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The treatment of HCV in patients with haemoglobinopathy in Kurdistan Region, Iraq: a single centre experience

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SUMMARY

Various variables that might influence the rapid and sustained virological response to recombinant PEG-IFN-α-2a were explored in Iraqi HCV-infected patients with haemoglobinopathy. Forty-three patients were evaluated for the relationship between rapid virological response (RVR), IL-28B polymorphism, viral load, liver enzyme levels, blood group, ultrasound findings, or HCV genotype and the sustained virological response (SVR) achievement. The overall RVR was 55·81% while the overall SVR was 53·49%. SVR in patients that achieved RVR was 82·61% ($P = 0{\cdot}0004$). A significant association was found between initial alanine transaminase levels and viral load with SVR achievement ($P = 0{\cdot}025$) and ($P = 0{\cdot}004$), respectively. Thirty-two (74%) out of 43 of our samples were host genotyped at the IL-28B locus as CC, a significant association was found between CC group and SVR achievement ($P = 0{\cdot}04$). Of our samples, 23/43 (53%) were typed as HCV genotype 4, 10/43 (23%) as genotype 1, 9/43 (20·9%) as genotype 3 and 1/43 (2·3%) as genotype 2. A significant association was found between genotype 3 and SVR achievement ($P = 0{\cdot}006$). Multivariate analysis showed that only RVR achievement independently associated with SVR in the Iraqi population ($P = 0{\cdot}00$). These results can be used to classify the patients requiring the more expensive new direct-acting antiviral drugs.

Key words: Haemoglobinopathy, HCV, Iraqi population, RVR, SVR.

INTRODUCTION

Hepatitis C virus (HCV) infects millions of people worldwide and is a leading cause of cirrhosis and liver cancer [1]. Chronic infection is associated with hepatic inflammation and fibrosis [2]. The combination of pegylated interferon alpha (PEG-IFN-α) and ribavirin is the approved standard, effective and well accepted treatment for chronic hepatitis C [3, 4]. However, the use of ribavirin is controversial in haemoglobinopathic patients because of the haemolytic complications; therefore, interferon alpha (INF-α) monotherapy is used as the best treatment for haemoglobinopathic patients [5]. New antiviral drugs have not been approved yet for the treatment of patients with haemoglobinopathy.

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INF-α induces the JAK/STAT pathway, which up-regulates genes with antiviral effects against HCV [6, 7]. The essential objective of HCV therapy is to cure the infection leading to the elimination of detectable circulating HCV after stopping the treatment. Rapid virological response (RVR) is defined as undetected HCV RNA level at week 4 of treatment [8], while sustained virological response (SVR) is defined as an undetectable HCV RNA level 24 weeks after the end of treatment. HCV genotype has been reported as an important predictor in determining SVR in HCV patients. Treatment has shown greater efficacy in genotypes 2 and 3 than in genotypes 1 and 4 [9, 10]. Genotype 4 is common in the Middle East with an SVR achievement of up to 70% [10, 11]. The SVR rate is about 50–79% for HCV genotype 1 and 75–94% for HCV genotypes 2 and 3 with the advised regimens, in Western and Eastern countries, respectively [12]. This indicates that HCV embodies genetic diversity which demands association study of RVR and SVR with HCV genotypes at the country level.

Moreover, genome-wide association studies (GWAS) have revealed a single nucleotide polymorphism (SNP) located upstream of IL-28B (also called interferon-lambda 3) to be associated with SVR [13, 14]. The SNP plausibly represents a functional variant influencing response of HCV to IFN-α and therefore it also needs to be studied at the country level.

As inference of positive predictive factors of SVR in HCV patients is of clinical importance [2], several predictors of SVR were analysed in this study. The study was designed specifically to study HCV treatment effectiveness using PEG-INF-α in patients with mixed HCV genotype and IL-28B polymorphism, suffering from haemoglobinopathy (thalassaemia or sickle cell anaemia) in the Iraqi population.

MATERIAL AND METHODS

Ethical approval

The study protocol was approved by the Ethics and Research Committee of Azadi Teaching Hospital and the School of Medicine, University of Duhok. Written informed consent was obtained from the participants (or their guardians when aged <18 years) of this study.

