Medical Journal of the
Islamic Republic of Iran

Volume 17
Number 2
Summer 1382
August 2003

Original Articles

SAFETY AND EFFICACY OF INTERFERON ALFA FOR THE TREATMENT OF CHRONIC HEPATITIS C INFECTED SUBJECTS WITH TRANSFUSION DEPENDENT THALASSEMIA IN IRAN

S. MIRMOMEN, H. GHOFRANI, H. FOROOTAN PISHBIJARY, N. EBRAHIMI DARYANI, M. JAFAR FARAHVASH, R.A. SHARIFIAN, F. AZMODEH AND R. MALEKZADEH

From the Digestive Disease Research Unit of Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, I.R. Iran.

ABSTRACT

Up to 30% of Iranian adult multi-transfused thalassemic patients are infected with hepatitis C virus (HCV) which can intensify the progression of liver disease caused by iron overload in this group of patients. Our aim was to assess the biochemical and virological response of interferon alfa (INF-α) and its safety in thalassemic patients with chronic HCV infection. This trial was a single center, open label, single treatment prospective study of INF-α (Heberon alfa R, 3 MU, every other day) for a period of 12 months. 29 subjects, 13 to 56 years old (mean ± SD: 25.1 ± 10.4 years), whose serum HCV-RNA was positive and mean ALT remained greater than 1.5 times upper limit of normal in the last 6 months before the study were enrolled. A percutaneous liver biopsy was performed before treatment and all patients underwent monthly assessment for adverse events and monitoring of serum ALT. Qualitative serum HCV-RNA was obtained in months 3 and 6 and at the end of therapy.

Pretreatment liver biopsy showed mild fibrosis in 33.3%, moderate fibrosis in 56.7% and cirrhosis in 10% of patients. Siderosis was severe in 14 patients (46.7%). Two nonsplenectomized patients discontinued INF because of mild cytopenia, which resolved in less than one week after interruption of therapy. The following were some of the important adverse events observed during the study period: Flu syndrome in 29(100%), chills or fever>39°C in 14(48%), local pain in 14(48%), transient gastrointestinal symptoms in 13(44%), weakness in 5(17%), local induration in 3(10%) and edema in 2(7%) of the patients. By the end of 12 months of therapy, 15 patients out of 27 (55.6%) had a normal ALT and negative HCV-RNA (complete end-treatment response), they were followed up for a mean duration of 10.5 months (range: 6 to 22 months) and in 8 of them (53.3%) the

E. mail: mirmomen@ams.ac.ir

condition relapsed (abnormal ALT with positive PCR). Viral clearance was a delayed event in our patients (29% by the end of month 3 and 63% by month 7) but ALT normalization occurred in 94% of responders by the end of month 3.

Our experience indicates that the cure of HCV-related liver disease in thalassemic patients is not an unrealistic aim and may be achieved with a safe and inexpensive INF preparation (Heberon Alfa R) in a sizeable portion of cases. As opposed to non-thalassemic patients, in whom most viral responses happen in the first 3 months of therapy, in this group of thalassemic patients we found that maximum virologic response happened between 3 to 6 months of therapy. Although INF-α is an effective drug for initial treatment in thalassemic patients infected with HCV, its efficacy with the above dose and duration, for maintaining long term remission is under question.

MJIRI, Vol. 17, No. 2, 87-95, 2003.

Keywords: Thalassemia, Interferon, Hepatitis C, Heberon

INTRODUCTION

Patients with major thalassemia require blood transfusion throughout their life to sustain their growth and development. Transfusion not only places these patients at increased risk of HCV infection but also causes an inevitable accumulation of body iron.[1] Iron overload and HCV infection are the main causes of chronic liver disease in patients with transfusion dependent thalassemia.[2,5]

INF-α Therapy has been proven to induce sustained normalization of serum ALT levels and disappearance of serum HCV-RNA in 10-30% of non-thalassemic patients.[6,9] Several trials have demonstrated that combination therapy with INF and ribavirin can improve the rate of sustained response compared to INF monotherapy in non-thalassemic patients[10-13] and a combination of INF and ribavirin is currently first-line treatment for non-thalassemic patients with HCV related chronic hepatitis.[14] There are few studies about the use of ribavirin in thalassemic patients.[15,16] It may induce hemolysis, which intensifies anemia and increases the transfusion requirement of thalassemic patients.[15] Currently INF monotherapy is the only approved treatment for thalassemic patients with HCV related liver disease and a combination of INF and ribavirin is preserved for INF non-responders only under investigational situations.[17]

The presence of liver siderosis has been shown to be related to poor response to INF in non-thalassemic patients,[18-21] hence one can expect a poor response to INF monotherapy due to transfusion related siderosis in thalassemic patients. However studies have shown that in thalassemic subjects, there is a promising response to INF monotherapy (as high as 50% sustained response in some series).[22-28] However, most of these studies are small, preliminary and performed upon pediatric patients and there is a lack of adult, long term prospective studies.[24]

The current study was designed to evaluate the long-term safety and effectiveness of INF-α for the treatment of chronic hepatitis C infected adult subjects with transfusion dependent thalassemia in Iran.

