatment outcome; however, some recent studies suggested several hosts and viral variables for predictive models. As there was not enough information about positive and negative predictors of achievement of SVR in Iranian patients, the present study was performed to find the association between pretreatment demographic, clinical, and viral characteristics of Iranian CHC patients and treatment success due to combination therapy of peg-IFN-α plus RBV.

Materials and Methods

Patients

Two hundred forty adult Iranian CHC patients with no background disease were visited in the clinic of Shariati

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Hospital (Tehran University of Medical Sciences, Tehran, Iran) from 2012 to 2014. CHC infection was confirmed by a positive polymerase chain reaction (PCR). The inclusion criteria were being IFN-naïve CHC patients infected with genotype 1 or 3. All cases were negative for anti-HIV antibody and HBsAg. Pregnant women, active drug users, patients were having previous treatment for CHC, those with liver disease of the origins other than HCV infection, and those aged <18 or >70 years were excluded. Finally, 200 patients meeting our criteria were enrolled in this cohort study (Figure 1). The study protocol was approved by the local Ethics Committee of Shiraz University of Medical Sciences (Approval no. IR.SUMS.REC.1395.S1107; Approval date: 2017-2-18). Written informed consent for using clinical data and blood samples was obtained from all patients prior to the study.

Study design

Pretreatment variables consisted of 2 variables for patient characteristics (age and gender), 3 of the blood chemistry test (AST, ALT, and FBS), and two virologic factors, HCV genotype and serum level of HCV-RNA. The serum level of ALT and AST were measured before treatment, monthly during treatment, at the end of treatment, and six months after the end of treatment. Viral RNA was extracted using the investors spin virus mini kit (Stratec, Germany) and quantified by the use of real time PCR assay (Roche Diagnostics, Germany). HCV genotyping was performed by a commercially available INNO-LiPA HCV II kit (Innogenetics, Belgium). The patterns of ALT and AST change were analyzed throughout the treatment and follow-up period. High HCV load and low HCV load were defined as HCV-RNA ≥400,000 IU/mL and <400,000 IU/mL, respectively.

The duration of therapy was carried out according to the HCV genotype: 48 weeks for genotype 1 and 24 weeks for genotype 3. Treatment consisted of 180 µg of peg-IFN-α2a weekly for all patients. Patients with genotype one infection received RBV at a dose of 1000 mg daily if their body weight was less than 75 kg and 1200 mg/day for those more than 75 kg. Patients with genotype three infection received 800 mg RBV daily in two divided doses. All the patients were followed-up for six months after the completion of treatment.

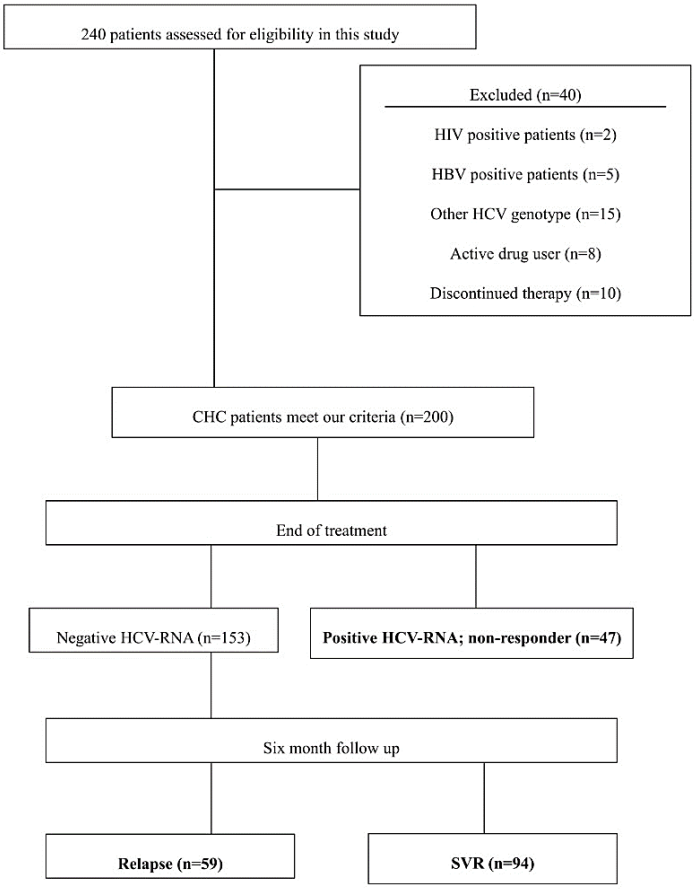

Figure 1. Flowchart of the CHC patient selection.

