Clinical, Biochemical and Histopathological outcome of six months of Interferon therapy in thalassemic patients with chronic hepatitis C viral infection

Ghadban J. M. *
Sayah H. A. *

* Al-Hussein general hospital- karbala - Iraq

Abstract

Background: Hepatitis C viral infection is a common cause of liver disease among polytransfused patients.
Aim of the study: To find out short term efficacy of Interferon therapy in thalassemic chronic viral hepatitis-C patients.
Setting: This study was carried out in Gastroenterology and Hepatology Teaching hospital in Baghdad – Iraq
Patients and methods: Twenty four thalassemic patients who were anti HCV antibody positive with elevated liver enzyme for more than one year enrolled in this study . All patients were submitted to liver biopsy to assess the degree of the inflammation and fibrosis .All patients were treated with interferon alpha (3×10^6 units/m^2) tree times a week for six months.
Result: Three out of twenty four patients stopped treatments because of sever side effect. Out of twenty one patients; 10(47 %) of patients showed complete biochemical response, 7(33%) of patients showed partial biochemical response, 4(19%) of patients showed no response.
Young age patients responded better to interferon therapy .patients with initial high serum alanine trasaminase responded less favorably than those who had lower initial pretreatment serum alanine transaminase ,this was statistically significant .The higher the initial serum ferritine the worst the biochemical and histological response .All patients who responded biochemically and submitted to another liver biopsy showed decrease in severity of histological activity index after six months of Interferon therapy ;there was strong correlation between biochemical response and histological response. There was no improvement in degree of fibrosis in those treated with interferon therapy.Splenectomized patient tolerated better and responded better than those who were not splenectomized .
Conclution: Thalassemic patients with chronic viral hepatitis C can benefit at least in short term from Interferon therapy especially in younger age patients and those who have lowest elevation of liver enzymes and those with lower serum ferritin level.
Keywords: Hepatitis,Hepatitis C,Thalassemia,Thalassemic patients ,Interferon

Introduction

Hepatitis C virus(HCV) is a single stranded virus related to Flavivirus and Pestivirus [1].The natural targets of HCV are hepatocytes and possibly B-lymphocytes [2,3] . Hepatitis C virus is highly heterogeneous with respect to sources of infection and clinicopathological features [4]. It has been found in every country in which it has been sought and accounts for the majority of cases 80-90% of post transfusion hepatitis .Community acquired or sporadic non A ,non B

hepatitis at least in the USA is also predominantly (around 70% of cases)due to HCV[5] . The world wide incidence of antibody to HCV in blood donors ranges from 0.3% to more than 10%;the highest number of infection reported in Egypt mean 22% among population[6] .

Histological findings in patient with chronic HCV hepatitis:

The finding include chronic active hepatitis, chronic persistent hepatitis, chronic lobular hepatitis and cirrhosis with their correspondence score according to the Knodel-Ishak score [7].

Methods of serodiagnosis of HCV infection:

1.1st, 2nd, and 3rd generation anti HCV ELISA ([8, 9, 10]).

2. Several immunoblot assays have been developed to confirm the presence of HCV antibody (Ab) [10].

3. PCR permits detection of viral RNA by amplifying reverse transcribed c-DNA [10].

Treatment:

1. Interferons(INF)(usual and long acting pegalated Interferon).
2. Ribavirin; it is a nucleoside analogue that is well absorbed orally and has broad antiviral activity against a variety of DNA and RNA viruses.

Indication:

1. Patient with acute hepatitis should be considered for antiviral therapy at the time of diagnosis.
2. Patient with chronic hepatitis C at fibrotic stage 2or 3.
3. Compensated liver cirrhosis. Combination therapy with interferon and ribavirin are recommended if there is no contraindications for neither drugs as the first treatment for patient with chronic hepatitis C; the course is six months for genotype 2 and 3 or type 1 with low viral load (2×10^6 virus equivalent/ml)and twelve months for type 1 and 4 with high viral load ($>2\times10^6$virus equivalents /ml)[11]

Thalassemia and Hepatitis C:

Patients with hematological disorders such as thalassemia, who receive repeatedly blood transfusions, have a high risk of exposure to HCV and may have persistent infection with associated chronic liver disease [12].