PATIENTS AND METHODS

The trial was a single center, open label, single treatment study. This study provided the opportunity to prospectively evaluate the efficacy and safety of INF-α in a cohort of subjects with major thalassemia treated in Tehran.

Patients

The study took place in Tehran Adult Thalassemic Clinic, which serves over 500 adult patients with transfusion dependent thalassemia. Subjects were included in the study if they met the following inclusion criteria: (1) diagnosis of β-thalassemia and being under a regular transfusion program (2) age above 13 years (3) positive serum HCV-RNA measured by qualitative PCR method (4) serum ALT 1.5 times above normal value at least in two different occasions in the 6 months prior to the study (5) histological grading (assessed by percutaneous liver biopsy) more than 3/18 or staging more than 2/6 according to Modified Histo-Activity-Index(HAI) scoring system.[29] The main exclusion criteria were: unsuccessful prior treatment with INF, decompensated cirrhosis, HIV infection or any other immunocompromised condition, critically ill patients, thrombocytopenia ($PLT < 50 \times 10^9 / L$), using medication which may cause bone marrow suppression (Hydroxyurea, cotrimoxazole,...), positive HBSAg or HBV-DNA.

The clinical trial was devised in accordance with the ethical principles outlined in the declaration of Helsinki

and laws and regulations of the Islamic Republic of Iran. Informed consent was obtained from each eligible subject prior to any procedure.

38 patients were screened concerning the possibility for participation in the clinical trial and 32 were enrolled. Three subjects were withdrawn from the study during the pretreatment period (one patient met an exclusion criteria and the other two voluntarily withdrew upon their request).[29] subjects with mean age of 25.1 years ± 10.44 (range: 13 to 56 years) were included in the study. Seventeen subjects (58%) were female.

Histological evaluation

A percutaneous liver biopsy was performed for all patients prior to start of therapy; samples were preserved in formalin solution and reviewed by a single pathologist. Hepatic inflammation was reported according to modified histo-activity index scoring system[29] (Modified HAI), which expresses inflammation from a minimum (grade 1) to a maximum (grade 18). We also considered those less than grade 4 to have mild and those above grade 4 to have severe hepatic inflammation.

According to the above scoring system hepatic fibrosis is expressed from stage 1 (no fibrosis) to stage 6 (cirrhosis) and we considered those with stages 1 and 2 as mild, stages 3 and 4 as moderate and stages 5 and 6 as severe fibrosis. Iron was shown using the Perls' stain procedure. Grading was based on the method of Scheuler modified by Rowe,[30] which classified parenchymal and mesenchymal hemosiderin deposition as follows: grade 0, no hemosiderin; grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, severe.

Treatment

Heberon Alfa R (Interferon a-2b) which has been developed by the Center for Genetic Engineering and Biotechnology (CIGB), Havana/Heber Biotech Company was administered as 3 million units, every other day, subcutaneously injected in the deltoid region. A trained nurse injected the first dose of medication and patients were allowed to inject later doses by themselves upon request.

Monitoring schedule

The treatment phase of the study lasted one year. During this phase patients received regular clinical and laboratory evaluation as follows:

Safety monitoring

All patients underwent assessment for any adverse events or use of concurrent medication at day 15 and then monthly in the first three months of the study and then at months 5, 7, 9 and 12 of the study. White cell differential counts, platelet counts, serum ALT, AST, T_3 and TSH were also measured in each visit. Patients were also asked to report any significant adverse event by telephone during the study duration.

Efficacy monitoring

Serum ALT and AST were analyzed at one, 3,7 and 12 months after therapy and blood was obtained for qualitative HCV-RNA assay at 3 and 12 months after therapy.

We considered those subjects whose serum ALT returned to normal in at least two consecutive visits as having a biochemical response and those whose serum HCV-RNA changed to negative as having a virological response. Subjects with both a biochemical and virological response were considered to have a complete response and if the complete response was durable for at least 6 months after drug discontinuation it was considered a sustained response.

