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HEPATITIS C;
EARLY PREDICTION OF THERAPY RESPONSE IN PATIENTS AND THE BENEFITS OF TREATMENT EXTENSION IN NON RESPONDERS

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ABSTRACT… Hepatitis C virus (HCV) has infected about 200 million individuals across the world and is known as the major cause of liver disease . **Objectives:** Viral load measurement at early stages of the therapy in Hepatitis C patients is believed to be a more effective tool to predict the sustained virological response (SVR). The primary aim of the present study was to evaluate whether the decline in viral load of HCV at early stages of the therapy may predict the treatment response. Another objective was to see the benefits of therapy extension in non-responders. **Study Design:** Descriptive, analytical study. **Setting:** Shalamar Hospital Lahore. **Period:** November 2010 to October 2013. **Methods:** Four hundred and thirty patients, chronically infected with different genotypes of Hepatitis C virus were treated with Interferon alpha 2b plus Ribavirin (IFNα-2b + RBV). Viral load was assessed at day zero, week four, in the mid time of therapy and at the end of therapy. The treatment duration was extended 12-24 weeks (according to HCV genotypes) in non-responders. **Results:** The patients with <2 MIU/mL viral load at day zero, able to drop ≥2 log viral load at week-4 or showed no virus at the time of half therapy completion, exhibited better response. The extension of therapy was more beneficial for those non-responder who had <0.05 MIU/mL viral load at the end point of therapy than those who had ≥0.05 MIU/mL at that stage. **Conclusions:** The viral load detection at early stages of the therapy will be useful in clinical practice. Moreover, the patients with <0.05 MIU/mL viral load at the end of therapy are suitable candidates for the therapy extension.

Key words: Hepatitis C, early response prediction, therapy extension benefits.

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INTRODUCTION

Hepatitis C virus (HCV) has infected about 200 million individuals across the world and is known as the major cause of liver disease[1,2]. To reduce the frequency of HCV infection and to prevent its progression to liver failure are the major health related problems now a days[3,4]. Due to the lack of vaccine against HCV infection, the only way to get rid of this menace is to improve and properly manage the therapeutic options. Unfortunately at this moment, limited options are available to treat the Hepatitis C patients. Only two standard treatment combinations (Interferon alpha plus Ribavirin (IFNα+RBV) and Pegylated Interferon alpha plus Ribavirin (PegIFNα+RBV) are approved by FDA to treat the Hepatitis C patients[5,6].

Despite of the good results of IFNα+RBV therapy in Pakistan, most of the HCV infected patients failed to eliminate the virus from their body even after the completion of therapy or relapsed within six months after the therapy termination[7,8]. These patients have to face the high cost of therapy and its adverse side effects rather getting the expected results from the therapy. For these non-responders the prediction of therapy response at early stages can evade them from the hazards and complications of this squandered therapy.

The inadequate success rate of the currently available therapies for Hepatitis C patients has motivated the researchers to develop the methods for the prediction of therapy outcome and to individualize the responding and non-responding patients at early stages. Although these options are taken under consideration by the practitioners during their routine clinical practice, however many researchers have reported that antiviral therapy response could be predicted with a reasonable level of accuracy

at early stages of therapy[9,10]. Current believe is that if the patient succeed to drop the viral load more than or equal to 2 log or became HCV RNA negative at 12th week of therapy, may clinically be taken under consideration to short the therapy duration[11]. It has also been recently suggested that the therapy duration may be reduced in the patients who became HCV RNA negative at 4th week of the treatment[12,13].

The Interferon therapy is not only costly but has its well-known adverse side effects; therefore, the prediction of treatment response is very important to characterize the responders and non responders at the early stages. The primary aim of this study was to analyze the influence of viral load at different stages of therapy on treatment response of the patients infected with different HCV genotypes and additionally, to see the significance of therapy extension in non-responders HCV patients. The findings of present study may help the clinicians to decide the continuation or termination of the therapy at early stages and to save the patients from the futile use of therapy, its adverse side effects and cost burden.

MATERIALS AND METHODS

Patient selection

A total of 430 patients with chronic HCV infection were enrolled at Shalamar Hospital Lahore, Pakistan. All the patients were naïve (with no previous Interferon therapy history). Out of 430 patients 233 were males and 197 females with mean age 36±6 years (range, 10-60), weight 65±11.5 Kg (range, 35-102), Hemoglobin (Hb) 12.5±2.4 g/dl (range, 10-17), and platelet count 178,566±54,156 mm^3 (range, 98,000-355,000). All the patients were serologically evaluated to exclude the chances of Hepatitis B and Acquire Immunodeficiency Syndrome (AIDS) in the enrolled patients before starting the therapy.

