

# A randomized trial of a 4- vs 12-week daily interferon dose regimen combined with ribavirin in treatment of patients with chronic hepatitis C

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**BACKGROUND:** Standard combination-therapy of ribavirin with alternate day interferon (IFN) in patients with chronic hepatitis C (CHC) has been reported to achieve 30%–55% sustained viral response. Early reduction of viral load by daily dosage of IFN could enhance viral clearance. However, the duration of daily dosage protocol and the likely side-effects have not been well studied. We compared the efficacy and safety of a 4- vs 12-week daily IFN dosage in patients with CHC.

**METHODS:** Fifty-nine, histologically proven CHC patients having ALT levels  $>1.5 \times \text{ULN}$  were divided randomly into 2 groups, group I was given IFN 3 MIU daily for 4 weeks, followed by tiw up to 12 months and group II was given IFN 3 MIU daily for 12 weeks, followed by tiw up to 12 months. Ribavirin was given in a dose of 800–1200 mg/d for 12 months.

**RESULTS:** Fifty-two of the 59 patients (group I = 28; group II = 24) completed the study. The pretreatment variables and the prevalence of HCV genotype 1 were comparable between the groups. Nine patients (29%) in group I and 6 (25%) in group II had stage 3, 4 fibrosis. At the end of 4, 12, 24 and 52 weeks, HCV RNA negativity was observed in 27%, 54%, 65% and 71% in group I and 38%, 54%, 71% and 75% in group II, respectively ( $P = \text{ns}$ ). Four of the eight (50%) patients with genotype 1 and 30 (69.8%) of 43 patients with genotype non-1 responded to therapy ( $P = \text{ns}$ ). Sustained viral response was achieved in 61% and 71% in groups I and II, respectively. None of the variables predicted non-response precisely. No serious adverse effects were observed and they were comparable between the two groups.

**CONCLUSION:** Daily IFN dosage with ribavirin is safe and can achieve response in up to 65% patients. Since the efficacy of a 4-week daily dosage of IFN is comparable to a 12-week schedule, we recommend the former regimen.

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**KEY WORDS:** hepatitis C virus; hepatitis C; interferon; ribavirin; induction dosage; sustained viral response; chronic hepatitis; combination therapy

## Introduction

The standard treatment using interferon 3 MIU, tiw, in combination with ribavirin can achieve sustained viral response (SVR) in 38%–43% of patients.<sup>[1,2]</sup> Mathematical models developed to study HCV kinetics indicate that initial high dose of interferon (IFN) can rapidly reduce viral load and improve efficacy.<sup>[3]</sup> However, therapy with high doses of IFN is associated with more side-effects and treatment suspensions. In patients receiving standard treatment three times weekly, an intermittent increase in viral load has been observed on treatment free days. There is evidence to suggest that daily IFN dose therapy improves the efficacy as compared with a three times a week regimen.<sup>[4]</sup> Similarly, pegylated IFNs have been used to treat chronic hepatitis C. When they are used in combination with ribavirin, SVR may be achieved in 46%–54% of the patients.<sup>[5]</sup> But it is not significantly different from the standard IFN combination regimen specially in patients with genotype 2 and 3. Moreover, the daily IFN dose regimen as well as pegylated IFN therapy have higher risks of neutropenia and cytopenia and greater cost.<sup>[5]</sup> Although attempts have been made to overcome these difficulties by decreasing the dose of pegylated IFN from 1.5 to 0.5  $\mu\text{g}/\text{kg}$  per week, there is no difference in the side-effects and the cost still remains higher than the tiw interferon regimen. Hence, an urgent need exists to

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determine the duration till when daily IFN dose is needed. Since 90% of the patients who become HCV RNA negative at 4 weeks may achieve sustained viral response, it would be worthwhile to give daily IFN therapy for at least 4 weeks. In the study by McHutchison et al, however, 59% of the patients who were treated with combination regimen for 48 weeks remained HCV RNA positive until week 12 or 24 of the therapy.<sup>[1]</sup> A proportion of these patients did lose HCV RNA with prolonged therapy.

