Treatment of Chronic Hepatitis due to Hepatitis C Virus (CH-C) in India: A Randomized Controlled Trial Comparing Daily Interferon-alfa-2b and Ribavirin with Daily Interferon-alfa-2b and Glycyrrhizin—A Multicenter Study

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Background and Aim: **Pegylated-interferon-alfa (PEG-IFN-α) with ribavirin is an established treatment in chronic hepatitis due to hepatitis C virus (HCV) (CH-C). Such treatment is expensive and in resource-poor countries such as India, alternative less expensive therapy is needed.** ***Methods:*** **Multicenter randomized controlled trial comparing two treatment regimens (interferon-alfa-2b [IFN-α-2b] 3 million unit/day [MU/day] and ribavirin 1000 mg/day [I+R] vs IFN-α-2b 3 MU/day and glycyrrhizin 250 mg [I+G]) in CH-C. Viral, host characteristics and therapeutic responses were assessed (ICMR—6 months trial for chronic hepatitis—CTRI/2008/091/000105).** ***Results:*** **One hundred and thirty-one patients meeting the inclusion criteria were randomized to I+G ($n=64$) or I+R ($n=67$) during the period February 2002 to May 2005. About 85% (I+G=53, I+R=58) completed 6 months of treatment and 89% of them (I+G=46, I+R=53) completed 6 months of follow-up after completion of treatment. Hepatitis C virus genotype 3 was the major type detected (71% patients). The mean $\log_{10}$ viral load (copies/mL), histological activity index, and fibrosis stage for all patients were 5.1±0.98, 5±2, and 2±1.5, respectively. Sustained viral response (SVR) was significantly higher in I+R group than in I+G group (65.7% vs 46.9%, OR=2.2, $P=0.03$). Treatment with I+G was associated with significantly lower frequencies of leukopenia (2% vs 17%, $P<0.01$) and anemia (8% vs 40%, $P<0.001$) as compared to treatment with I+R.** ***Conclusion:*** **Genotype 3 HCV infection with low viral load is prevalent in India. Daily IFN with ribavirin showed significantly better responses. Leukopenia and anemia were significantly more in ribavirin group. Responses observed with IFN+ribavirin were similar to the reported response rates with PEG-IFN suggesting that this modality may be considered as a cheaper alternative of treatment for chronic hepatitis C. (J Clin Exp Hepatol 2012;2:10–18)**

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Abbreviations: ALT: alanine aminotransferase; CHC: chronic hepatitis C; CLD: chronic liver disease; ELISA: enzyme-linked immunosorbent assay; ETVR: end of treatment viral response; HAI: histological activity index; HCV: hepatitis C virus; HR: histological response; PEG-IFN: pegylated-interferon; RVR: rapid viral response; SVR: sustained viral response
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Hepatitis C virus (HCV) is a known cause of chronic hepatitis, cirrhosis, and hepatocellular cancer.[1] In India, about 15–25% of patients with chronic liver disease (CLD) are due to HCV.[2,3] Hepatitis C virus is predominantly transmitted parenterally and donor screening for HCV has reduced transfusion-related transmission of HCV considerably.[4] However, regular donor screening for anti-HCV to exclude HCV-infected transfusion has started in India only during 2002. Therefore, the magnitude of HCV-associated CLD is likely to increase before the effect of donor screening starts showing. Till date, information on the prevalence of HCV genotypes, HCV disease characteristics, and therapeutic response has emanated from various single center reports in India.[2,3,5–10]

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Globally, pegylated-interferon (PEG-IFN) with ribavirin is the recommended therapy for chronic hepatitis C (CHC) infections.[11] Most of the reports on the efficacy of this therapy are from the Western literature, where HCV genotype 1 is more prevalent. However, this expensive therapy has limited success rates and also considerable side effects that can reduce the patient compliance to therapy.[11]

A symposium conducted by the Indian Council of Medical Research at New Delhi and reports from single center studies indicate that genotype 3 HCV is the prevalent genotype in most parts of India[3,6,7] and one Indian report indicates that treatment using conventional IFN-α-2b (3 million unit/day) and ribavirin (1000 mg/day) for 6 months was associated with 90% sustained viral response (SVR).[6] The cost of such therapy is almost half of PEG-IFN and ribavirin.[11] With a prevalence of about 1%, India has a large number of HCV population.[3] Treatment involving PEG-IFN is not affordable by about two-thirds of the treatable patients in India (experience of experts involved in this study). Further, state funding or insurance cover for such treatment is nonexistent in India, and hence it is necessary to establish appropriate schedule of therapy which is affordable by a large segment of treatable patients with chronic hepatitis due to HCV.

