Iraqi Journal of

Original Article

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

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Abstract

Bachgruond: Hepatitis C viral infection is a common cause of liver disease among poly transfused patients .Interferon alpha was the first accepted treatment of chronic hepatitis C.

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 these patients were assessed clinically and biochemically .All patients were submitted to liver biopsy to assess the degree of the inflammation and fibrosis .All these patients were treated with interferon alpha 3 x 10^6 units/m2 three time a week for six months .

They were followed up by regular evaluation of complete blood picture and biochemical evaluation; monthly liver enzymes to assess the biochemical response.

Those who completed six months of therapy were submitted to another liver biopsy to assess the degree of histological improvement after therapy.

Result: Three out of twenty four patient stopped treatment because of severe side effects.

Out of twenty one patients; (16)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 is strong correlation between biochemical response and histological response .there was no improvement in degree of fibrosis in those treated with interferon therapy noted .Splenectomized patient tolerated better and responded better than those who was not splenectomized .

Conclution:Thalassemic patient with chronic viral hepatitis C can benefit at least in short term from Interferon therapy especially so in younger age patients and those who have lowest elevation of liver enzymes and those with lower serum ferritin level .

Introduction

Hepatitis C virus is a single stranded virus related to Flavivirus and Pestivirus (1).The natural targets of HCV are hepatocytes and possibly B-lymphocytes (2,3) Viral replication is extremely robust and its estimated that more than 10 million virion particles are produced per day even in the chronic phase of infection (4) there are at least six distinct genotype have been recognized warld wide . some of these appears to be
limited in distribution to a particular geographic area where as others are more wide spread within a genotype ,there may be a considerable variation among isolates, subgenotypes(5) . in the United State and western Europe ,Genotype Ia and Ib are most common type followed by genotype 2and 3 .the other genotypes are virtually never found in these countries but are more common in other areas ,such as Egypt in case of genotype 4 ;South Africa in the case of genotype 5 and South East Asia in the case of genotype 6 . Knowledge of these genotypes is important because it has predictive value in terms of the response to antiviral therapy (6) ;with better response associated with genotype 2 and 3 than with genotype 1(7)

Epidemiology, risk groups and modes of transmission :
Hepatitis C virus is highly heterogeneous with respect to sources of infection and clinicopathological features(8).Hepatitis C virus remains a significant public health problems .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(9) . 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(10) . The factors most strongly associated with infection are injection drug use and receipt of a blood transfusion before 1990 .Poverty high risk sexual behavior are linked to an increased risk of infection(11) .Maternal fetal transmission occurs but is infrequent and often associated with co-infection with HIV in the mother (12).Casual house hold contact and contact with

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saliva of infected persons also appear to be inefficient modes of transmission(13).Nosocomial transmission has been documented such as from patient to patient by a colonoscope (14),during dialysis (15) and during surgery(16) .Blood transfusion posed a major risk of HCV infection in developed countries (17).Needle stick injuries in the health care setting continue to result in nosocomial transmission of the virus , the rate of transmission after a needle stick injury involving blood known to be infected ranged from 0-10% in various studies (18)

Histological findings in patient with chronic HCV hepatitis : The range of histological presentations of chronic hepatitis is almost identical to that described for chronic hepatitis B .The finding include chronic active hepatitis , chronic persistent hepatitis , chronic lobular hepatitis and cirrhosis with their correspondence score according to the Knodel-Ishak score(19).

Methods of serodiagnosis of HCV infection:

1.1st generation anti HCV ELISA developed by Ortho and Abbott, utilized the antigen expressed by C-100-3 core from the NS4 non structural region of the virus genome (20) .

2. second generation anti HCV ELIZA test ,which is produced by incorporation of recombinant or synthetic antigens (C22 and C33) which where derived from two conserved regions :the structural region (core)and NS3 region respectively (21).

3. 3rd generation Anti HCV ELIZA test which additionally detect NS5 Ab have been developed ,it has high sensitivity and specificity.

4. several immunoblot assays have been developed to confirmed the presence of HCV Abs these assays detect separately antibody reactivates to several HCV antigens (22).

5. PCR permits detection of viral RNA by amplifying reverse transcribed c-DNA ,

Treatment:

1. Interferons(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 is 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)(23)

Thalassemia and Hepatitis C:

Patients with hematological disorders such as have a high risk of exposure to HCV and may have persistent infection with associated chronic liver disease (17). Approximately 25% of patients in the United Kingdom with thalassemia major have antibodies to HCV (24) and parts of Italy the prevalence of HCV is more than 75%,in Iraq in a study done 1996 ,the prevalence of anti HCV Ab among thalassemic patients was 66.6% (25)which is nearly comparative to the prevalence among Saudi Arabia thalassemic patients which was 70% (26).The majority of cases of HCV infection gives rise to chronic hepatitis with the risk of progression to cirrhosis and hepatocellular carcinoma (27) .In the case of patients with thalassemia major the hepatic damage due to HCV infection is exacerbated by transfusional Iron overload and liver disease is a recognized cause of mortality (28) .