Statistical methods

A perprotocol approach was used for statistical analysis of the data: serum ALT and HCV-RNA were used for efficacy analysis. The statistical analyses were performed using SPSS for windows (ver.10).

RESULTS

Mean medication exposure in 29 patients was 11.3 months/patient, ranging from one month to a maximum of 13 months. Mean duration of follow-up after drug discontinuation was 10.5 months (range: 6 to 22 months). The pretreatment clinical profiles are shown in Table I.

Histological evaluation of all 30 patients prior to the start of treatment showed that 17 patients (56.7%) had mild hepatic inflammation (grade <4) while 13 patients had moderate to severe hepatic inflammation (grade >4). Liver fibrosis was severe in 3 patients (10%), moderate in 17 patients (56.7%) and mild in10 patients (33.3). Histological iron grading showed that only one patient had mild iron deposits (Grade <2). Iron grading was severe in 14 (46.7%) and moderate in 15 (50%) patients. Mean serum ferritin value, prior to start of therapy, was 1538±1400g/L and histological iron staging relatively correlated with serum ferritin value (Figure 1). All patients were anti-HIV negative at the time of enrollment. Ten patients were reported to be HBc-antibody positive but all patients were HBS-Ag negative at baseline. Serum anti-HCV (Elisa II) was positive in all patients and was confirmed both by RIBA II assay and HCV RNA (PCR) in all. Serum globulin value was more than 3 g/L in 22 patients (73%) and mean serum globulin value was 3.4±0.7 (ranging from 2.5 to 5.2 g/L). We also checked serum autoantibodies in all 30 patients before the start of therapy and results showed that Antinuclear Antibody (ANA) was negative in 27 (90%) patients while one patient showed a titer of 1/20 and two patients showed a

Table I. Pretreatment characteristics of 29 multi-transfused thalassemic patients treated with Heberon Alfa.

	Mean	Std. Deviation
Age (years)	25.24	±10.67
Sex (female: male)	17:12	
Weight (Kg)	53.97	±10.69
Height (cm)	160.62	±9.18
Mean transfusion years	19.62	±7.14
Duration of documented HCV infection (months)	48.72	±26.07
Splenectomized patients	60%	
Liver Biopsy		
Histological grade*	5.45	±3.18
Histological staging*	3.24	±1.21
Iron grading[30]	2.55	±0.74
serum ALT(IU/L)	97.45	±39.22
serum Albumin (gr/L)	4.50	±0.513
serum Globulin (gr/L)	3.48	±0.730
serum Prothrombin time (%)	94.21	±12.22
serum Ferritin (μg/L)	1585.9	±1407.30

*According to modified HAI scoring system[29]

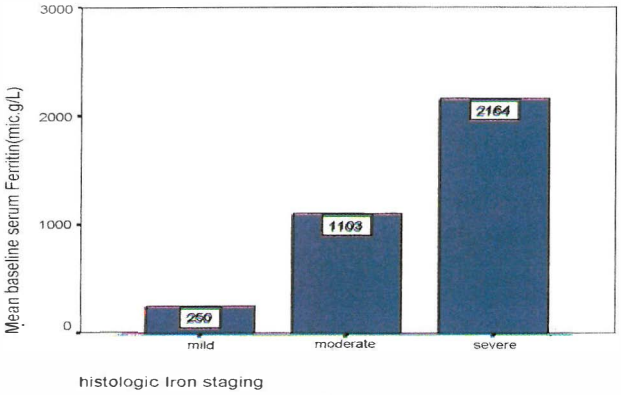

Fig. 1. Correlation between mean serum ferritin and histological iron grading.

titer of 1/40. Anti-smooth muscle antibody (ASMA) was negative in 28 patients (93.3%), and positive in two patients (1/20 and 1/40). But none of the patients had a Revised International Autoimmune Hepatitis Score (IAH group) of more than 15.[31]

Evaluation of safety

Early withdrawal: From 29 patients, in whom Heberon Alfa R was started, 4 patients didn't complete the 12 months period of study and withdrew from the study earlier. One of these withdrawals was because the patient started using Hydroxyurea in month 10 of the therapy (a drug that was not allowed to be used in this study) and was excluded from the study after 10 months of successful Heberon use. Two others (a 22 y/o male and a 17 y/o female) were nonsplenectomized patients with borderline cytopenia prior to the start of therapy and both were advised by a hematologist to undergo splenectomy which they had postponed. These two patients experienced mild cytopenia [Neutropenia (<1500, >500×10^9/L) and thrombocytopenia (<150, >50×10^9/L), respectively] in the first three months of therapy which was nonresponsive to dose reduction but resolved in less than one week after interruption of therapy and reappeared after start of Heberon, so they were withdrawn from the study and advised to undergo splenectomy.