A plant product glycyrrhizin derived from roots of *Glycyrrhiza glabra* has been reported to induce endogenous IFN.[12] It is also documented to have hepatocyte protective effect against various hepatotoxic injuries, both in humans and in vitro.[13–16] Clinical trials have shown that glycyrrhizin therapy is associated with normalization or decrease in alanine transferase (ALT) associated with histological improvement in HCV-induced CLD.[17–19] Glycyrrhizin has also been documented to inhibit replication of RNA viruses, through an unknown mechanism[20] and is reported to be safe in humans with negligible side effects. Besides, the cost of glycyrrhizin is about one-third of ribavirin's cost. Accordingly, IFN and glycyrrhizin combinations in the treatment CH-C may be associated with fewer side effects than IFN and ribavirin combinations.

Therefore, this multicenter study was designed to evaluate therapeutic efficacy of two cheaper treatment regimens, namely, regular IFN-α-2b and ribavirin with IFN-α-2b and glycyrrhizin.

PATIENTS AND METHODS

Framework of Multicentric Study (Trial Registration—CTRI/2008/091/000105—Registered in Clinical Trial Registry of India)

Nine tertiary care medical centers located in various regions of the country participated in the study. The details of these nine centers are All India Institute of Medical Sciences (AIIMS), Maulana Azad Medical College (MAMC), Gobind Ballabh Pant Hospital (GBPH), and Metro Centre for Liver and Digestive Diseases (MCLDD) all located in Delhi; Postgraduate Institute of Medical Education and Research (PGIMER) in Chandigarh and Sanjay Gandhi Postgraduate Institute of Medical Education and Research (SGPGI) in Lucknow, all from northern India; Institute of Postgraduate Medical Education and Research (IPGMER), Kolkata, in eastern India; Bombay Hospital and Medical Research Centre (BHMRC), Mumbai, in western India, and Deccan College of Medical Sciences and Allied Hospital (DCMSH), Hyderabad, in southern India.

Each participating center obtained ethical clearance from the respective Institute's Ethics Committee. The individual patient's data, from inclusion to last follow-up, were collated and scrutinized continuously at the National Institute of Epidemiology (NIE), Chennai. All virological studies which included genotyping and viral load estimation were performed at one center using the same technology at the National Institute of Virology, Pune. The liver histology in each patient was initially evaluated at each center and subsequently re-evaluated by a single experienced pathologist at the AIIMS, New Delhi. The analysis of the data was carried out at the AIIMS. Each center used similar methodology and therapeutic schedule. Randomization to individual treatment arm for each patient at each center was performed centrally at the NIE, Chennai. Regular communication among various study centers was made through e-mails, telephones, and postal services to monitor the progress of the study. Progress of the study was monitored annually by the task force group of the sponsor, the Indian Council of Medical Research.

Patients

Consecutive patients diagnosed as HCV-induced CLD were screened to include patients who satisfied the inclusion criteria. Chronic liver disease was diagnosed using conventional clinical, biochemical, and histological criteria.[21] The HCV infection was diagnosed if anti-HCV and/or HCV-RNA were detectable in the sera.

Inclusion Criteria

Patients of CLD with detectable HCV-RNA in their sera, with ALT ≥ 60 IU/L (normal 40 IU/L), having histological activity index (HAI) ≥ 3,[21] willing to undergo liver biopsy at inclusion and at the end of follow-up and willing for collection of sera at inclusion, and at 4, 12, 24, and 48 weeks after starting therapy were included in the study.

Exclusion Criteria

Patients having the following criteria were excluded from the study: presence of ascites, large varices or liver histology showing cirrhosis,[21] serum bilirubin > 3 mg/dL, prothrombin time ≥ 6 sec prolonged, presence of hepatocellular cancer, presence of comorbid illnesses like coronary artery disease, pulmonary diseases, chronic renal failure and renal

allograft recipients, depressive illness, thyroid gland disorders, autoimmune disorders, Wilson's disease, co-infection with human immunodeficiency virus (HIV) and/or hepatitis B virus (HBV), multiple transfusion requirement such as in thalassemics and hemophiliacs, intravenous (i.v.) drug abuse, immunosuppressive therapies, use of IFNs or any indigenous drug or any hepatotoxic drug anytime during the previous 6 months, alcohol ingestion >80 g/day for 1 year or more, pregnancy and lactating state.