Patients and antiviral treatment

We recruited all patients with thalassaemia or sickle cell anaemia and HCV who were referred to the infectious disease unit in Azadi Teaching Hospital, Duhok, Kurdistan Region, Iraq during the period from June 2011 to June 2012. In this period, 47 patients visited the unit and all were treated with recombinant interferon monotherapy (recombinant PEG-IFN-α-2a) at a dose of 180 µg/1·73 m^2. No patients were treated with PEG-IFN plus ribavirin. During treatment, RVR, early virological response (EVR) (as defined by >2 log fall in the viral load by week 12) and as well as post-treatment SVR rates were determined. Three patients did not attend for treatment and one patient refused to continue treatment due to severe myalgia, those patients were therefore excluded from further study. All patients infected with HCV genotypes 1 and 4 received treatment for 48 weeks, while patients infected with genotypes 2 and 3 received treatment for 24 weeks. All patients were followed up by measuring the viral load, alanine transaminase (ALT) and aspartate transaminase (AST) levels at weeks 4, 12, 24 (for genotypes 2 and 3) and week 48 (for genotypes 1 and 4). These investigations were performed at week 24 after stopping treatment. All the patients who achieved SVR were tested for HCV RNA by reverse transcriptase–polymerase chain reaction (RT–PCR) 6 months later (48 weeks after ceasing treatment).

HCV quantification and genotyping

Automated nucleic acid purification was performed using the Qiagen QIAsymphony (Qiagen, USA). The Qiagen RT–PCR assay was performed using the artus HCV QS-RGQ kit (Qiagen) and was run on a Rotor-Gene Q thermocycler. The value for the 95% lower limit of detection given by the manufacturer was 36·2 IU/ml for HCV. The linear ranges of quantification were 67·6 to $1{\cdot}8 \times 10^7$ IU/ml. HCV genotyping was performed by GEN-C 2.0 reverse hybridization strip assay (Nuclear Laser Medicine, Italy). The assay discriminates between HCV genotypes on the basis of variations in the 5′-UTR and core regions.

DNA extraction and IL-28B genotyping

Frozen whole blood was used for IL-28B genotyping after genomic DNA extraction using QIAamp DNA Blood Mini kit (Qiagen). IL-28B genotyping was performed by PCR with confronting two-pair primers (CTPP) PCR as described previously [15].

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Table 1. *The predictive variables in accordance with the response achievement (SVR)*

		Response achieved ($n = 23$)		No response achieved ($n = 20$)	
Predictive variable	Group	Count (n)	(%)	Count (n)	(%)
1. IL-28 type	CC	20	87	12	60
	(non-CC) CT or TT	3	13	8	40
2. RVR	Achieved	21	91·3	4	20
	Not achieved	2	8·7	16	80
3. Ultrasound finding	Normal	20	87	12	60
	Abnormal (hepatomegally or nodules)	3	13	8	40
4. Gender	Male	11	47·8	13	65
	Female	12	52·2	7	35
5. Genotype	1	6	26·1	4	20
	2	0	0	1	5
	3	9	39·1	0	0
	4	8	34·8	15	75
6. Blood group	O	15	65·2	9	45
	A	4	17·3	5	25
	B	3	13	5	25
	AB	1	4·3	1	5

SVR, Sustained virological response; RVR, rapid virological response.

The primer sequences used for IL-28B were (F1: GAC GAGAGGGCGTTAGAGCG, R1: GGAGTGCAA TTCAACCCTGGTTCG; F2: GAGCTCCCCGAA GGCGT, R2: AACGCAGGCTCAGGGTCAAT) [15]. The reactions consisted of a total volume of 50 µl containing 1·5 U Hotstart DNA polymerase (Takara, China), 0·5 µM of primers F1, F2, and R2, 0·75 µM of primer R1, 1·0 µM dNTP and 50 ng genomic DNA. Thermocycler conditions were as follows: preheating at 95 °C for 5 min followed by 40 cycles of 95 °C for 30 s, 69·2 °C for 1 min, and 72 °C for 3 min. PCR products were separated by standard electrophoresis on 2% agarose gels containing ethidium bromide. The homozygous C/C gives bands of 400 bp and 312 bp, the homozygous T/T gives bands of 400 bp and 128 bp, and the heterozygote genotype yields bands of 400 bp, 312 bp, and 128 bp.

Statistical analysis

Obtained data were completely anonymized before statistical analysis. Univariate and multivariate analysis was conducted by logistic regression. All variables that achieved a P value <0·2 were included in the multivariate study. Step-down regression was used where the variable with the lowest partial correlation was eliminated first. Variable confounder was determined if its deletion changed the coefficient by 20% of a statistically significant predictor. The statistical analysis was conducted with SPSS version 21 (SPSS Inc., USA).