We therefore initiated a randomized, controlled clinical trial in chronic HCV patients to compare the efficacy, safety and tolerability of a 4- vs 12-week daily IFN dose regimen followed by an alternate daily IFN dose in combination with ribavirin for a year in patients with chronic hepatitis C.

## Methods

### Study population

Enrolled were 59 patients with histologically proven chronic HCV infection who had been subjected to antiviral therapy during January 1998 to April 2001. The inclusion criteria were chronic hepatitis confirmed by liver biopsy, raised alanine aminotransferase (ALT) to  $>1.5 \times$  upper limit normal (ULN) and HCV RNA positive on two occasions at baseline. Patients were excluded if they had hepatitis B virus (HBV) or HIV infection, history of heavy alcohol consumption ( $>80$  g/d for  $>5$  y), positivity for antinuclear or antismooth muscle antibody (in 1:80 dilution), autoimmune liver disease, thyroid disease, associated hepatocellular carcinoma, decompensated liver disease, pregnancy, lactation, bronchial asthma or a psychiatric disorder. The patients who had received antiviral or immunosuppressive therapy were excluded. The study protocol conformed to the ethical guidelines of the Helsinki Declaration and was approved by the Institutional Ethical Committee.

A detailed history and clinical examination was done in every patient according to a predefined protocol. Hematological and biochemical tests were done by the central laboratory using an automated analyzer. Ultrasonography of the upper abdomen and upper gastrointestinal endoscopy were carried out in every patient.

### Serological and virological assays

Antibodies to HCV (anti-HCV) were tested by the third generation enzyme-linked immunosorbent assay. HCV RNA was detected by an in-house nested reverse transcription polymerase chain reaction (RT-PCR) using conserved primers in the 5'-noncoding region of the viral genome.<sup>[13]</sup> The lower limit of detection of the RNA was up to 600 viral copies/ml.

HCV viral load was determined by a commercial assay (Roche-Amplicor, Monitor assay). HCV genotyping

was performed with the reverse hybridization line probe assay (LIPA; Innogenetics, Ghent, Belgium).<sup>[14]</sup>

### Histological assessment

Liver biopsies were evaluated both for the stage of fibrosis and the degree of necroinflammatory activity according to Knodell et al.<sup>[6]</sup> All pre- and post-therapy liver biopsies were evaluated by two pathologists who were unaware of the treatment regimen and the response to therapy. Sections were routinely stained with hematoxylin-eosin, reticulin and orcein-stains.

### Study design

The patients who fulfilled the inclusion criteria were randomly assigned using a table of random numbers to either of the two groups:

#### Group I

The patients received interferon alfa 2b (Viraferon, Fulford India Ltd., Bombay, India), 3 MIU, subcutaneously plus oral ribavirin (800–1200 mg) daily for 4 weeks. This was followed by IFN 3 MIU given every alternate day with ribavirin for another 11 months.

#### Group II

The patients received interferon alfa 2b, 3 MIU subcutaneously plus oral ribavirin (800–1200 mg) daily for 12 weeks. The IFN was then continued on a 3 MIU alternate day dose for another 9 months along with ribavirin.

After completion of 12 months of therapy, the patients were followed for another 6 months to assess the response.

During the treatment, the patients were assessed at the outpatient liver clinic every week for 4 weeks and then at week 4, 8, 12, and subsequently every 4–6 weeks for the duration of the treatment. After completion of the therapy they were seen at week 4, 12, and 24. Tests of liver function and HCV RNA were done before treatment, at serial intervals during the therapy as well as during the follow-up period.

### Assessment of therapeutic efficacy

The response to therapy was assessed at various time intervals.

#### End-of-therapy response (ETR)

This was defined as undetectable HCV RNA and normal ALT level at month 12.

#### Sustained viral response (SVR)

This was defined as undetectable HCV RNA and normal ALT in serum at the end of follow-up, i. e. 6 months after the completion of the therapy. This was considered as the primary end-point of the therapy.

Secondary end-point was the improvement in the

liver histology, which was defined as reduction in the histological activity index (HAI) by at least two points as compared with the score in the pre-treatment biopsy.