Patient Evaluation

All included were subjected to a thorough clinical evaluation including assessment of body mass index (BMI),[22] and were subjected to routine hematological, biochemical (serum bilirubin, ALT, alkaline phosphatase, serum total protein, serum albumin, and prothrombin time estimation, blood urea, serum creatinine, fasting blood glucose), alfa fetoprotein and ultrasonography, upper gastrointestinal endoscopy evaluation, using conventional techniques. Each patient was also tested for Wilson's disease, autoimmune liver disease, using appropriate tests. Hepatitis B virus surface antigen positivity (using commercial enzyme-linked immunosorbent assay [ELISA]) and HIV positivity (HIV 1 and 2 using ELISA) were also performed on all patients. At inclusion, each patient was evaluated for depressive illness and had serum levels of T_3, T_4, and TSH estimated using conventional methods available at each center. Liver biopsy was done using Menghini's aspiration biopsy needle or Tru-cut liver biopsy needle on all those with normal coagulogram after informed consent at the time of inclusion. Liver biopsy was repeated at the end of follow-up in patients who consented for the same.

Randomization, Blinding, and Treatment Regimens

Randomization of patients to the two treatment arms was carried out centrally at the NIE, Chennai. Random permuted blocks were used to randomize the patients. The two treatment regimens included either combination of IFN-α-2b with ribavirin or IFN-α-2b with glycyrrhizin. While IFN-α-2b (Fulford, India) in both arms was used at a dose of 3 million units daily subcutaneously for 6 months, ribavirin (Lupin, India) was given 1000 mg/day in two divided doses orally for 6 months and glycyrrhizin (Curewell, India) was given 250 mg/day in two divided doses orally for the same duration. Ribavirin and glycyrrhizin were provided in similar capsules for oral consumption without any label of the drug on the capsules. Both ribavirin and glycyrrhizin were given in such a manner that each patient received three capsules in the morning and two capsules in the night (each capsule contained either 200 mg ribavirin or 50 mg glycyrrhizin). Investigators and the patients included in the study were blinded about the adjuvant drug (ribavirin or glycyrrhizin). The dose of IFN-α-2b used in this study was based on the high SVR reported using similar therapeutic schedule in Indian patients with chronic hepatitis due to HCV.[6] Using random allocation lists, NIE, Chennai, packaged the trial drug for individual patients and supplied to each trial center in batches. The IFN-α-2b which is commercially available was procured centrally and distributed to individual centers. At each center, compliance for ingestion of oral drug ribavirin or glycyrrhizin was carried out by pill-counting method. Each patient was given the oral capsules for 4 weeks in a container containing 150 capsules of either ribavirin or glycyrrhizin.

Informed written consent was obtained from all patients included in the study.

Withdrawal Criteria

If any patient after starting the allotted therapy developed persistent leukopenia ($<2600/mm^3$), thrombocytopenia ($<40{,}000/mm^3$), or anemia (<8 g/dL) despite discontinuation or modification of doses (half the schedule dose) for >2 weeks, he/she was withdrawn from the study. Patients were also withdrawn from the study if they developed behavioral abnormalities or if they became clinically intolerant to therapy as evidenced by the inability to perform daily routine activities.

Follow-up Schedule

During the therapy, each patient was followed up at least once in 2 weeks, during which the clinical, psychological, and hematological evaluations were performed. Liver function tests were performed every 4 weeks. Hepatitis C virus RNA (both qualitative and quantitative) testing was done at inclusion and at 4, 12, 24, and 48 weeks after starting the therapy. Liver biopsy was done at inclusion and at follow-up. At the end of treatment, T3, T4, and TSH were repeated.

End Point

End point was achieved if a patient completed the treatment and follow-up period or withdrawn or dropped out from the study or died.

Outcome Measures

Primary Outcome Measures:

1. Sustained viral response (SVR) defined as HCV-RNA negativity at the end of 24 weeks follow-up after the cessation of treatment.
2. Histological response (HR) defined as an improvement in HAI by a factor of 2 and/or improvement in fibrosis score by a factor of 1.