Approximately 25% of patients in the United Kingdom with thalassemia major have antibodies to HCV [13] and in parts of Italy the prevalence of HCV was more than 75%,in Iraq in a study done 1996 ,the prevalence of anti HCV Ab among thalassemic patients was 66.6% [14]which was nearly comparative to the prevalence among Saudi Arabia thalassemic patients which was 70% [15].The majority of cases of HCV infection gave rise to chronic hepatitis with the risk of progression to cirrhosis and hepatocellular carcinoma [16] .In the case of patients with thalassemia major the hepatic damage due to HCV infection was exacerbated by transfusional Iron overload and liver disease is a recognized cause of mortality [17] .

Aim of the study:

-To determine the clinical, biochemical and histopathological changes in thalassemic patients with chronic hepatitis C viral infection after six-month therapy with Interferon

-To identified the efficacy of six- month treatment with Interferon monotherapy.

Patients and methods:

A prospective study performed in Gastroint-erology and Hepatology Teaching Hospital – Baghdad –Iraq for the period between Feb.2003-April, 2004 .Twenty-four thalassemic patients were involved, all patients have at least 2 folds elevation of liver enzymes in more than three occasions for more than one year.

All patients were thalassemic major were referred from thalasemic center .The patients and their relatives were interviewed properly .All patients were anti HCV Ab positive (screening and confirmatory). All patients were assessed biochemical by liver function tests, serum Iron, total Iron binding capacity, serum ferritin.All patients were subjected to liver biopsy using Menigine needle. Histopathological assessment was assessed by Knodell score [18] .The liver biopsies were evaluated by single pathologist who was aware of the diagnosis .Interferon monotherapy were given to all patients with Knodell score more than 3 and fibrosis more than stage 1 fibrosis .The dose of INFα-2b was calculated according to the surface area (3×10^6 units/m^2) three times a week for six months .Patients were monitored for the development of side effects of INF therapy during their treatment period for those who developed sever adverse events , the dose of the drug was lowered to the next lower dose after being held up for two weeks .Sever adverse events included an event that interfered with patient daily activities or resulted in admission to the hospital , a platelet count <30000/mm^3 or a WBC count <1200/mm^2 or a granulocyte count <500/mm^2 [19] .

These patients were monitored by complete blood count every week for 1st 2 months then monthly for the last 4 months liver enzymes monitoring every month to assess biochemical response.

Biochemical responses: Three patterns of responses have been recognized [20].

1. Compleat response; is defined as normalization of ALT levels which is usually occurs rapidly (generally within 2 months of initiation of treatment).

2. Partial response; is defined as a decrease in ALT of more than 50% from baseline (mean of all pre treatment value).

3. Non responder; showed no effect of treatment on ALT levels.

Patients who complete six months of INF therapy submitted to another liver Biopsy to show the effect of therapy.

Statistical analysis:

All data tabulated and arranged in number , percentage ,range (minimum ,maximum)and mean ±stander deviation, association between variables measured by using chi-square ,paired t-test and student t-test and analysis of variable (ANOVA)which is appropriate the differences considered to be significant statistically when $p<0.05$.

Result:

A total of twenty-four patients mean age 14.3 years (range 6-50 years), male =15 (62.5%), female =9(37.5%) were enrolled in the study

Mean ALT level before the Interferon therapy was 68 IU/L with a range of (42 -99 IU/L) (Normal value < 20 IU/L).

Mean serum ferritin level at the starting of Interferon therapy was 1571.8 ng/ml with a range of (1000-2425 ng/ml).

Mean histological activity index (HAI) for all patients before starting therapy excluding (fibrosis) was 6.9 with a range of (4-12).

Mean fibrosis stage of histopathologic specimens of liver biopsies in all enrolled patients was 2.8 before starting therapy. Three of the twenty- four patients was stage 4 fibrosis (12.5%), fifteen of the twenty –four patients were stage 3 fibrosis (62.5%), and six of the twenty-four patients were stage 2 fibrosis (25%).

Ten patients out of the twenty-four had splenectomy before starting therapy .During Interferon therapy, three patients failed to complete first three months of therapy, because of development of sever hematological side effects.