HCV RNA detection, genotyping and quantification

HCV RNA detection and quantification was performed on Real-time PCR. Firstly the HCV RNA was extracted by column based, Roboscreen RNA isolation kit (Instant virus RNA kit: AJ ROBOSCREEN, Germany). For the amplification of HCV RNA, commercially available HCV RNA Real-time amplification kit (Robogene® HCV RNA qualitative and quantification kit: AJ ROBOSCREEN, Germany) was used that had <100 IU/ml or <200 copies/ml detection limit. For the identification of HCV genotypes a multiplex PCR was established using type specific primers and protocol of previous study reported by Ohno and coworkers in 199714.

Viral kinetics

To compare the viral loads, measured at different stages of therapy with treatment response, HCV RNA quantification was done at day-0 (baseline viral load or BVL), week-4 (rapid virological response or RVR), in the middle of therapy (early virological response or EVR) and at the end of therapy (end of therapy response or ETR).

Treatment

On the basis of HCV genotypes, the patients included in this study were categorized into two groups (G1 and G2). The patients infected with HCV genotype 2 and/or 3 were placed in G1 and those infected with genotype 1 and/or 4 were members of G2. The treatment duration was 24 weeks for G1 patients and 48 weeks for G2 patients. All the patients were treated with IFNα-2b+RBV combination therapy under the supervision of an expert gastroenterologist in the hospital. IFNα-2b was given at a dosage of 3MU (Injected), thrice a week and RBV 1000-1200 mg/day (Orally) according to the patient's body weight. To see the late response in non-responders, the duration of therapy was extended (12 weeks in G1 patients and 24 weeks in G2 patients).

STATISTICAL ANALYSIS

Simple descriptive statistics were used to evaluate the individual characteristic distribution. To compare the overall distribution of response and its association with viral kinetics at different stages of therapy, Chi-Square test was used. SPSS 11 software was applied for statistical analysis. The $p<0.05$ was taken as statistical significance.

RESULTS

Out of 430 treated patients 299 (69.53%) showed no HCV RNA at the end of therapy. The patient with normal Alanine aminotransferase (ALT) and undetectable HCV RNA at end point of therapy were defined as end of therapy responders (ETR). 243 (81.27%) out of 299 ETR could sustain their response twelve months after the treatment completion and were declared as sustained virological responders (SVR). Patients of G1 showed 74.05% ETR and 85.74% SVR, while the ETR and SVR of G2 patients was 41.67% and 32% respectively (Table-I).

During the treatment, viral load was measured at different time points in all the patients of both groups (G1 and G2). To see the impact of baseline viral load (BVL) on ETR and SVR of the patient, HCV viral load was analyzed at day-0. For the analysis of rapid and early virological response (RVR and EVR) the viral kinetics were examined at week-4 and week-12 in G1 patients and at week-4 and week-24 in G2 patients respectively, according to their treatment duration (24 weeks in G1 patients and 48 weeks in G2) (Table-II & III).

The patients of G1 who had BVL less than two million international units per milliliter (<2MIU/mL), revealed a significantly higher response (ETR=84% and SVR=93.76%) as compared to those who had BVL more than or equal to two million international units per mL (≥2MIU/mL) (ETR=64.53% and SVR=83.38%) (p=0.0001) as illustrated in table-2. In G2, the patients with viral load <2MIU/mL at day-0, showed 50.15% ETR and 43.28% SVR. The patients of G2 with ≥2MIU/mL viral load at day-0, showed 34.45% ETR and 23.38% SVR (Table-III). All the patients (either from G1 or from G2) who were able to reduce the viral load more than or equal to two logs at week-4, revealed a better ETR and SVR (80.76% and 92.22% in G1; 53.92% and 49.56% in G2 patients) as compared to those who could decrease the HCV viral load less than two logs from their bodies at that time (68.92% and 78.56% in G1; 30.68% and 17.10% in G2 patients) (Table-II & III).