### Relapse

It was defined as reappearance of HCV RNA during the 24-week follow-up after being undetectable at the end-of-therapy at month 12.

### Safety evaluation

Safety was monitored by clinical and laboratory evaluations. Data collected on adverse events included the severity, frequency and length of each event, as well as the impact on study treatment and outcome. The severity of adverse effects was graded as mild, moderate or severe or life threatening.<sup>[9]</sup> The therapy was temporarily withheld or modified when severe adverse events developed. The dose of IFN alfa-2b was reduced to 1.5–2 MIU and increased gradually. The dose of ribavirin was adjusted if the hemoglobin level decreased to 9 g/dl, and the drug was discontinued if it dropped below 8.0 g/dl.

### Statistical analysis

The data were carefully analyzed using the statistical package SPSS (version 10.0, SPSS Inc, Chicago). The Mann-Whitney test was used for comparison of quantitative variables between the groups, Wilcoxon's signed-rank test for evaluation of changes of variables within the same group, and the corrected  $\chi^2$  or two-tailed Fisher's exact test for the qualitative data. In all analyses, a 2-tailed *P* value less than 0.05 was considered statistically significant.

## Results

### Baseline characteristics of the patients

The study design and the randomization of the patients to the treatment group are shown in Fig. The mode of

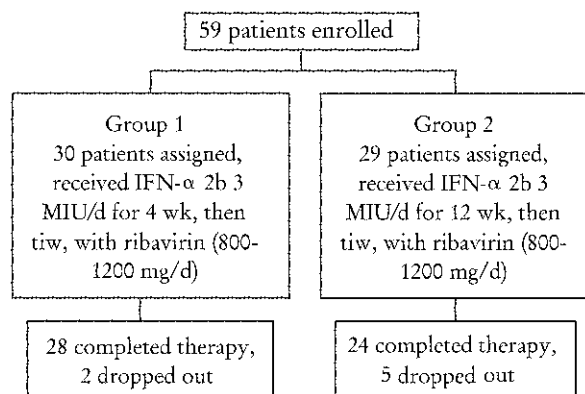


Fig. Study design.

acquisition of HCV infection and the demographic profile of the patients belonging to the two groups are shown in Table 1. Of the 59 patients enrolled, 52 completed the 12-month therapy and the follow-up protocol. The seven patients who did not complete the therapy had taken none ( $n=4$ ) or less than 3 weeks ( $n=3$ ) of treatment and did not come for further medication. The baseline characteristics of the patients in the two groups were comparable. Forty-three of the 51 (84.3%) patients, in whom genotypes were available, had HCV genotypes 2 or 3. In one patient, the genotype was not determined.

### Biochemical and virological response

The primary end-points were carefully assessed in individual patients at several points during the course of therapy and during the follow-up (Table 1). While there was an initial slightly higher biochemical response than the virological response in group II patients, the difference was not significant (Table 2). Subsequently, the biochemical and virological response correlated in the patients during and at the end of the therapy. An initial virologic response with undetectable serum HCV RNA at week 4 of the therapy was seen in 27% patients in group I and 38% patients in group II ( $P=ns$ ). At week 24, the virologic response rates were 65% and 71% respectively (Table 2). The end-of-treatment response, i. e. HCV RNA undetectable at the end of the therapy was seen in 20 patients (71%) in group I and 18 patients (75%) in group II ( $P=ns$ ). The primary end-point, a sustained virologic response at the end of 24 week after cessation of the therapy was achieved in 17 (61%) and

Table 1. Baseline demographic and disease characteristics

Parameter	Group I ( $n=28$ )	Group II ( $n=24$ )
Age (y, mean $\pm$ SD)	39 $\pm$ 14	38 $\pm$ 14.2
Male/Female	23:5	14:10
ALT (IU/L)	131 $\pm$ 91	125 $\pm$ 111
S. Albumin (g/dl)	4.3 $\pm$ 0.9	4 $\pm$ 0.4
HCV genotype		
1	5 (23%)	3 (14%)
Non-1	23 (77%)	20 (86%)*
HCV RNA (genome copies/ml)	14138 $\pm$ 4406	13082 $\pm$ 5644
Liver biopsy		
HAI	6.6 $\pm$ 3.3	6.6 $\pm$ 2.3
Fibrosis	2 $\pm$ 1.4	2 $\pm$ 1.5
Mode of acquisition of HCV		
Blood transfusion	8	10
Needle stick injury	11	7
Intravenous drug use	3	2
Medical procedure	2	3
Unclear	4	2

\*: In one patient, the genotype was not determined. There was no significant difference between the two groups.