One of them underwent splenectomy and has now been using Heberon for 6 months and hasn't experienced any cytopenia this time, her last serum PLT count being 568×10^9/L. It seems that Interferon has just exaggerated these two patients' prior hypersplenism status (cytopenia) rather than primarily causing it. The last withdrawal was because of hypothyroidism which occurred at month 11 of the study in a 45 y/o female. Although she did not complete the 12 month period of the study, she had a complete *sustained response* to Interferon 12 months after drug discontinuation.

Table II shows all the adverse events which have been reported during this study.

General adverse events including: flu syndrome, chills, high grade fever (temperature above 39°C), weakness, headache and severe flu-like syndrome were the most frequent adverse reactions observed during the study period. No serious or unexpected adverse event

was noted during this study.

Efficacy results

Except for those 2 patients who discontinued INF before three months of therapy, there were 27 patients that used Heberon for at least 10 months and the efficacy results for these 27 patients are reported here:

Biochemical response

17 patients out of 27(62.9%) had a biochemical response by the end of therapy. Biochemical response at months 3 and 7 of the study were 59% and 62.9%, respectively. Most of the biochemical responses happened in the first 3 months of the study (Figure 2). Mean baseline and end of therapy serum ALT values in 27 patients who completed at least 10 months of therapy were 98.3±40 and 41.8±32 IU/L, respectively ($p<0.001$). But we did not find any significant difference between mean serum ALT at month three and at the end of therapy.

At the end of therapy there were only two patients with biochemical response but positive serum HCV-RNA; in other words 88% of biochemical responders had a virological response, too.

Virological response

17 patients out of 27 (62.9%) had a virological response by the end of therapy. Virological response at months 3 and 7 of the study were 33.3% and 63% respectively. In contrast to biochemical response, virological response was a delayed event in our patients and nearly 50% of the responses occurred after the third month of therapy (Figure 2).

There were two patients who had increased serum ALT despite a complete virological response.

Complete response

Fifteen patients out of 27 (55.6%) had a complete biochemical and virological response at the completion of the study. Only 29.6% of complete responses occurred by the third month of the study but, 100% of them occurred by the end of month 7. Among 15 responders, 7 patients (46%) had a complete sustained response (CSR), at least 6 months after discontinuation of INF. Overall 25.9% (out of 27) attained a CSR in this study.

Predictors of complete response

To minimize potential effects of cofactors of liver damage such as siderosis, only patients with complete end-treatment response (CER) or complete sustained response (CSR) were chosen as end-point. Lower mean duration of HCV infection and lower histological iron grading were significantly more common among subjects with CER. Multivariate analysis showed that only lower mean duration of HCV infection was independently associated with attaining both CER and CSR.

DISCUSSION

Our trial confirms and extends the results of previous studies in thalassemic patients with chronic HCV infection,[22,28] which reported that a course of IFN could produce a high rate of end-of-treatment response comparable with non-thalassemic patients.

Although transfusional iron overload in the liver acts as a cofactor in determining the severity of liver damage in thalassemic patients, it seems that at least in this small population it dose not affect the efficacy of Interferon Alfa in thalassemic patients infected with HCV virus. Over a long follow-up period (mean:10.6 months), we showed that the relapse rate was as high as 53.5% in our thalassemic patients, which was in contradiction with the findings of Di-Marco et al. that showed a very low relapse rate (18%)[24] in thalassemic patients. This could be due to our small sample size. To date, INF is an approved therapy for chronic HCV infected subjects and meta-analysis of clinical trials has shown that INF alone leads to CSR in about 10 to 30% of non-thalassemic subjects,[32]