RESULTS

Analysis was performed for patients [males ($n = 27$, 57·45%); females ($n = 20$, 42·55%)] suffering from HCV (genotypes 1–4) and treated with recombinant PEG-INF-α-2a monotherapy (Table 1). The ages of the subjects ranged from 6 to 36 years (mean 14·4 years).

SVR achievement

Of our patients, 53·49% (23/43) achieved SVR as defined as undetectable levels of viral load 24 weeks after the end of the treatment. All the patients who achieved SVR tested negative for HCV RNA via RT–PCR results 24 weeks later (at 48 weeks post-treatment).

The association between different factors and SVR achievement

Univariate analysis

The data of SVR achievement were stratified according to initial viral load, ALT, AST, RVR, gender, IL-28 polymorphism, HCV genotype, blood group

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Table 2. *The relationship between response achievement (SVR) and the study independent variables by univariate regression analysis*

Variable	Type of variable	Type of relationship with SVR (*P* value)	OR	95% CI
Viral load	Scale	Significant (0·004)	0·3145	0·1321–0·7487
ALT	Scale	Significant (0·025)	0·9896	0·9806–0·9987
AST	Scale	Not significant (0·054)	0·9887	0·9770–1·0006
RVR	Categorical	Significant (0·000)	26·2500	5·1071–134·9222
Gender	Categorical	Not significant (0·258)	0·5833	0·1708–1·9927
IL-28	Categorical	Significant (0·04)	0·1964	0·0432–0·8929
Genotype	Categorical	Significant (0·006)	2·134	3·2861–86·396
Ultrasound finding	Categorical	Not significant (0·14)	0·3429	0·0827–1·4209
Blood group	Categorical	Not significant (0·591)	0·6154	0·1827–2·0722

OR, Odds ratio; CI, confidence interval; SVR, sustained virological response; ALT, alanine transaminase; AST, aspartate aminotransferase; RVR, rapid virological response;

Table 3. *Significance of sustained virological response (SVR) achievement in HCV genotypes*

HCV	SVR achieved*	95% CI	*P* value
Genotype 1	(6/10), 60%	(26·24–87·84)	0·006
Genotype 3	(8/9), 88·89%	(51·75–99·72)	
Genotype 4	(9/23), 43·48%	(23·19–65·51)	

CI, Confidence interval.
A significant association was found between genotype 3 and SVR achievement. One patient had genotype 2 infection. RVR was achieved but not SVR.
* SVR achieved = (no. of patients/total no. of patients with specified genotype).

and ultrasound findings. Univariate logistic regression analysis was conducted to study the association between individual factor and SVR (Table 2).

RVR and EVR

All the patients who achieved EVR achieved SVR. RVR for all subjects was 55·81% while SVR was 53·49%. SVR in patients that achieved RVR was 82·61% ($P = 0·0004$) (Table 3).

IL-28B polymorphisms

Thirty-two (74%) out of 43 of our sample were genotyped as CC and 20/32 (62%) of these CC samples achieved RVR and along with SVR. Eleven (25%) out of 43 of our samples were typed as TT/CT and 3/11 (27%) of these subjects achieved RVR and SVR. A significant difference was found between CC group and TT/CT group in SVR achievement ($P = 0·04$) (Table 2).

HCV genotype

Twenty-three (53%) out of 43 of our samples were typed as HCV genotype 4, 10/43 (23%) as genotype 1, 9/43 (20·9%) as genotype 3 and 1/43 (2·3%) as genotype 2. SVR was achieved in 88·89% of the patients infected with HCV genotype 3 ($P = 0·006$) (Table 3) (Fig. 1).

Baseline viral load, ALT and AST

Regression analysis was conducted for elucidating independent associations between each of ALT, AST or viral load prior to the initiation of treatment and SVR. There was a significant association between ALT levels at baseline and the attainment of SVR (mean ± S.D.: 67 ± 68 *vs.* $144 \pm 83·7$, $P = 0·025$) (Fig. 2) while no significant association was found between AST and SVR achievement ($83 \pm 53·4$ *vs.* 114 ± 55, $P > 0·05$). In addition, a significant association was found between the levels of viral load and SVR achievement (log mean ± S.D.: $4·5 \pm 0·8$ *vs.* $5·2 \pm 0·83$, $P = 0·004$) (Fig. 3).

Multivariate analysis

Logistic regression was used to perform the multivariate analysis. In this test a dichotomous outcome is predicted by one or more variables. We used logistic regression to model the effects of RVR, AST, ALT, viral load, IL-28 polymorphism and genotype on SVR achievement. It was found that RVR is the only independent factor associated with SVR achievement ($P = 0·00$) (Table 4). None of the variables could achieve the 20% reduction in the coeffiecient of relationship and therefore could not be regarded as a confounder.