Biochemical response:

1. Complete response: Ten patients out of twenty one patients (47%) showed complete response.
2. Partial response: Seven patients out of twenty-one patients (33%) showed partial response 3. No response: four patients out of twenty-one patients (19%) showed any response to INF therapy. Table-1-

Table -1-. Biochemical response after Interferon therapy

Biochemical response	ALT** level Before therapy (mean ±SD)	ALT level After therapy (mean ±SD)	P Value
Complete (n=10)	70.6±16.4	19.3±5.5	<0.05
Partial (n=7)	75±21.1	27.1±4.7	<0.05
No response (n=4)	79.5±8.7	73.8±11.1	NS*

** Alanine aminotransferase ,* Not significant

There was no significant difference in response to INF therapy according to gender of the patient. Table -2-

Table -2- Relation between biochemical response and gender

Biochemical response	Gender		P value
	Female no. (%)	Male no. (%)	
Complete (n=10)	4 (50 %)	6 (46 %)	NS*
Partial(n=7)	2 (25%)	5 (38%)	NS
No response(n=4)	2 (25%)	2 (15%)	NS
Total 21	8 (100%)	13 (100%)	

* Not significant

All patients who showed biochemical response during first two months continues to do so till the end of six months of therapy. The younger patients responded more than older one, the difference was significant p. value <0.05 .Table -3-

Table -3- Relation between biochemical response and age

Biochemical response	Patients no. (%)	Age (Mean ±SD)
Complete	10 (47)	12.5±2.1
Partial	7 (33)	13±0.31
No response	4 (19)	17.5±2.01

P is <0.05 significant using ANOVA test.

The rate of response related inversely to the level of serum ferritin, this relation was significant, p.value <0.05 using ANOVA test. Table -4-

Table (4).The relation between serum Ferritin before therapy and biochemical response

Biochemical response	Serum Ferritin mean ± SD
No response (n = 4)	2000 ± 150.1
Partial response (n = 7)	1561 ± 50.1
Complete response (n =10)	1345 ± 43.2

P value is <0.05 using ANOVA test.

Seventy percent of patients who had complete response were splenectomized, while 42% of those with partial response were splenectomized.Table -5-

Table (5) Relation between splenectomy and biochemical response

Biochemical response	Patient who had splenectomy No. (%)
No response (n =4)	0(0)
Partial response (n= 7)	3(42)
Complete response(n=10)	7(70)

All patients who had been splenectomized tolerate medication and their platelets and WBC never fall below the normal range.

Histopathological response:

Twelve patients who completed six months of therapy were submitted to another liver biopsy. The rest (12 patients) were not submitted to another liver biopsy because of:

1) Four of them were non responder.
2) Three of them developed severe side effects.
3) Five patients declined to do another liver biopsy.

Six of the twelve patients who had another liver biopsy had complete biochemical response, the other six patients showed partial biochemical response .In both groups the histopathological response to the therapy were statistically significant. Table -6- . The difference in Knodell score before treatment and after six – month course of INF was statistically significant for male but non significant for female patients .Table -6-

The fibrosis stage was not affected by the therapy in both genders .Table -6-

Table -6- Relation of biochemical response and gender of patients to Knodell score before and after therapy (12 patients who had 2 liver biopsy)

Patients have Biochemical response		Knodell score before therapy (mean ± SD)	Knodell score after therapy (mean ± SD)	P value
Partial response (n = 6)		(7.8 ± 2.9)	(4.3±1.8)	<0.05
Complete response (n = 6)		(6.8±2.3)	(4.3±1.6)	<0.05
Gender	*Female (n =4)*	(7±2.3)	(5±1.4)	NS*
	Male (n =8)	(7.6±2.7)	(3.9±1.5)	<0.05
	Total (n = 12)	(7.4 ±2.54)	(4.3 ± 1.5)	< 0.05

*Not significant.

The side effects during Interferon therapy.

Four patients developed hematological side effects, three of them developed bleeding tendency only one of these three responded to dose reduction of treatment while the other two patients did not responded to dose reduction.

The fourth patient had sever thrombocytopenia (below 30000/cc).The three patients who didn't respond to dose reduction ,discontinue medication because of the severe side effects .Nearly all patients developed constitutional symptoms .