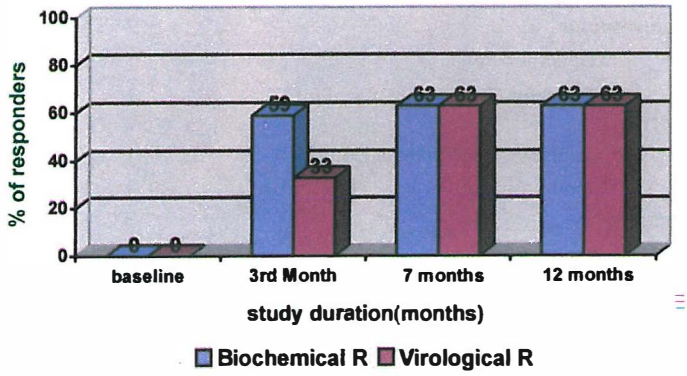

Fig. 2. Time profile of biochemical and virological response rate (%) in 27 thalassemic patients treated with INF-α.

so achieving a CSR of 25% in this study is a promising finding and could be due to:

(1) As an accepted international rule, thalassemic patients should be checked yearly for all viral markers including HCV antibody,[38] so early detection and treatment of HCV infection might be one reason for this promising result. Jackel and colleagues have shown that treatment of acute HCV infection in non-thalassemic subjects can results in over 90% CSR.[39] The mean duration of HCV infection in this study was 4 years (Table I), which is less than what we usually see in non-thalassemic subjects.

(2) Genotype of the HCV virus is a very important determinant of response to therapy. Studies have shown

Table II. Frequency of adverse events in 29 thalassemic patients treated with Heberon Alfa R.

Body System / Adverse Events	Patients No.	(%)
General	**29**	**100**
Flu Syndrome	29	100
Chills	14	48.2
Weakness	5	17.2
T>39	9	31
Headache	3	10.3
Severe Flu Like Syndrome	2	6.8
Skin	**21**	**72.4**
Alopecia	14	48.2
Local pain	14	48.2
Local induration	3	10.3
Generalized rash	2	6.8
Utricaria	2	6.8
Pruritus	2	6.8
Exaggerated skin reaction to Desferal	2	6.8
Vitiligo	1	3.4
Acne (Comedones)	1	3.4
Local cellulitis	1	3.4
Gastrointestinal	**13**	**44.8**
Anorexia	8	27.5
Weight loss	7	24.1
Dry mouth	5	17.2
Diarrhea	1	3.4
Nervous system	**5**	**17.2**
Mood change	8	27.5
Somnolence	4	13.7
Morning headache	1	3.4
Cardiovascular	**2**	**6.8**
Peripheral edema	1	3.4
Periorbital edema	1	3.4
Endocrine	**2**	**6.8**
Exaggerated prior hypocalcemia	1	3.4
Hypothyroidism	1	3.4
Hematology	**3**	**10.3**
Leukopenia	2	6.8
Thrombocytopenia	1	3.4
Musculoskeletal	**1**	**3.4**
Arthralgia	1	3.4
Ocular	**1**	**0.7**
Photosensitivity	1	0.7

Table III. Differential features among complete responders (CR) and non-CRs.

Variable	CER	Non-CER	*p*-value	CSR**	Non-CSR	*p*-value
	N=15	N=12		N=7	N=20	
Age(Years)	26.73	24.33	N/S	30.57	23.97	N/S
Sex(M/F)	8/15	8/12	N/S	2/5	9/11	N/S
BMI	55.83	51.1	N/S	22.2	20.2	N/S
Splenectomized	9 (60%)	8 (66.7%)	N/S	5	12	N/S
Mean transfusions per year (mL/year)	161.33	181.67	N/S	17.8	20.3	N/S
Positive serum HVcAb	77.3%	58.8%	N/S	14.3%	40%	N/S(0.2)
Mean duration of HCV infection (months)	181.67	32.93	0.000	27.14	54.65	0.007
Mean histologic grade (baseline)	6.47	4.50	N/S	7	5	N/S
Mean histologic staging (baseline)	3.20	3.25	N/S	3.14	3.25	N/S
Mean histological iron grading (baseline)	2.20	3.00	0.004	2.29	2.65	N/S
Mean baseline serum ALT (IU/L)	99.07	97.42	N/S	89.1	101.55	N/S
Mean baseline serum globulin (gr/L)	3.427	3.583	N/S	3.34	3.55	N/S
Mean baseline serum ferritin (µg/L)	1281.40	1747.50	N/S	1311	1550	N/S

*Complete end-treatment response

**Complete sustained response

that genotypes other than 1(mostly 2 and 3) have a more favorable response to INF than genotype 1.[40, 41] Unfortunately we did not have HCV genotyping facilities available during this study and the non-genotype I dominancy in our patients could be one of the important reasons for the high response rate in our patients.