Secondary Outcome Measures:

1. Frequency of rapid viral response (RVR) (HCV-RNA negativity at 4 weeks of starting therapy) and the influence of RVR on SVR.

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2. End of treatment viral response (ETVR): Hepatitis C virus-RNA negativity at the end of 24 weeks of treatment.
3. Side effects and frequency of discontinuation of therapy in both treatment groups.

Virological Study Methods

Anti-HCV and HCV-RNA (qualitative and quantitative): Anti-HCV at each center was tested using a third-generation commercial ELISA (Xcyton, Bangalore or Organon Taknika, the Netherlands and Abbott Lab, USA). Hepatitis C virus RNA qualitative test was performed centrally at the National Institute of Virology (NIV), Pune, employing nested reverse transcriptase polymerase chain reaction (RT-PCR) with primers from the highly conserved 5′-noncoding region (5′-NCR) as described by Bukh et al.[23] Briefly, the total RNA was extracted from 100 µL of serum with trizol LS reagent (GIBCO BRL, Life Technologies) according to manufacturer's instructions. Single-tube nested RT-PCR was carried out, the expected size of the amplicon being 256 bp. For PCR, stringent measures were taken to avoid contamination. Negative controls were included between two samples and subjected to the entire protocol. Pre-PCR and post-PCR manipulations were performed on the different floors of the laboratory. For the quantification of HCV-RNA, Amplicor HCV Monitor Test, version 2.0 (Roche Molecular Systems, NJ, USA), was used.

Hepatitis C Virus Genotyping: For HCV genotyping, core gene-based phylogenetic analysis was carried out according to the method described earlier.[24] Core region positive PCR products (405 bp) were purified in a column with a gel extraction kit (QIAGEN, Valencia, California) and used as templates for sequencing in the Big-Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems). Samples were analyzed on an automated sequencer (ABI PRISM 3100-Avant genetic analyzer, Applied Biosystems) and both strands were sequenced.

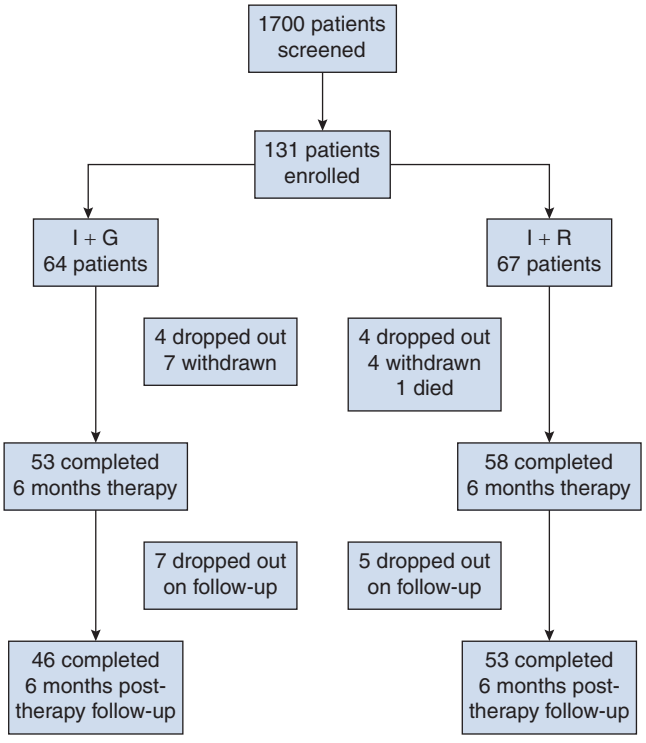
Liver Histology: Liver biopsy specimens were examined by a single pathologist blinded to the type of therapy. Specimens were stained by hematoxylin and eosin, reticulin stain, trichrome mason, orcein, and periodic acid-Schiff (PAS) stain. The grade and stage of each specimen was evaluated using Ishak–Knodell grading and staging system.[21]

Statistical Methods

Descriptive statistics for quantitative variables included mean ± SD (standard deviation) as well as median (range) and for categorical variables, the frequency distribution with percentages were calculated. To compare continuous variables among groups of patients, Student's *t*-test and non-parametric rank sum test were used as appropriate. Chi-square test or Fisher's exact test was used for comparing the proportions of categorical variables among different groups. A P value of < 0.05 was considered as statistically significant. All analyses were implemented on Stata 11.2. Intention to treat principle was adopted for the analysis.