Table 1. Baseline characteristics of HCV-infected patients enrolled in this cohort study

Baseline parameters		HCV genotypes		*P*
		Genotype 1 n (%)	Genotype 3 n (%)	
Sex	**Male, n=158**	108 (68.4%)	50 (31.6%)	0.931
	Female, n=42	29 (69%)	13 (31%)	
Age	**≤40 years, n=95**	64 (67.4%)	31 (32.6%)	0.743
	>40 years, n=105	73 (69.5%)	32 (30.5%)	
FBS	**Normal (<100 mg/dL), n=132**	86 (65.2%)	46 (34.8%)	0.156
	Abnormal (≥100 mg/dL), n=68	51 (75%)	17 (25%)	
AST	**Normal (<40 IU/L)**	61 (75.3%)	20 (24.7%)	0.166
	Abnormal (≥40 IU/L)	76 (66.1%)	39 (33.9%)	
ALT	**Normal (<40 IU/L)**	41 (80.4%)	10 (19.6%)	0.057
	Abnormal (≥40 IU/L)	96 (66.2%)	49 (33.8%)	
HCV viral load	**Low (<400,000 IU/mL)**	31 (64.6%)	17 (35.4%)	0.012
	High (≥400,000 IU/mL)	80 (83.3%)	16 (16.7%)	

Definition of virological responses

Patients were visited monthly following the initiation of therapy to assess the efficacy of treatment and possible adverse events. Non-responder was defined as a patient having detectable HCV-RNA at the end of treatment. The second endpoint was a virological relapse, defined as undetectable HCV-RNA at the end of the treatment and detectable HCV-RNA during the follow-up period. SVR was defined as undetectable serum HCV-RNA at the end of the treatment and six months later.

Statistical analysis

All statistical analyses were carried out using SPSS version 15. Variables were checked for normality by the Kolmogorov-Smirnov test. In the case of repeated measurement variable, GEE regression was used for variables without normal distribution. Categorical variables were given as the number and percentage and compared with a $\chi 2$ test. Comparison of mean values between the groups was performed by *t*-test. For identification of the factors associated with response to therapy, multivariable logistic regression analysis was performed. A *P* of less than 0.05 was considered statistically significant.

Results

The overall SVR rate was 47% (94 patients), and virological relapse was occurred in 59 patients (29.5%). Moreover, there were 47 patients (23.5%) with no response to combination therapy. The baseline characteristics of HCV-infected patients are shown in Table 1.

To identify the factors that were predicting SVR, we assessed the following variables: sex, age, HCV genotype, FBS level, pretreatment viral load, and AST and ALT activity (Table 2). Sex and HCV genotypes did not significantly affect the outcome of treatment, but older patients (aged >40 years) were more likely experienced relapse, while in those aged ≤40 years, the SVR rate was significantly high (Chi-square, $P<0.0001$). The mean of the baseline FBS level in the non-responder group was significantly higher than that of the SVR (ANOVA, $P=0.002$). Older patients had a higher baseline FBS (independent *t*-test, $P=0.002$), and the normal level of FBS was significantly associated with SVR (Chi-square, $P=0.004$). Pretreatment HCV loads had a wide range of distribution, but the mean value was not different among the SVR, relapse, and non-responder groups (ANOVA, $P=0.997$). The majority of patients (66.7%) had a high baseline HCV-RNA; however, HCV-1 infected patients had a higher HCV load compared to those infected with HCV-3 ($P=0.011$). In both groups of non-responder and relapse, HCV-RNA was significantly higher than SVR ($P=0.033$).