**Table 2.** Biochemical and virological response to therapy

Parameters and time	Group I number (%), n=28	Group II number (%), n=24	P value
ALT (normalization)			
wk 4	7 (25)	14 (59)	ns
wk 12	13 (46)	14 (59)	ns
wk 24	15 (54)	19 (63)	ns
wk 52	20 (71)	14 (59)	ns
wk 76	20 (65)	16 (6)	ns
HCV RNA negative			
wk 4	7 (27)	9 (38)	ns
wk 12	14 (54)	13 (54)	ns
wk 24	17 (65)	17 (71)	ns
wk 52	20 (71)	18 (75)	ns
wk 76	17 (61)	17 (71)	ns

**Table 3.** Comparison of predictors of virological response

	Responders	Non-responders	P value
Age	39±14	35.6±12.2	ns
Sex (M:F)	21:12	14:4	ns
Genotype			
1	4	4	ns
Non-1	26	11	ns
HAI			
Pre-therapy	6.1±2.6	9.6±3	<0.01
Fibrosis			
Pre-therapy	1.8±1.2	3.2±0.99	<0.01
ALT (IU/L)			
Baseline	131±91	125±111	ns
4-week	119±82	135±80	ns
HCV viral load (genome copies/ml)	13481±5115	15128±8070	ns

17 patients (71%), respectively. No significant difference was observed between the two treatment regimens.

When the virologic response was considered together with the biochemical response to treatment (combined response), the proportion of subjects with undetectable serum HCV RNA and normal ALT values were comparable between the two groups at the end of the treatment and the end of the follow-up.

### Histological response

Pre- and post-treatment liver biopsies were analyzed in a blind manner. Overall improvement in mean inflammation score as well as fibrosis score was observed in both groups (Table 3). The reduction in HAI and fibrosis score in the two groups was comparable.

### Factors associated with SVR

Of the 52 patients, 34 (65%) responded to the antiviral therapy in the two groups.

Subgroup analysis of ETR and SVR was done for

different variables. There was no significant difference in the mean age, gender and baseline ALT levels between responders and non-responders (Table 3). In group I, 5 patients had genotype 1 and 23 had genotype non-1 whereas in group II, 3 patients had genotype 1 and 20 had genotype non-1. In one patient, the genotype was not determined. Subjects with HCV genotype non-1 had a better sustained response rate in both the groups. Four of the eight patients (50%) with genotype 1 and 30 of 43 patients (69.8%) with genotype non-1 had responded to the therapy. The difference was however not significant. The time at which HCV RNA was first negative was analyzed in relation to subsequent development of sustained virologic response. This was conducted to assess the short- and long-term sustainability of the response to the treatment. In each treatment group, the likelihood of SVR was higher in subjects whose first negative HCV RNA occurred at the end of 4 weeks of treatment. At week 4, six patients in group I and 4 patients in group II displayed HCV RNA negativity and HCV RNA remained negative throughout the therapy. At week 12, four patients in group I and 3 patients in group II were additionally HCV RNA negative shown by PCR. The ETR was achieved in 38 of the 52 patients (73%). The rate of relapse was low in our study: 3 (11.5%) patients in group I and 1 (4%) in group II ( $P=ns$ ), who were HCV RNA negative at week 52 after cessation of the therapy. Overall, after completion of the therapy, 34 of the 52 patients were HCV RNA negative. This accounted for an overall 65% rate of response.