Patients characteristics	Mean ± SD	Median	Range
Age(years)	36±6	35.5	10-60
Weight (Kg)	65±11.5	68	35-102
Hb (g/dl)	12.5±2.4	12.8	10-17
Platelet count(mm^3)	178566±54156	175650	98000-355000
Viral response (n=430)			
ETR	274/370 (74.05%) in G1 patients 25/60 (41.67%) in G2 patients **(ETR of G1+G2= 69.53%)**		
SVR (out of ETR)	235/274 (85.76%) in G1 patients 8/25 (32%) in G2 patients **(SVR of G1+G2= 81.27%)**		

Table-I. Pre-treatment patient's characteristics and viral response

The ETR and SVR were significantly higher in those patients who had no HCV RNA in their blood in the middle of therapy point (95.20% and 96.24% in G1; 54.20% and 49.54% in G2 patients) than those who still had HCV RNA in their blood. The G1 patients with less than 0.2 MIU/mL viral load in the middle of therapy demonstrated comparatively better ETR and SVR than those who had more than or equal to 0.2 MIU/mL viral load at that time (ETR=45.32%, SVR=65.76% vs. ETR=7.1%, SVR=40.44%). Same conditions were noted in the response of G2 patients at this time point (ETR=20.75% and SVR=20% vs. ETR=9.65% and SVR=14.24%) (Table-II & III) (p=0.0004).

End of therapy non responders (HCV RNA positive at the end of therapy duration) were categorized in two groups on the bases of HCV viral load at that stage. The patients with ≥0.05 MIU/mL were included in first category and the patients with <0.05 MIU/mL in second category (Table II & III).

With therapy extension, the patients with viral load less than 0.05 MIU/mL, exhibited better response than those who had more than 0.05 MIU/mL at the end of therapy point (p=0.0003). With the extension of 12 weeks therapy, a total of 60.5% ETR non responders of G1, who had >0.05 MIU/mL viral load were able to eradicate the virus from their body. Out of these 90.43% also succeeded to sustain their response after the 12 months of therapy termination. On the other hand with therapy extension the ETR was seen only in 10% patients who had more than or equal to 0.05 MIU/mL at that stage and out of those 70.55% showed SVR (Table-II).

When the treatment duration was extended from 48 weeks to 72 weeks for the end of therapy non-responders of G2 patients who had less than 0.05 MIU/mL viral loads, a total of 40.23% became HCV RNA negative and out of those 43.42% could sustain their response. On the other hand, who had viral load more than or equal to 0.05 MIU/mL at the end of therapy, only 5% were able to eliminate the virus from their body and out of those 30.32% showed SVR (Table-III).

Time Point	Viral load		ETR	SVR (Out of ETR)
Day- 0 (BVL)	≥ 2 MIU/mL		64.53%	83.38%
	< 2 MIU/mL		84%	93.76%
Week-4 (RVR)	Dropped by ≥2 log		80.76%	92.22%
	Dropped by <2 log		68.92%	78.56%
Week-12 (EVR)	Negative <100 IU/mL		95.20%	96.24%
	Positive	≥0.2 MIU/mL	7.1%	40.44%
		<0.2 MIU/mL	45.32%	65.76%
Week-24 (ETR)	Negative<100 IU/mL		100%	89.23%
	Positive ≥100 IU/mL		00%	00%
12 weeks therapy extension (24-36 weeks)	≥0.05 MIU/mL (at week-24)		10%	70.55%
	<0.05 MIU/mL(at week-24)		60.5%	90.43%

Table-II. HCV viral load detection at different time points of treatment in G1 patients

Time Point	Viral load		ETR	SVR (Out of ETR)
Day- 0 (BVL)	≥2 MIU/mL		34.45%	23.38%
	<2 MIU/mL		50.15%	43.28%
Week-4 (RVR)	Dropped by ≥2 log		53.92%	49.56%
	Dropped by <2 log		30.68%	17.10%
Week-24 (EVR)	Negative <100 IU/mL		54.20%	49.54%
	Positive	≥0.2 MIU/mL	9.65%	14.24%
		<0.2 MIU/mL	20.75%	20%
Week-48 (ETR)	Negative<100 IU/mL		100%	33.33%
	Positive ≥100 IU/mL		0.00 %	0.00%
24 weeks therapy extension (48-72 weeks)	≥0.05 MIU/mL		5%	30.32%
	<0.05 MIU/mL		40.23%	43.42%

Table-III. HCV viral load detection at different time points of treatment in G2 patients

DISCUSSION

From the day of first Interferon based treatment in HCV infected patients; the early virological response prediction remains a burning issue both for the clinicians and the patient. Different virological (i.e HCV genotyping, baseline viral load) and host related factors (i.e Insoline resistance, obesity etc) have always been considered the predictors of the long lasting virological response of the Hepatitis C patients[5,6,15,16]. The measurement of viral load fluctuation at early stages of therapy is also considered a good predictor of therapy

response these days in HCV infected patient[10, 17], which is not addressed properly in Pakistan.