Overall, pretreatment AST and ALT activity had no predictive role in response to treatment (ANOVA, $P=0.062$, $P=0.967$, respectively). The baseline characteristics of the patients indicated that 42.5% and 27.5% of the patients had normal (<40 IU/L) AST and ALT activities, respectively. Those who had elevated levels of transaminases at baseline (≥40 IU/L) were followed up for six months, and if they showed ≥two times elevation in the ALT and/or AST activities, they were considered as having persistently elevated levels. In this regard, subjects were classified into four groups as follow: G1: patients with the initial normal level of AST and ALT and sustained normal level during treatment and follow-up periods; G2: patients with an initial normal level and sustained abnormality; G3: patients with an

initial abnormal level and sustained normal levels; and G4: patients with an initial abnormal level and sustained abnormality (Table 3).

The rapid decline of ALT and AST levels started at the beginning of the combination therapy in all groups of patients. The main differences appeared after week 12 when the three groups showed different behaviors. The patients in non-responder and relapse groups showed an abnormality during the treatment course as opposed to the SVRs who showed a persistent decline in AST and ALT activities (Figure 2).

Table 2. SVR rates of different baseline parameters in HCV-infected patients with genotype 1 and 3

Baseline Parameters		Genotype 1 n (%)	Genotype 3 n (%)
Sex	**Male**	47/108 (43.5%)	33/50 (66%)
	Female	10/29 (34.5%)	4/13 (30.8%)
Age	**≤40 years**	38/64 (59.4%)	25/31 (80.6%)
	>40 years	19/73 (26%)	12/32 (37.5%)
FBS	**Normal (<100 mg/dL)**	24/63 (38.1%)	25/39 (64.1%)
	Abnormal (≥100 mg/dL)	7/38 (18.4%)	9/17 (52.9%)
AST	**Normal (<40 IU/L)**	28/61 (45.9%)	16/24 (66.6%)
	Abnormal (≥40 IU/L)	29/76 (38.2%)	21/39 (53.8%)
ALT	**Normal (<40 IU/L)**	17/41 (41.5%)	8/14 (57.1%)
	Abnormal (≥40 IU/L)	40/96 (41.7%)	29/49 (59.2%)
HCV viral load	**Low (<400,000 IU/mL)**	15/31 (48.4%)	12/17 (70.6%)
	High (≥400,000 IU/mL)	30/80 (37.5%)	7/16 (43.8%)

Table 3. Treatment outcome on the basis of different ALT and AST conditions

Markers		Total n (%)	SVR n (%)	Relapse n (%)	Non-responder n (%)	*P*
ALT groups	**G1**	42 (21%)	20 (21.3%)	13 (22%)	9 (19.1%)	*P*=0.091
	G2	4 (2%)	1 (1%)	1 (1.7%)	2 (4.2%)	*P*=0.819
	G3	90 (45%)	62 (66%)	13 (22%)	15 (31.9%)	*P*<0.0001
	G4	64 (32%)	11 (11.7%)	32 (54.2%)	21 (44.6%)	*P*=0.002
AST groups	**G1**	66 (33%)	41 (43.7%)	13 (22.1%)	12 (25.5%)	*P*=0.003
	G2	12 (6%)	1 (1%)	4 (6.7%)	7 (14.8%)	*P*=0.247
	G3	63 (31.5%)	43 (45.8%)	11 (18.6%)	9 (19.1%)	*P*<0.0001
	G4	59 (29.5%)	9 (9.5%)	31 (52.5%)	19 (40.4%)	*P*<0.0001