RESULTS

The study was initiated in February 2002 and recruitment of patients was completed in May 2004. Six months post-therapy, the follow-up was completed in May 2005. A total of 1700 anti-HCV-positive patients with CLD were screened, of which 131 (7.7%) met the inclusion criteria. Sixty-four patients were randomized to IFN with glycyrrhizin (I+G) and 67 to IFN with ribavirin (I+R). The details of the number of patients included, dropped out during therapy and during follow-up, withdrawn from the study have been depicted in Figure 1. The main reasons for exclusion of screened patients were the presence of clinical and histological features of cirrhosis, the presence of comorbid conditions, previous treatment with IFN, the presence of minimal disease, and regular alcohol consumption.



I + G: Interferon-α-2b 3 MU daily + glycyrrhizin (250 mg daily in two divided doses)
I + R: Interferon-α-2b 3 MU daily + ribavirin (100 mg daily in two divided doses)

Figure 1 Descriptive details of patients with chronic hepatitis due to hepatitis C virus included in the study.

Table 1 Baseline characteristics of patients.

Characteristics	All patients (*N*=131)	Interferon+glycyrrhizin (*N*=64)	Interferon+ribavirin (*N*=67)	*P*
Age (yr)				
Mean±SD	40.8±11.07	43±9.93	38.8±11.76	0.03
Median	42	42.5	39	
Range	16–65	16–58	16–65	
Sex				
Male	99 (75.6%)	50 (78.1%)	49 (73.1%)	0.51
Duration of disease (months)				
Mean±SD	11.2±18.26	8.9±17.07	13.3±19.21	0.18
Median	6	4	6	
Range	1–120	1–96	1–120	
Source of infection				
BT	38 (29%)	18 (28.1%)	20 (29.9%)	0.73
Community	57 (43.5%)	30 (46.9%)	27 (40.3%)	
Others	36 (27.5%)	16 (25%)	20 (29.9%)	
Body mass index (Kg/m^2)				
Mean±SD	24.2±3.77	24.8±3.6	23.7±3.87	0.09
Median	24.2	24.4	23.6	
Range	15.4–34.7	17.8–34.7	15.4–33.9	
<23	49 (37.7%)	20 (31.2%)	29 (43.9%)	0.14
ALT (IU/L)				
Mean±SD	118.7±56.24	123.7±54.04	114±58.26	0.32
Median	101	111	95	
Range	60–385	60–320	60–385	
AST (IU/L)				
Mean±SD	95.1±53.77	96.4±50.84	93.8±56.78	0.78
Median	86	79.5	88	
Range	20–275	29–275	20–272	
Serum albumin (g/dL)				
Mean±SD	4±0.64	4±0.59	4±0.69	0.82
Median	4	4	4.2	
Range	2.1–6	2.2–5.1	2.1–6	
International normalized ratio				
Mean±SD	1.2±0.32	1.2±0.4	1.17±0.21	0.29
Median	1.1	1.1	1.1	
Range	0.81–3	0.81–3	0.85–1.8	
Hemoglobin (g%)				
Mean±SD	13.7±1.8	13.8±1.64	13.6±1.95	0.57
Median	13.9	14	13.7	
Range	8.6–17.3	9.7–17.1	8.6–17.3	
Serum bilirubin (mg%)				
Mean±SD	0.87±0.53	0.98±0.63	0.78±0.37	0.03
Median	0.7	0.8	0.7	
Range	0.1–3	0.1–3	0.3–2.5	
HAI (*N*: 62 and 67)				
Mean±SD	5±2.06	5.4±1.87	5.5±2.22	0.65
Median	5	5	5	
Range	3–12	3–11	3–12	
Fibrosis stage (*N*: 62 and 67)				
Mean±SD	2.2±1.52	2.2±1.56	2.2±1.49	0.87
Median	2	2.5	2	
Range	0–5	0–5	0–5	
≥3	61 (47.3%)	31 (50.0%)	30 (44.8%)	0.55
Genotype (*N*: 51 and 50)				
1	23 (22.8%)	9 (17.6%)	14 (28%)	0.43
3	72 (71.3%)	39 (76.5%)	33 (66%)	
4	5 (4.9%)	3 (5.9)	2 (4%)	
6	1 (1%)	0	1 (2%)	
Log viral load (*N*: 57 and 59)				
Mean±SD	5.1±0.98	5±0.97	5.2±0.98	0.16
Median	5.3	5.3	5.4	
Range	2.3–6.7	2.8–6.5	2.3–6.7	
≥2×10^6/m	8 (6.9%)	2 (3.5%)	6 (10.2%)	
<2×10^6/m	108 (93.1%)	55 (96.5%)	53 (89.8%)	0.27

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BT: blood transfusion; HAI: histological activity index; SD: standard deviation.