Aim of the study :

-To identifiyd the correlation between the clinical ,biochemical and histopathological abnormalities in thalassemic patients with chronic hepatitis C viral infection.

-To identifiyd the efficacy of six months of treatment with interferon monotherapy.

Patients and motheds:

This is a prospective study which was performed in Gastrointerology and Hepatology Teaching Hospital - Baghdad -Iraq for the period between Feb.2001-April ,2002 .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 their relatives were interviewed properly .All patients were anti HCV Ab (+ve)(screening and confirmatory) All patients were assessed biochemically by liver function tests (SAP,S.ALT,S.AST,and TSB).SAP were corrected to the age of patient. The normal value of S.ALT is below 20 IU/L.Serum Iron binding capacity, serum ferritin (normal range is 15-332ng/ml).All patients were subjected to liver biopsy using Menigine needle under light general anesthesia .Histopathological assessment was assessed by Knodell score (29) .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 INF^{α}-2bwas calculated according to the surface area (3×10^6 units/m2 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/mm3 or a WBC count <1200/mm2or agranulocyte count <500/mm2 (30) .
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(31) .
1.Complete response ;is defined as normalization of S.ALT levels which is usually occurs rapidly (generally with in 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 S.ALT levels.
Patients who complete six months of INF therapy submitted to another liver Bx to show the effect of therapy.

Statistical analysis used in the study:

All data tabulated and arranged in number , percent ,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 (24) patients mean age 14.3 years(range 6-50 years) ,male =15(62.5%),female =9 (37.5%) were enrolled in the study
Mean S.ALT level before the Interferon therapy was 68 IU/L with a range of (42 -99 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 on 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 was stage 3 fibrosis (62.5%),six of the twenty-four patients was 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 (60% male , 40% female).
2.Partial response :Seven patients out of twenty one patients (33%) showed partial response (male=71.4% ,female=28.6%).
3.No response :Four patients out of twenty-one patients (19%) showed no response to INF therapy (male=50%,female= 50%).
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 .Table (3).The rate of response related inversely to the level of serum ferritin .Table(4).
Seventy percent of patients who had complete response were splenectomized ,while 42% of those with partial response were splenectomized.Table (7).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 (5).
The histopathological response to the therapy were statistically significant for male patients. Table (5).
The fibrosis stage not effected by the therapy in both gender .Table(6).
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 not responded to dose reduction.
The fourth patient had sever thrombocytopenia (below 30000/cc).These 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 (17) .Interferon therapy is an effective treatment for patient with chronic hepatitis C .
In this study ;those who showed complete biochemical response had lower initial S.ALT levels in comparison with those who showed partial biochemical response .Non responder have higher initial S.ALT as shown in table (1) which showed strong correlation between pretreatment liver enzymes and biochemical response which consistent with other study (32).
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 B-

thalassemia , where complete response was 46% ,partial response 30% while no response was 23%(33). This study showed no significant correlation between gender and biochemical response and this is consistent with the British guide lines in the management of hepatitis (34).
As showed in table (3) complete response in younger age is higher than those who had partial response who were in turn younger than those who had no response which is statistically significant $p<0.05$.This result is consistent with other study(34) .
As showed in table (4) there is a significant correlation between pretreatment S.ferritin level and biochemical response .It confirmed that the lower the serum ferritin level the better the biochemical response and this is consistent with other study which showed the response to Interferon therapy was inversely related to the liver iron burden(35) .
As shown in table (5) there is a significant correlation between biochemical response and Knodell scor 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%)(36)
As shown in table (5) there is significant correlation between Knodell score after therapy for males and also for both male and female as shown in table (5) which statistically significant ,this is consistent with other study (34) while for female there is no significant correlation .
As shown in table (6)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 (37,38) .
Sever side effect may occur during therapy in patient with thalassemia that may 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(39) .

Tables:

Table (1) Biochemical response after Interferon therapy

Biochemical response	S.ALT level Before therapy (mean ±SD)	S.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*

* Not significant

Table (2)Relation between biochemical response and gender

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

* Not significant

Table (3) Relation between biochemical response and age

Biochemical response	Patients no.(%)	Age (Mean ±SD)
Complete	10 (47)	12.5±2.1
Partia	I7 (33)	13±0.31
No response	4 (19)	17.5±2.01

P is <0.05 significant using ANOVA test.

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

Biochemical response	S.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.

Table (5) Relation of biochemical response and gender of patients to Knodell score before and after therapy (12 patients who had 2 liver biopsy)

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

*Not significant.