(3) One of the differences between transfusion dependent thalassemic (TD-T) patients with non-thalassemics, in all studies, is that TD-T patients always use subcutaneous daily injections of desferroxamine to get rid of their extra body iron. Recently some investigators have shown an antiviral effect for desferroxamine, mostly evident on HBV[42,43] CMV[44] and HIV.[45,47] One theory is that the use of desferroxamine in thalassemic patients can intensify the antiviral effect of INF on HCV.

Although adding Ribavirin to INF increases the rate of sustained response to nearly 45%[33] in non-thalassemic subjects, for three reasons we are not eager to use it in thalassemic patients in Iran: Firstly because of the severe hemolytic anemia induced by ribavirin, there is a relative contraindication for its use in thalassemia; secondly it is still an expensive drug in Iran; and thirdly, there are still limited studies using ribavirin in thalassemic patients.[15]

Another interesting finding in our study was that persistent ALT normalization was not paralleled by serum HCV RNA clearance in the first 6 months of our study. Data obtained in the subset of patients for whom serial HCV RNA testing was available showed that in CSR subjects, biochemical response is usually an early event (occurring in the first 3 months), but viral clearance may be delayed up to 7 months. This data is also in contradiction with other studies in thalassemics[24] or non-thalassemics[6-9] and warrants further evaluation. We suggest that thalassemic subjects receive at least 6 months of continuous therapy and then check for serum HCV RNA.

Regarding the safety of INF-α (Heberon Alfa R), the adverse events reported during this study were in the same range of other widely used interferon products in the world.[34-37] The observation of cytopenia (neutropenia and thrombocytopenia) in two nonsplenectomized patients during this study suggests that INF might have an additive effect on the cytopenic consequence of hypersplenism in those multi-transfused thalassemic patients who have not undergone splenectomy yet, and in this group of patients we warrant a more close hematological observation. As a matter of fact, since thalassemic patients can have multi-system involvement (liver, endocrine, heart, hematopoietic system, etc.) as a consequence of iron overload and chronic anemia, it would not be unusual if some of the INF side effects are more prominent in these patients. In our opinion although the safety results of this study are very promising, one should not forget close monitoring of every adult thalassemic patient in whom INF is prescribed.

In conclusion, our experience indicates that the cure of HCV-related liver disease in thalassemic patients in not an unrealistic aim and may be reached with this safe and inexpensive INF preparation (Heberon Alfa R) in a sizeable proportion of cases, especially when HCV infection is diagnosed in early phase by patient screening programs.

ACKNOWLEDGEMENT

The authors wish to express special thanks to Dr Hamid

Zakeri for his support and assistance in conducting this study.