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Table 2 Therapeutic response between two treatment regimens.

Response category	Interferon+glycyrrhizin (*N*=64)	Interferon+ribavirin (*N*=67)	*P*	Overall (*N*=131)
Duration of fluorouracil (FU) (months)				
Mean+SD	9.5±4.4	10±4	0.49	9.8±4.2
Median	12	12		12
Range	0–12	0–12		0–12
Rapid virological response (RVR)	44/64 (68.8%)	54/67 (80.6%)	0.12	98/131 (74.8%)
End of treatment virological response (ETVR)	45/64 (70.3%)	55/67 (82.1%)	0.11	100/131 (76.3%)
Sustained virological response (SVR)	30/64 (46.9%)	44/67 (65.7%)	0.03	74/131 (56.5%)
End of treatment biochemical response (ETBR)	18/64 (28.1%)	26/67 (38.8%)	0.20	44/131 (33.6%)
Sustained biochemical response (SBR)	13/64 (20.3%)	20/67 (29.8%)	0.21	33/131 (25.2%)
Histological response (HAI improvement≥2)	25/64 (39.1%)	30/67 (44.8%)	0.51	55/131 (42.0%)
Fibrosis response (score improvement≥1)	19/64 (29.7%)	18/67 (26.9%)	0.72	37/131 (28.2%)

HAI: histological activity index; SD: standard deviation.

Baseline Characteristics of the Study Population

Table 1 depicts the baseline characteristics of the study subjects. The mean (±SD) age was 41±11 years with 75.6% males. The mean BMI was 24.2±3.8 Kg/m^2. Community-acquired HCV infections formed a major group (43%) while the transfusion-related and those due to other causes were equally frequent (28%). Genotyping of the HCV could be done in 101/131 (77.1%) of cases and majority (71.3%) were of genotype 3 followed by genotype 1 (22.8%). Of the 116 patients (88.5%) in whom baseline viral load estimations could be done, the mean $\log_{10}$ viral load was 5.1±0.98 copies/mL. All 131 patients were subjected to liver biopsy. However, adequate liver tissue (at least 5 portal tracts) was available in 129 patients. The mean HAI was 5±2 and the mean fibrosis stage was 2±1.5.

Patients on IFN with ribavirin were younger on the average (38.8 years vs 43 years) and had lower bilirubin (0.8 mg% vs 1 mg%). All other baseline characteristics between two treatment groups were similar.

Results of Therapy

Table 2 depicts the therapeutic response in the two study groups. The median post-treatment follow-up duration in both treatment arms was 24 weeks. The RVR and ETVR tended to be higher in I+R group, though statistically not significant. Sustained viral response was significantly higher in I+R group (65.7% vs 46.9%, P=0.03). Histological response, fibrosis improvement, and other responses were not significantly different between the two treatment groups (P ranges from 0.21 to 0.72).

Overall, 75% achieved RVR and 56% achieved SVR among the patients receiving IFN with either glycyrrhizin or ribavirin. Rapid viral response could be achieved in 68.8% of I+G group as against 80.6% in I+R group (P=0.12). The proportion of SVRs was 69.4% among those who attained RVR as compared with only 18.2% among those who could not achieve RVR (P<0.001). Although, the SVR among the RVR achieved cases was more in I+R group, it was statistically not significant (75.9% vs 61.4%, P=0.13).

About 42% of all patients had improvement of HAI and 28% had improvement in fibrosis score in both the arms of therapy.

The therapeutic responses were not significantly different in genotype 3 and the other genotypes; BMI groups and also patients in the two fibrosis stage groups.

Table 3 shows the details of side effects and withdrawal of study subjects. A total of 11 patients (7 in I+G group and 4 in I+R group) were withdrawn from the study. Eight patients (4 in each treatment group) had dropped out of the study and one died before completion of treatment. Overall, the distribution between the two treatment arms is not very different (P=0.71). No significant difference with respect to the median duration of discontinuation due to side effects was observed between the two treatment groups. Leukopenia (17% vs 2%) and anemia (40% vs 8%) were significantly more in I+R group as compared with those in I+G.