Table (6)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 .

Table (7) Relation between splenectomy and biochemical response

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

Conclusions:

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

2.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 .

3.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. Neumann AU,Lam NP,Dahari H.et al.Hepatitis C viral dynamics in vivo and the anti viral efficacy of Interferon-alpha therapy .Science1998;282:103-7.
5. Dienstag J.,ISSELBACHER.Acute viral hepatitis cited in Harrison's principle of internal medicine ,1994,13th edition :1459-1462.
6. Poynard T, Marcellin P,Lee SS et al. Randomized trial of interferon ? 2b plus placebo for 48 weeks for treatment for chronic infection with hepatitis C virus. Lancet1998;352:1426-32.
7. McHutchison JG,Gordon SC;Schiff ER et

al.Interferon alfa -2b alone or in combination to ribavirin as initial treatment for chronic Hepatitis C .New England Journal Medicine 1998;339:1485-92.
8. Alter J.et al .The natural history of community acquired HCVin the United States .Hepatology Feb. 1995; vol. 21 ,No. 2 : 285.
9. 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.
10. Naramy A. et al. Sero prevalence of HCV-blood donors India.Gastrointerology Oct.1994;2-7:1667.
11. Frank C,Mohamed MK .Strickland GT et al .The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt .Lancet 2000;355:887-91.
12. Alter MJ,Kruszon-Moran D ,Nainan UV et al .The prevalence of hepatitis C virus infection in the United States 1988-1994.New England J Med 1999;341:556-62.
13. Ohto H.,Terazawa S,Sasaki N.et al .Transmission of hepatitis Cvirus from mother to infants New England J Medicine 1994;330:744-50.
14. Cauzijou P.,Richard L,Dumas F,Schouler L.et al .Detection of HCV RNA in saliva of patient with chronic hepatitis C.Gut 1998,34:supplement.
15. Bronowicki J.P.,Venard V.,Botte C et al.Patient to patient transmission of hepatitis C virus during colonoscopy .New England J Med 1997;337:237-40.
16. Katsoulidow A,Paraskevis D et al .Molecule epidemiology of a hepatitis C virus outbreak in a hemodialysis unit :Multicenter hemodialysis cohort study in viral hepatitis .Nephrology Dialy.Transplant 1999;14:1188-94.
17. 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.
18. Williams AE,Dodd RY.The serology of hepatitis C virus in relation to post transfusion hepatitis .Annals and laboratory science 1990;20:192-199.
19. 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.
20. Gerber MA.Chronic hepatitis C :the beginning of the nomenclature.Hepatology1992;15:733-734.
21. 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.
22. Marcellin P,Mantinot-Peignonx M,Boyer N ,et al.Second generation (RIBA)test in diagnosis of chronic hepatitis C. Lancet 1991;337:551-552.
23. Consensus statements on the prevention and management of hepatitis B and hepatitis C in the Asia - Pacific region. Journal of Gastroenterology and Hepatology 2000.
24. Wouke B,Hoffbrand A.V. , Brown D.C.and Dushieko G.(1990).Antibody to hepatitis C virus in gy multiply transfused patients with thalassemia major .Journal of clinical pathology ,43;638-640.
25. 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.
26. Al-Faleh F,Ramia S.Hepatitis C virus infection in Saudia , review article Annalsof Saudia Medicine Jan 1997,vol.17 ,No.1;77-82.
27. Alter HJ(1995) to C or not to C these are the questions .Blood 85,1681-1695.
28. Zurio MG,Destetano C.,DiGregorio E.,Buratimmi MG and Toroli S.(1989).Survive and causes of death in thalassemia major .Lancet ;3:I:2730.
29. Dienstag J.,Issebacher K .Chronic hepatitis in Harrison's principles of internal medicine ,1998,14th edition ; 297: 1696 -1703.
30. Jacyna MR,Thonas HC. Antiviral therapy give medical glossitis British medical Bulleten 1990;46:368-382.
31. 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.
32. 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.
33. 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.
34. Booth L. ,Graly J., Newbeyer J. Guide lines on the management of hepatitis C .GUT Data 2001; vol. 49: supplement I.
35. 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.
36. Dibisceglie et al.Recombinant interferon alpha therapy for chronic hepatitis C .N.Engl.J.Med.1989 Nov. vol 32,No.22.
37. Piccinino F, Felac FM,Aprea-L,Messina V,et al .Non responders to interferon therapy among chronic hepatitis patients infected with HCV.Arch - Virolo-supplement 1993;8:257-63.
38. 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.
39. Zatelci,S.,Cappelimi M.D. et al .(Alpha)interferon treatment in (beta)thalassemia major - patients with chronic hepatitis C.European Journal of clinical investigation ,vol.30,suppl.June 2000 ,P.24