The most important predictors of non-response were the HAI and hepatic fibrosis score. The responders had a lower HAI ( $P<0.01$ ) and fibrosis score ( $P<0.01$ ) than did the non-responders (Table 3). There was no significant difference in the baseline HCV RNA levels between the responders and non-responders. The HCV RNA levels between patients with genotypes 1 and non-1 were also comparable.

A higher response was noted in patients with genotypes 2 and 3 than in those with genotype 1. However, the difference was not significant, possibly due to a smaller number of patients. At six months, 65% of the patients in group I and 71% in group II responded to the treatment. It was not significantly different from the response rates at month 12.

### Safety evaluation

With respect to safety and tolerability, the incidence and severity of adverse events were similar among the two groups. The total leukocyte counts and platelet counts decreased from baseline in the first few weeks of the treatment in both groups. However, they stabilized during the remainder of the treatment and reverted to baseline levels upon cessation of the treatment. In 4 (14%) and 5 patients (21%), the IFN therapy had to be

temporarily withheld for 2 days to 2 weeks. In none of the patients, the therapy had to be discontinued or had any serious adverse effects. The patients in whom the total leucocyte count decreased to  $<3000 \text{ mm}^3$ ,  $1 \mu\text{g}/\text{kg}$  of granulocyte-macrophage colony stimulating factor was given subcutaneously; it was repeated when required. None of the patients who had taken initial 4 weeks of therapy withdrew from the treatment protocol. The mean Hb values were reduced in the two groups of patients to the same extent. Except for 3 patients in whom ribavirin had to be discontinued for 2–4 weeks, dose modification acid iron and folic and supplementation allowed uninterrupted therapy. None of the patients had symptoms due to anemia or required blood transfusion.

## Discussion

The results of this randomized controlled trial indicate that initial daily dose with IFN in combination with ribavirin achieves high SVR rates. ETR could be achieved in 73% of the patients, while SVR could be achieved in 65% of the patients. It was 71% in the group which received daily IFN for 12 weeks and 65% in the group which received daily IFN for 4 weeks. The difference between the two groups was not significant. The results of this study clearly suggest that since a 4- or 12-week course of daily IFN dose has comparable efficacy, the former regimen could be adopted specially for cost consideration.

Among the several factors associated with sustained clearance of HCV infection, HCV genotype has been reported to be relevant in almost all therapeutic trials; genotype 1 is more resistant than others to antiviral therapies. Recent reports<sup>[19,20]</sup> showed that monotherapy with peg-IFN  $\alpha$ -2b or  $\alpha$ -2a has led to sustained virologic clearance varying from 10% to 28% respectively in patients with genotype 1, indicating that monotherapy with peg-IFN might not be optimal for the treatment of patients with genotype 1. In therapy combined peg-IFN  $\alpha$ -2b with ribavirin, a dose dependent effect on the rate of sustained virologic response was evident for genotype 1: 42% vs 32% by using peg-IFN  $\alpha$ -2b high or low dose, respectively. In two randomized trials, a high daily dose of IFN  $\alpha$ -2b (at a dose of 5 MIU daily) showed no better benefit than 3 MIU tiw.<sup>[19,20]</sup> In the induction therapy group, the HCV RNA levels decreased more rapidly to lower levels, but the rate of sustained viral response was comparable among the groups since many of the patients on the daily high dose relapsed after stopping the therapy. Combined IFN induction (10 MIU or 5 MIU daily) with ribavirin did not show any significant difference in the rate of ETR or SVR as compared with standard and combination therapy.<sup>[21]</sup>

Unlike the west, in the Indian continent, genotypes 2 and 3 are more common than genotype 1. The

present study confirms the previous observations that 43 of 51 patients with chronic HCV (84%) were infected with genotype non-1. We could therefore achieve an overall response rate of 65% in the two treatment groups, higher than that reported recently.<sup>[10–12,19]</sup> Patients with genotypes 2 and 3 responded better than genotype 1 (70% vs 50%,  $P = \text{ns}$ ). The response rates in the 4- vs 12-week daily IFN therapy was not different in the genotype 1 patients, mainly because of the limited number of genotype 1 patients available in our study.