DISCUSSION

This study revealed that patients of CH-C in India are young with a mean age of 41±11 years and 57 (44%) were below 40 years. Our results indicate that majority of HCV infections are community-acquired which is in concurrence with the finding from a large community-based study in India.[25] It is also reported that the usage of unsterile needles and glass syringes had an odds ratio of about 4 for transmission of HCV.[25] This study supports this finding further. In most rural and suburban areas of India, institution of drugs for common and minor ailments is made through parenteral route. Many children and young adults from rural and suburban areas receive injections for minor ailments, which are forgotten in later

Table 3 Details of side effects and withdrawals of the study subjects.

Characteristics	I+G (*N*=64)	I+R (*N*=67)	*P*
Status of the patient			
Completed therapy without discontinuation	45 (70.3%)	47 (70.1%)	
Completed with discontinuation	8 (12.5%)	11 (16.4%)	
Withdrawn	7 (10.9%)	4 (6.0%)	
Dropped out/died before completion	4 (6.3%)	5 (7.5%)	0.71
Reasons for withdrawal			
Increase in AST/ALT	1	0	
Liver failure	1	0	
Nausea and vomiting	1	0	
Severe irritability	2	0	
Anemia	2	2	
Incoherent behavior	0	1	
Severe depression	0	1	
Duration of discontinuation (days)			
Mean	5.7±8.17	12.5±16.19	0.32
Median	4	5	0.16
Range	1–24	2–57	
Reason for discontinued therapy			
Thrombocytopenia (<60,000)	6/53 (11.3%)	7/58 (12.1%)	0.99
Leukopenia (<2600)	1/53 (1.9%)	10/58 (17.2%)	<0.01
Anemia (<10)	4/53 (7.6%)	23/58 (39.7%)	<0.001
	2/53 (3.8%)	4/58 (6.9%)	0.68
Behavioral abnormality			
Clinical intolerance	7/53 (13.2%)	10/58 (17.2%)	0.61
Cardiac toxicity	3/53 (5.7%)	1/58 (1.7%)	0.35
Pulmonary toxicity	2/53 (3.8%)	2/58 (3.4%)	0.99
Neurotoxicity (CNS)	2/53 (3.8%)	4/58 (6.9%)	0.68
Neurotoxicity (peripheral)	0/53 (0.0%)	1/58 (1.7%)	0.99
Oral mucositis	1/53 (1.9%)	4/58 (6.9%)	0.37
GM-CSF use	0	2/5 (3.4%)	0.50
Erythropoietin use	0	0	–

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CNS: central nervous system.

life. Such socio-cultural practice is likely to be responsible for the younger mean age of CH-C patients observed in this study. These facts provide a lead for a necessary intervention strategy for the prevention of HCV disease in the community.

Our study also revealed that HCV genotype 3 infection was prevalent in almost three-fourths of our patients, which is considered to be IFN-sensitive. The viral load among the CH-C patients in this study was low, being <2 million copies/mL in 93% patients. In contrast, reports from USA and Europe indicated that >60% of patients had high viral load >2 million copies/mL.[26–28] The median fibrosis score of patients included in this study was 2, indicating that most patients included in this study had progressive disease and therefore needed treatment. Thus, predominantly genotype 3 infection together with low viral loads and yet requiring therapy makes an Indian CH-C scenario different from the West.

Overall, IFN in combination with ribavirin showed significantly higher SVR than IFN with glycyrrhizin (65.7% vs 46.9%, $P=0.03$). The odds ratio of SVR with I+R was 2.2 (95% CI: 1.07 to 4.38). Even among cases of genotype 3, the proportion of SVRs was higher in I+R (66.7% vs 46.2%, $P=0.1$). A recent study from a single center in India documented an SVR of 67% among patient with genotype 3 HCV-induced CHC treated using the recommended PEG-IFN-α and ribavirin.[29] Further, a multicenter trial in Europe and Asia (including India) was conducted to evaluate the dose and duration of PEG-IFN-α-2b with weight-based ribavirin dosing in patients with genotype 2 and 3 HCV-associated chronic hepatitis.[34] In this study, PEG-IFN-α-2b (1.5 μg/Kg/wk) and weight-based ribavirin (800–1400 mg/day) for 6 months duration achieved an SVR of about 62% among Indian patients with CHC infection of whom about 80% were due to genotype 3 HCV infection. Therefore, it seems that in Indian patients who predominantly suffer from genotype 3 HCV infection, the SVR with recommended PEG-IFN and regular IFN with ribavirin is likely to be similar. A few reports even from the West indicate that treatment with regular IFN with ribavirin in comparison to PEG-IFN with ribavirin had similar SVR in patients with genotype 3 CHC.[11,26,27] Thus, the cheaper treatment regimen involving IFN-α-2b used in this study showed an SVR that is comparable with the