This study was started with the aim to evaluate the association of viral load fluctuation at different stages of therapy with sustained virological response of the patients infected with different types of HCV in our population. Many researchers who worked on different HCV genotypes and different combination of treatments also believed that the analysis of viral kinetics could clinically be a constructive to predict the treatment response at the initial stages of therapy[10,17,18]. In this study, the viral load was measured at four different time points (Day-0, Week-4, in the middle and at the end of therapy) to compare the effect of viral kinetics with ETR and SVR at these time points (Table-II and III).

All the patients either from G1 or G2 who had baseline viral load (BVL) <2 MIU/mL at day zero presented better ETR and SVR as compared to those who had more than or equal to 2 MIU/mL baseline viral load (Table-II and III). It indicates that the Interferon therapy response in Hepatitis C patients can be predicted at the start of therapy with the measurement of baseline viral load (BVL). Although these results were in harmony with previous results, however their findings were based on different HCV genotypes and different therapy combinations[11,16].

The patients who dropped the quantity of HCV RNA more than or equal to two logs at week-4 presented better ETR and SVR than those who could decrease the viral load less than two logs at that time point and were referred as rapid virological responders (RVR) (Table-2, 3). It indicates that rapid viral response at week-4 has the association with ETR or SVR. It is important to note here that the HCV RNA quantity can increase again to detectable level in late therapy period as seen in few cases of this study. This reappearance of previously undetectable HCV RNA in the patients during the treatment is generally regarded as “breakthrough”[19, 20].

Previously, the viral load detection at 12th week of Interferon therapy in the patients infected either with HCV genotype 2 and/or 3 or 1and/or 4 had been used as an early virological response (EVR) predictor to decide the continuation or termination of therapy at that stage[21]. In the present study, to see the early virological response, viral load was analyzed at 12th week of therapy in the patients infected with HCV genotype 2 and/or 3 and at 24th week in those infected with genotype 1and/or 4 according to their treatment duration (24 weeks in the patients infected with genotype 2 and/or 3 and 48 weeks in the patients infected with genotype 1 and/or 4 infected patients).

Most of the patients who had no HCV RNA in their blood in the middle of therapy and had normal ALT, showed better ETR than those who showed HCV RNA at that stage. Most of these patients were also able to sustain their response after the termination of therapy. But it was also interesting to see that few cases which were negative in the middle of therapy again showed HCV RNA in their blood during the late therapy period and were defied as relapsed cases. This clearly indicates that, although the patient becomes HCV RNA negative in the middle of therapy or shows early virological response even though, the chances of reappearance of the virus could not be ruled out. It strengthened the fact that the standard therapy period in Hepatitis C patients should be completed in any case[6, 22].

Benefits of therapy extension had also been described in previous studies where significantly higher rates of sustained response were noted with prolonged therapy as compared to the standard therapy duration[23]. In some other studies the results of therapy extension were inharmonious[24,25], while in contrast, others did not agree the benefits of prolonged therapy in end of therapy non responders[26]. However, in all those previous studies the therapy was prolonged in all the patients either they were HCV RNA negative or positive at the end of therapy. In this way they were unable to categories the true candidates for therapy extension. In the present study the therapy duration was extended only in those patients who were HCV RNA positive at the end

of therapy. These non responding patients were divided in two groups according to their viral load at that stage. In first group those patients were included who had ≥0.05 MIU/mL viral load while the viral load of second group patients was <0.05 MIU/mL. The extension of treatment duration was found useful in those patients who had viral load <0.05 MIU/mL at the end of therapy time (Table-II and III). It reveals that the patients with viral load less than 0.05MIU/mL might be taken under consideration for therapy extension.

CONCLUSIONS

The viral load measurement at different time points of therapy (Day-0, Week-4, Week-12 or 24) could be clinically constructive to predict the treatment response at the earliest stages of the therapy to decide the treatment continuation or termination. Additionally the extension of therapy duration could be useful in the patients with low viral load (<0.05MIU/mL) at the end of standard therapy period especially those infected with HCV genotype 2 and 3.

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"Have patience
All things are difficult
before they become easy."

Saadi

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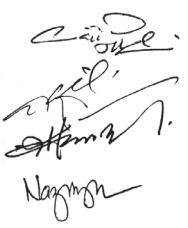
1	Dr. Sajjad Iqbal	Main researcher and corresponding author	
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