There was no significant difference in the mean HCV RNA load between patients in the two groups. Furthermore, the response rate between the patients with high and low viral loads was comparable because most of our patients had genotype non-1 and there was a smaller number of patients.

Fried et al have found that in patients with genotypes 2 and 3, the response rates in patients with high or low viral load are not significantly different.<sup>[5]</sup> Their observations remain questionable whether HCV genotyping is more relevant than HCV viral load. In Western countries and Japan, where genotype 1 is predominant, it would be prudent to do a pretreatment genotype analysis to rationalize the duration of therapy. We believe that this approach is also justified in the Indian continent because about 70%–80% of the patients could be of non-1 genotype. In the southern India, equal frequency of genotype 1 and non-1 has been reported.<sup>[22,23]</sup> Again, HCV genotyping for patients in this region would therefore be justified.

Whether the combination therapy should be given for 6 or 12 months remains debatable. It was reported that in patients with genotypes 2 and 3, a 6-month regimen could be as effective as a 12-month one.<sup>[22]</sup> The two largest trials on the combination therapy with 3 MIU of IFN plus ribavirin, both given for 6 or 12 months, have indicated that patients with genotype non-1 may be treated effectively with a 6-month regimen, whereas responded patients with genotype 1 would need to prolong the therapy to 12 months.<sup>[1,2]</sup> Our study in which 84% patients had genotypes 2 and 3 supports this concept. Sixty-five and 71% patients in groups I and II respectively had response at 6 months. This is almost similar to what we observed at 52 weeks. A recent report<sup>[16]</sup> indicated that a high dose of IFN  $\alpha$ -2b (6 MIU tiw) plus ribavirin given for 12 months induces a significantly higher SVR.<sup>[16]</sup> However, this study included the patients who had relapsed after the initial treatment. In another report, even in relapsed patients with genotypes 2 and 3, a 6-month course of combination therapy has been advocated.<sup>[15]</sup> Rationalizing a 6-month therapy would avoid side-effects and unnecessary costs.<sup>[24]</sup> In the present study the patients were treated for 48 weeks regardless of HCV genotyping. With regard to the benefits of shorter treatment period, we do support a

6-month course of combination therapy for genotypes 2 and 3 patients.

The need for pre-treatment liver biopsy is controversial.<sup>[18]</sup> Like other reports<sup>[7,8]</sup> we support the concept that pre-treatment liver biopsy helps in predicting the outcome of antiviral therapy. The most important predictors of non-response in our study were high fibrosis score and histological activity index. Unfortunately, there are no studies tailored for liver histology to define the dose and duration of therapy. It is conceivable that if therapeutic trials are designed according to liver histology instead of viral load and genotypes, the overall outcome of therapy in unselected patients might be better.

ALT levels did not help accurately predict the outcome of antiviral therapy in the present study. No significant difference was seen in the pretreatment ALT levels in responders and non-responders as observed by others.<sup>[18]</sup> It was reported that the patients who became HCV RNA negative at week 4 in either group achieved SVR at the end. These patients, however, constituted only a small proportion of the total cohort of patients. In our study, it was at week 12 or 24 that a significant proportion of patients were first detected to be HCV RNA negative. Since we tested the blood for RNA at week 12 and 24 and not in-between, some patients were likely to become RNA negative soon after week 12. Based on our findings, we recommend that the initial decision for continuation of antiviral therapy should not be based on week 4 assessment. This would prevent denial of therapy to potential responders. Our recommendation is supported by Fried et al's study, in which 97% of subjects, who showed no response at week 12, did not have SVR at last. They suggested that the incremental benefit of continuing therapy beyond 12 weeks for patients without early virological response must be considered for each individual patient. In our series, 79% of responders had response at 12 weeks, while those who became HCV RNA negative at 24 weeks were HCV RNA negative and showed SVR. We therefore propose that it is justified to give therapy for at least 24 weeks to patients with chronic hepatitis C and the initial response should be assessed at week 12.

In summary, our results support the concept of a short daily IFN dose with ribavirin in patients with chronic hepatitis C. This regimen is safe, relatively low cost and effective.

### Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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