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SVR observed in the study using expensive PEG-IFN, particularly in the Indian scenario.

In India, all patients with CH-C bear the treatment expenses themselves due to limited state funding and unavailability of insurance cover. Cost of PEG-IFN treatment for 6 months in India would be ₹300,000 (US$ 7500) compared with ₹150,000 (US$ 3750) of the cost of IFN-α-2b. Thus, the treatment costs for a patient are halved with similar success, if PEG-IFN is replaced with IFN-α-2b in the treatment of CH-C, especially in the Indian scenario. Lower cost will also contribute to better patient compliance for the treatment. About 20 million people in India fall below the poverty line each year because of indebtedness due to healthcare needs.[31] In view of this, the findings of the study are highly relevant in social perspective also. Further, if one were to compare the two, with an assumption of 10% more successes with PEG-IFN than the 66% observed with I+R, we need to randomize at least 700 patients. Such a study may neither be necessary nor be feasible in view of the numbers and costs involved. In the light of the results of this study and previous studies cited, head-to-head comparison of IFN-α-2b with PEG-IFN may not be essential.

Although, multicenter studies are the need of time with increasing drug regimens, such an approach is not common in India. This study is the first multicenter study on liver disease from the Indian subcontinent which registered patients from all parts across the country. Racial and ethnic factors influence the therapeutic results of PEG-IFN+ribavirin in CHC. African-Americans and Latinos in USA with CHC infection had significantly lower SVR than similar White American patients, subsequent to adequate treatment with PEG-IFN and ribavirin.[32,33] Our results are unlikely to be influenced by such factors as we included patients from different parts of the country representing various socioeconomic and cultural spectrums and represents the cross-section of the Indian subcontinent.

Patients registered in this trial had similar characteristics of young age, community-acquired infection of virus C, mostly genotype 3 infection, low viral load (<2 million copies/mL in 93%), minimal fibrosis with moderate necro-inflammation, no associated HIV or HBV infection, and nondiabetics without history of alcohol consumption. These could have helped in achieving a success rate of about 60% with either of the two modalities.

Histological improvement could be seen in a sizable proportion of patients (39% with I+G and 45% with I+R) in our study, with no significant differences between the two arms. Similarly, improvement in fibrosis score could be seen in 30% and 27% of patients treated with I+G and I+R, respectively. Reports from the West also indicate that combined therapy of PEG-IFN with ribavirin had similar histological improvements.[26–28,30]

Among the two, the combination with glycyrrhizin was found to be less toxic than that with ribavirin. Therefore, it is suggestive that glycyrrhizin can replace ribavirin to combine with IFN for the treatment of CHC, if ribavirin produces side effects.

Like most previous studies,[27] this study could not establish any relationship between pre-treatment viral load, genotype, histological severity, source of infection, BMI, and sex distribution with SVR.

The strength of this study lies in the fact that it is a multicenter investigation in which the adjuvant drugs ribavirin and glycyrrhizin were instituted in a double-blind manner and the histopathological evaluation was performed in a blinded manner. The results show that IFN-α-2b with ribavirin is associated with better therapeutic responses than with glycyrrhizin. Ribavirin is associated with more toxicity than glycyrrhizin. Looked in another way, IFN with ribavirin is an effective treatment in Indian patients of CH-C, with an SVR of 65% and an HR of 45% with marked reduction in the cost of treatment.

To conclude, this study establishes that IFN with ribavirin is associated with better therapeutic responses than IFN with glycyrrhizin. It also suggests that IFN therapy with ribavirin could be a successful alternative treatment modality for CHC in India with considerable reduction in the treatment costs. Glycyrrhizin showed fewer side effects than ribavirin.

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CONFLICTS OF INTEREST

All authors have none to declare.

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