REFERENCES

1. Gabutti V, Piga A: Results of long term iron-chelation therapy. Act Haematol 95: 26-36, 1996.
2. Lai ME, De Virgilis S, Argiolu F, Farci P, Mazzoleni AP, Lisci V, Rapicetta M, Clemente MG, Nurchis P, Arnone M, Balistreri A, Cao A: Evaluation of antibodies to hepatitis C virus in a long-term prospective study of post-transfusion hepatitis among thalassemic children: comparison between first and second-generation assay. J Pediatr Gastroenterol Nutrition 16: 458-64, 1993.
3. Resti M, Azzari C, Rossi ME, Vullo C, Zammarchi E, Vierucci A: Hepatitis C virus antibodies in a long-term follow-up of beta-thalassaemic children with acute and chronic non-A, non-B hepatitis. Eur J Pediatr 15: 573, 1992.
4. Angelucci E: Antibodies to hepatitis C virus in thalassemia subjects with persistently abnormal ALT.Haematologica 79: 353, 1994.
5. Mirmomen S, Zakeri H: Prevalence of hepatitic C virus infection in adult multi-transfused thalassemic patients in Iran. Unpublished data, presented in 9th Iranian Congress on Infectious Diseases and Tropical Medicine, 14-18 Jan. 2001.
6. Carithers RL Jr, Emerson SS: Therapy of hepatitis C. Meta-analysis of INF alfa-2b trials. Hepatology 26(Suppl 1): 83S, 1997.
7. Camma C, Giunta M, Pinzello G, et al: Chronic hepatitis C and INF alpha: conventional and cumulative meta-analyses of randomized controlled trials. Am J Gastroenterol 94: 581, 1999.
8. Davis GL, Balart LA, Schiff ER, et al: Treatment of chronic hepatitis C with recombinant INF alfa: a multicenter randomized, controlled trial. N Engl J Med 321: 1501, 1989.
9. Niederau C, Heintges T, Haussinger D: Treatment of chronic hepatitis C with alpha INF: an analysis of the literature. Hepatogastroenterology 43:1544, 1996
10. Reichard O, Norkrans G, Fryden A, et al: Randomised, double-blind, placebo-controlled trial of INF alfa-2b with and without ribavirin for chronic hepatitis C. Lancet 351: 83, 1998.
11. Poynard T, Marcellin P, Lee SS, et al: Randomised trial of INF alfa 2b plus ribavirin for 48 weeks or for 24 weeks versus INF alfa 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 352: 1426, 1998.
12. Schalm S, Hansen B, Chemello L, et al: Ribavirin enhances the efficacy but not the adverse effects of INF in chronic hepatitis C: a meta-analysis of individual patients data from European centers. J Hepatol 26: 961, 1997.
13. McHutchison J, Gordon S, Schiff E, et al: INF alfa 2b alone or in combination with ribavirin in naive chronic HCV patients: A US multicenter trial. N Engl J Med 339: 1485, 1998.
14. Booth JCL, O'Gray J, Neuberger J, et al: Clinical guidelines on the management of hepatitis C. Gut 49(suppl I):i1-i21, 2001.
15. Telfer PT, Garson JA, Whitby K, Grant PR, Yardumian A, Hoffbrand AV, Wonke B: Combination therapy with INF alpha and ribavirin for chronic hepatitis C virus infection in thalassaemic patients. Br J Haematol Sep 98(4): 850-5, 1997.
16. Li CK, Chan PKS, LingSC, Luk CW, Ha SY: Combination therapy as front-line treatment for chronic hepatitis C in thalassemia. Presented in 8th International Conference on Thalassemia and the Hemoglobinopathies (abstract form). October 18-21, Athens-Greece.
17. Wonke B, Hoffbrand AV, Bouloux P, Jensen C, Telfer P: New approaches to the management of hepatitis and endocrine disorders in Cooley's anemia. Ann N Y Acad Sci Jun 30; 850: 232-41, 1998.
18. Van Thiel DH, Friedlander L, Fagiuoli S, Wright HI, Irish W, Gavaler JS: Response to INF-a therapy is influenced by the iron content of the liver. J Hepatol 20: 410, 1994.
19. Olynyk JK, Reddy KR, Di Bisceglie AM, Jeffers LJ, Parker TI, Radick JL, Schiff ER, Bacon BR: Hepatic iron concentration as a predictor of response to INF alfa therapy in chronic hepatitis C. Gastroenterology 108: 1104, 1995.
20. Girelli CM, Mirata C, Lesinigo E: Iron overload and response to alpha-INF in chronic hepatitis C. Am J Gastroenterol 90: 170, 1995.
21. Barton AL, Banner BF, Cable EE, Bonkovsky HL: Distribution of iron in the liver predicts the response of chronic hepatitis C infection to INF therapy. Am J Clin Pathol 103: 419, 1995.
22. Donohue SM, Wonke B, Hoffbrand AV, Reittie J, Ganeshaguurun K, Scheuer PJ, Brown D, Dusheiko G: Alfa INF in the treatment of chronic infection in major thalassemia. Biochemical response. J Haematol 83(3): 491-497, 1993.
23. Di Marco V, Lo Lanco O, et al: Alfa-INF treatment of chronic hepatitis C in young patients with homozygous beta-thalassemia. Haematologica Nov-Dec 77(6): 502-6, 1992.
24. Di Marco V, Lo Lanco O, Almasio C, Ciaccio M, Capra M, Rizzo R, Malizia A, Maggio C, Fabiano F, Barbaria A, Crax D: Long-term efficacy of a-INF in b-thalassemics with chronic hepatitis C. Blood 90(6) (September 15): 2207-2212, 1997.
25. Clement MJ, Congia M, Lai ME, et al. Effect of iron overload on the response to recombinant interferon-alfa treatment in transfusion-dependent patients with major thalassemia and chronic hepatitis C. J Pediatr Jul 125(1): 123-128, 1994.
26. Wonke B, Donohue SM, Hoffbrand AV, Scheuer PJ, Brown D, Dusheiko G: Recombinant alpha 2b interferon(INF) in