Discussion:

Hepatitis C virus is responsible for the majority of cases of post transfusion non A, non B hepatitis in patients with Thalassemia major [12]. Interferon therapy is an effective treatment for patient with chronic hepatitis C.

In this study;those patients, who showed complete biochemical response, had lower initial ALT levels in comparison with those who showed partial biochemical response .Non responder have higher initial ALT as shown in Table (1) which showed strong correlation

between pretreatment liver enzymes and biochemical response which consistent with other study [21].

Patients who showed complete response were 47% ,partial response 33% and no response were 19% ,this is consistent with other study done on response to Interferon therapy in multitransfused children with β-thalassemia , where complete response was 46% ,partial response 30% while no response was 23%[22].

This study showed no significant correlation between gender and biochemical response and this was consistent with the British guide lines in the management of hepatitis [23].

As showed in table (3) complete response in younger age was higher than those who had partial response who were in turn younger than those who had no response which was statistically significant $p<0.05$.This result is consistent with other study[23] .

As showed in table (4) there is a significant correlation between pretreatment serum ferritin level and biochemical response .It confirmed that the lower the serum ferritin level the better the biochemical response and this was consistent with other study which showed the response to Interferon therapy was inversely related to the liver iron burden[24] .

As shown in table (6) there was a significant correlation between biochemical response and Knodell score after therapy for both partial and complete bipchemical response , this had been found also in other study which showed that the chronic persistent hepatitis and mild chronic aggressive hepatitis strongly correlate with biochemical response to Interferon therapy ,the prevalence of non responder was lower in chronic persistent hepatitis (9.7%)than in chronic active hepatitis (mild form 13.9% ,sever form 20.9%) and significantly higher in patient with cirrhosis (53.9%)[25].

As shown in table (6) there was significant difference between Knodell score before and after therapy for males and also for both male and female .This is consistent with other study [23] while for female there is no significant correlation.

As shown in table -7- there is no relation between fibrosis stage after treatment with gender .This is expected because the degree of hepatic fibrosis or portal inflammation dose not change with therapy [26,27] .

Sever side effect may occur during therapy in patient with thalassemia that may br required cessation of therapy , in this study three patients were stopped treatment because of sever hematological side effects this lower than what showed by Zatelli,S. et al study who showed that 5/16 thalassemic patients had stopped treatment due to severe side effects[28] .

Table (7).The relation between fibrosis stage (before and after therapy) and gender

Gender	Fibrosis stage before therapy (mean±SD)	Fibrosis stage after therapy (mean ±SD)	P value
F (n=4)	3.25 ± 0.5	3.25± 0.5	NS*
M (n = 8)	2.75 ± 2.8	2.75± 2.8	NS

* Not significant.

Conclusions:

All thalassemic patients should be screened for anti HCV Ab and liver enzymes in order to pickup early those who required Interferon therapy.

1. Interferon therapy is more effective in thalassemic patients who are at younger age, who have modest initial elevation of liver enzyme and those who have lower level of serum ferritin.
2. Splenectomized patient showed good tolerance and response to Interferon therapy.

References:

1. Parti,D.et al .Influence of different HCV genotype on the course of asymptomatic HCV infection .G.E Jan 1996;vol.110,No.1:178-190.
2. Okuda M,Hino K,Korenaga M,Yamaguchi Y,Katoh Y,Okita K. Differences in hyper variable region 1 quasispecies of HCV in human sera ,peripheral blood mononuclear cells and liver .Hepatology 1999;29:217-22.
3. Zignego AL,DeCarli M,Monti M et al .Hepatitis C virus infection of mononuclear cells from peripheral blood and liver infiltrates in chronically infected patient.J.Medical virology 1995;47:58-64.
4. Alter J.et al .The natural history of community acquired HCVin the United States .Hepatology Feb. 1995; vol. 21, No. 2: 285.
5. Alten MJ,Hadler SC,Tudson FN et al. Risk factors for acute NANB hepatitis in the United States and association with hepatitis virus Antibody .Journal of the American Medical association 1990;264:2331-2235.
6. Naramy A. et al. Sero prevalence of HCV-blood donors India.Gastrointerology Oct.1994; 2-7:1667.
7. Alter HJ .Chronic consequences of non A, non B in current perfectives in Hepatology .Seef LB;Leeis JH (eds).plenum Publishing, New York ;1989,P.83.
8. Gerber MA.Chronic hepatitis C: the beginning of the nomenclature.Hepatology1992; 15:733-734.
9. Alter HJ,Purcell RH,Shin JW, et al.Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non A ,non B hepatitis .N. Engl.J med. 1989;329:1494-1500.
10. Marcellin P,Mantinot-Peignonx M,Boyer N ,et al.Second generation (RIBA)test in diagnosis of chronic hepatitis C. Lancet 1991;337:551-552.
11. Consensus statements on the prevention and management of hepatitis B and hepatitis C in the Asia –Pacific region. Journal of Gastroenterology and Hepatology 2000.
12. Ross RS,Viazov S,Gross T,Hofmann F,Seipp H-M;Roggandorf M .Transmission of HCV from patient to an anesthesiology assistant to five patients .N.Engl.J Med.2000;343:1851-4.
13. Wouke B,Hoffbrand A.V. , Brown D.C.and Dushieko G.(1990).Antibody to hepatitis C virus in multiply transfused patients with thalassemia major .Journal of clinical pathology ;43:638-640.
14. Hussein A. Al-Hilli,Jassim M. Ghadhban. Prevalence of serological markers of hepatitis B virus (HBsAg) and hepatitis C virus (HCV AB) among blood donors and certain risk groups .J.Fac.Med.Baghdad 2000, vol.42, No.1.
15. Al-Faleh F,Ramia S.Hepatitis C virus infection in Saudia , review article Annals of Saudia Medicine Jan 1997;vol.17 ,No.1:77-82.
16. Alter HJ (1995) to C or not to C these are the questions .Blood; 85: 1681-1695.
17. Zurio MG,Destetano C.,DiGregorio E.,Buratimmi MG and Toroli S.(1989).Survive and causes of death in thalassemia major .Lancet ;3,I:2730.
18. Dienstag J.,Issebacher K .Chronic hepatitis in Harrison's principles of internal medicine ,1998,14th edition ; 297: 1696 -1703.
19. Jacyna MR,Thonas HC. Antiviral therapies give medical glossitis British medical Bulleten 1990; 46:368-382.
20. Sarracco G,Rosina F,Torrani C.et al .A randomized controlled trial of interferon alpha -2b as therapy for chronic non A, non B hepatitis.Hepatology1990;11:543-549.
21. Simon D, et al .Treatment of chronic hepatitis C with interferon alpha: A multicenter randomized open label trial .Hepatology Feb.1997; vol.25: No.2.
22. Spiliopulou I. Response to interferon alpha-2b therapy in multi transfused children with B-thalasssemia and chronic hepatitis C .European J.clinical Microbiology –Infect-Dise.1999; Oct.18 (10):709-15.
23. Booth L. ,Graly J., Newbeyer J. Guide lines on the management of hepatitis C .GUT Data 2001; vol. 49: supplement I.
24. Clemente MG, Congia M,Cai ME et al .Effect of Iron overload on the response to recombinant interferon alpha treatment in transfusion dependent patients with thalassemia major and chronic hepatitis C. Journal of pediatrics 1994 July;125(1):123-8.
25. Dibisceglie et al.Recombinant interferon alpha therapy for chronic hepatitis C .N.Engl.J.Med.1989 Nov; vol 32:No.22.
26. Piccinino F, Felac FM,Aprea-L,Messina V,et al .Non responders to interferons therapy among chronic hepatitis patients infected with HCV.Arch –Virolo-supplement 1993;8:257-63.
27. Tsubot A,Chayam-K,Arase-Y et al .Factors useful in predicting the response to interferon therapy in chronic hepatitis C.J. Gastroenterology and Hepatology 1993 Nov-Dec.;8(6):536-9.
Zatelci,S.,Cappelimi M.D. et al .(Alpha)interferon treatment in (beta) thalassemia major – patients with chronic hepatitis C. European Journal of clinical investigation 2000June;vol.30,suppl:24-29.