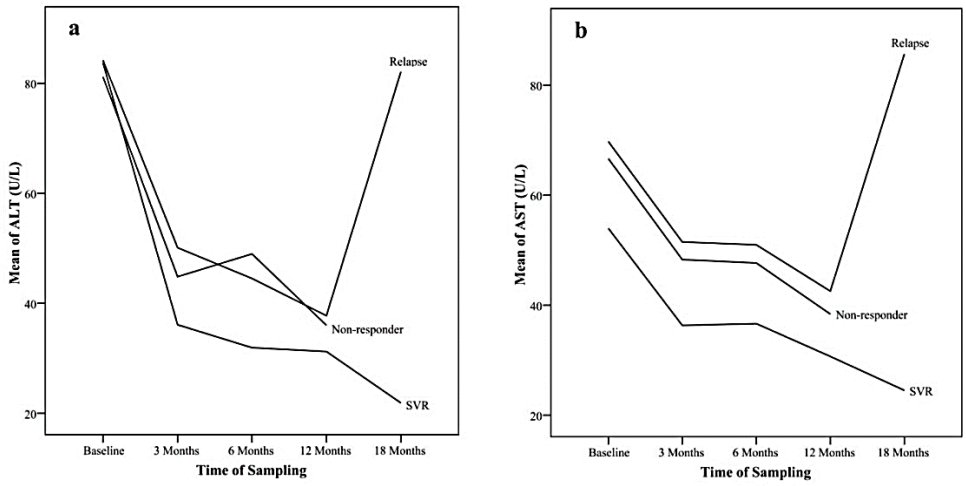

Figure 2. The ALT and AST patterns of different groups of CHC patients throughout the course of the treatment and follow-up period; a) ALT, b) AST. Patients in SVR group had persistent decline in the level of ALT and AST

At the end of the follow-up (week 72), 91 patients (96.8%) showed a biochemical response in the SVR group manifested as a normalization of ALT and AST levels. During combination therapy, the serum ALT and AST levels were decreased significantly to the normal range in patients who achieved SVR (paired t-test, $P<0.0001$), while the mean of these markers increased in the relapse and non-responder groups. Rapid normalization of ALT in patients with initial abnormal ALT level was significantly associated with response to the treatment (Chi-square, $P=0.035$). SVR rate in patients with normalized ALT levels was significantly higher than that in patients who could not normalize ALT during the first three months of therapy (Table 3).

Discussion

Although the viral response is not always associated with the biochemical response (7), in general, decreased production of ALT is the accepted basic indicator of interferon therapeutic effect in CHC patients and several studies have shown that delayed normalization of ALT level may indicate poor response to interferon therapy (20).

In this study, the SVR rate was found to be high in patients who could normalize the liver enzyme level during the course of the treatment. Although the pretreatment level of ALT and AST had no predictive role in the treatment outcome, rapid normalization of ALT in the first three months after initiation of combination therapy could be an indicator of response to the treatment. In addition, during the course of the treatment, the level of AST and ALT decreased significantly to the normal range in all patients who achieved SVR. In accordance with this study, some researchers believe that rapid normalization of ALT after combination therapy with PEG-IFN plus RBV may have an impact on treatment response and SVR rates were found to be significantly higher in patients with normalized ALT at week four and thereafter (15,21). Dogan *et al*., (21) found that biochemical response and normalization of ALT during the first eight weeks in CHC patients infected with genotype one could predict the viral response. A similar result was found by Kim *et al*., (15) at week four after initiation of the treatment. Hung *et al*., observed that 13% of CHC patients who obtained SVR showed persistent elevated ALT during treatment (19). On the other hand, Zeuzem *et al*., showed that 41% of the patients did not achieve ALT normalization until ETR (22). These findings seem to suggest that the lack of ALT normalization is not necessarily associated with a decreased efficacy of the treatment (18). However, this phenomenon has not been characterized systematically, and little is known about its incidence, clinical characteristics, longitudinal pattern, and clinical relevance in CHC patients treated with combination therapy.

To the best of our knowledge, this study was the first survey evaluating the pattern of ALT and AST in the outcome of treatment in Iranian CHC patients. Besides, our data showed that eradication of HCV infection and the achievement of SVR were significantly associated with the pretreatment FBS level. SVR is more common in CHC patients with pretreatment normal FBS level. Although we measured the serum FBS level at the beginning of the study only, the high level of FBS suggests that these patients had diabetes, and several investigations have shown that diabetic patients achieve a lower SVR rate (23,24).

Although some studies indicate that pretreatment viral load is correlated with the treatment response (25), we found that the mean of baseline HCV viral load was similar among patients in the SVR, relapse and non-responder groups; however, the higher viral load (≥400,000 IU/ml) was more common in non-responder and relapse groups. In this study, we showed that the rate of SVR significantly increased in patients younger or equal to 40 years, and the SVR rate was not significantly different between males and females; this is compatible with the finding of Namazee et al. in Iran (26).

Our results demonstrated that rapid normalization of ALT/AST, age, and pretreatment FBS level were strong predictive factors associated with SVR among Iranian HCV mono-infected patients treated with pegylated interferon and ribavirin. In addition, we showed that the pattern of ALT/AST is related to response to treatment; however, the role of normalization in the outcome of therapy still needs to be elucidated.

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