the treatment of chronic hepatitis C disease in major thalassemia (TM). Bone Marrow Transplant 12: 24-25, 1993.
27. Sipiliopoulou I, Repanti M, et al: Response to interferon alfa-2b therapy in multitransfused children with beta-thalassemia and chronic hepatitis C. Eur J Clin Microbiol Infect Dis Oct 18(10): 709-15, 1999.
28. Katamis C, Kostaridou S, et al: Efficacy of INFα treatment in thalassemic patients with chronic hepatitis C. Presented in 8th International Conference on Thalassemia and the Hemoglobinopathies (abstract form). October 18-21, Athens-Greece.
29. Ishak K, Baptista A, Bianchi L, et al: Histological grading and staging of chronic hepatitis. J Hepatol 22: 696-9, 1995.
30. Searle JW, Kerr JFR, Halliday JW, et al: Iron storage disease. In: MacSween RNM, Anthony PP, Scheuer PJ, (eds.), Pathology of Liver, 2nd ed., Edinburgh: Churchill Livingstone, pp. 185-6, 1987.
31. International Autoimmune Hepatitis Group Report. Review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 18: 929-938, 1998.
32. Poynard T, Leroy V, Cohard M, et al: Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effect of dose and duration. Hepatology 24: 778-789, 1996.
33. Ogana G, Tocco A, Garrucciu G, Branca B, Azara V, Manzoni NE, Deplano A, Solinas A: Patterns of response during therapy with interferon and outcome of retreatment with interferon plus ribavirin for chronic hepatitis C. J Clin Gastroenterol Sep 33(3): 254, 2001.
34. Roche: Roferon-ᴿA (interferon alfa-2a, recombinant) prescribing information (dated 1996 Oct). In: Physicians' desk reference. 51st ed. Montvale, NJ: Medical Economics Company Inc (Suppl A): A276-80, 1997.
35. Schering: Intron-A (interferon alfa-2b, recombinant for injection) prescribing information (dated 1996 Jan). In: Physicians' desk reference. 51st ed. Montvale, NJ: Medical Economics Company, pp. 2506-14, 1997.
36. Roche: Roferon-ᴿA, brand of interferon alfa-2a, recombinant/Roche: comprehensive product information. Nutley, NJ; Roche, 1986.
37. Scott GM: The toxic effects of interferon in man. In: Gresser I, (ed.), Interferon 5. New York: Academic Press, pp. 87-99. 1983.
38. Thalassemic international federation: Guidelines for the clinical management of thalassemia, April, p98-99(www.thalassemia.org), 2000.
39- Jackel E, et al: Early treatment of acute HCV infection with Interferon α2b monotherapy prevents development of chronic HCV infection. Presented at 51st American Association for Study of Liver Disease (AASLD) Oct 2001.
40- Romeo R, Tommasini MA, Rumi MG, et al: Genotypes in the progression of hepatitis C related cirrhosis and development of hepatocellular carcinoma (abstract). Hepatology 24: 153A, 1996.
41- McHutchison JG, et al: Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. NEJM 339: 1485-92, 1998.
42- Chouteau P, Le Seyec J, Saulier-Le Drean B Cannie I, Brissot P, Lescoat G, Guguen-uillouzo C, Gripon P: Inhibition of hepatitis B virus production associated with high levels of intracellular viral DNA intermediates in iron-depleted HepG2.2.15 cells. J Hepatol Jan 34(1): 108-13, 2001.
43- Bayraktar Y, Koseoglu T, Somner C, Kayhan B, Temizer A, Uzunalimoglu B, De Maria N, Van Thiel DH: The use of deferoxamine infusions to enhance the response rate to interferon-alpha treatment of chronic viral hepatitis B. J Viral Hepat May 3(3): 129-35, 1996.
44- Gumbel H, Cinatl J, Rabenau H, Vogel JU, Doerr HW, Ohrloff C: Selective inhibition of replication of human cytomegalovirus by desferrioxamine *in vitro* and *in vivo* (case report). Ophthalmol Dec 92(6): 840-3, 1995.
45- Georgiou NA, van der Bruggen T, Oudshoorn M, Nottet HS, Marx JJ, van Asbeck BS: Inhibition of human immunodeficiency virus type 1 replication in human mononuclear blood cells by the iron chelators deferoxamine, deferiprone, and bleomycin. J Infect Dis Feb 181(2): 484-90, 2000.
46- Sappey C, Boelaert JR, Legrand-Poels S, Forceille C, Favier A, Piette J: Iron chelation decreases NF-kappa B and HIV type 1 activation due to oxidative stress. AIDS Res Hum Retroviruses Sep 11(9): 1049-61, 1995.
47- Gordeuk VR, Delanghe JR, Langlois MR, Boelaert JR: Iron status and the outcome of HIV infection: an overview. J Clin Virol Feb 20(3): 111-5, 2001.