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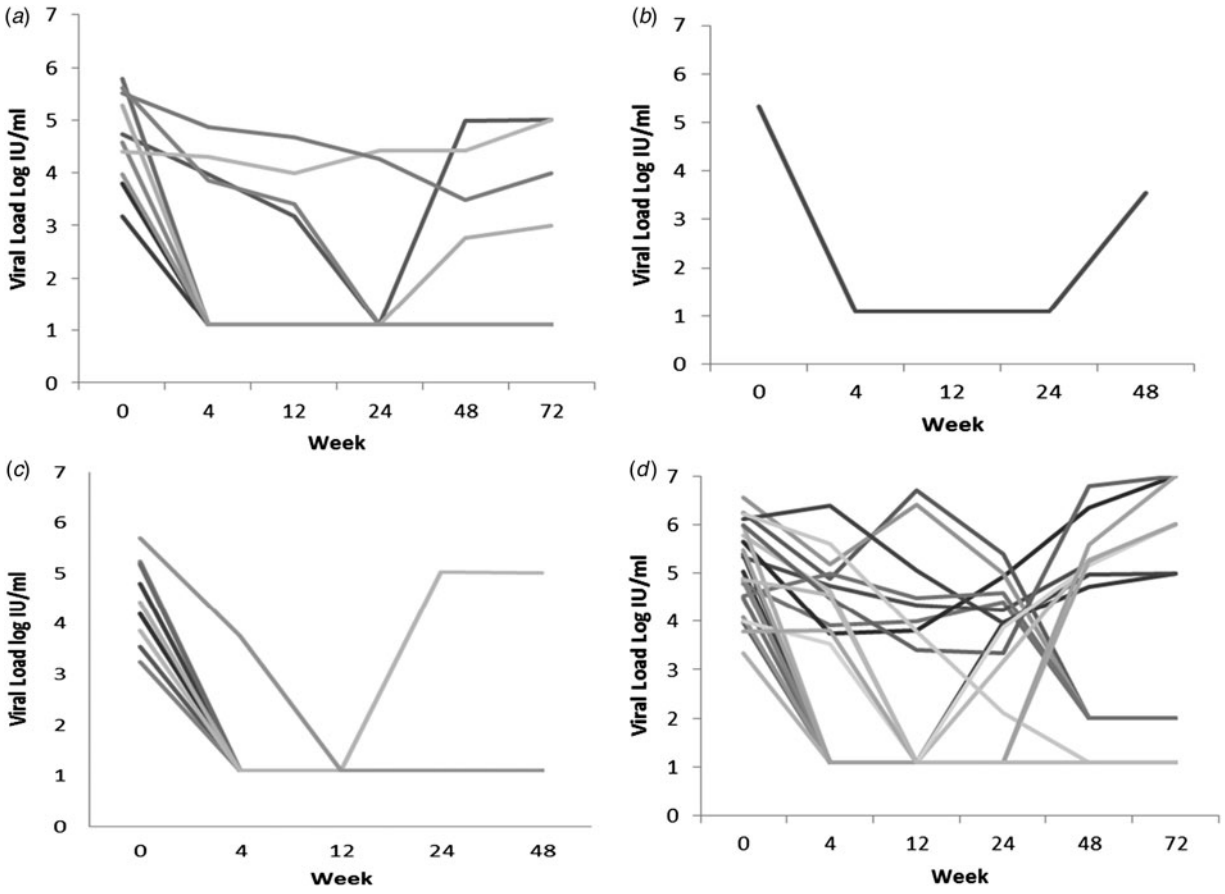

Fig. 1. The RNA $\log_{10}$ IU/ml changes in subjects chronically infected with HCV genotype 1 (*a*), genotype 2 (*b*), genotype 3 (*c*) and genotype 4 (*d*) following PEG-IFN monotherapy.

DISCUSSION

The relationship between SVR achievement and the baseline characteristics blood group, hepatomegaly, gender, genotype, IL-28B polymorphism and viral load was assessed in the recruited patients.

Several previous clinical studies [16] showed that IFN monotherapy administered for 6–15 months induced a sustained biochemical response and SVR in 40–50% of thalassemia patients with HCV-related chronic hepatitis. In a study conducted in Iran comprising thalassaemia patients SVR was achieved, ranging from 24% for IFN monotherapy to 51% in patients receiving combined therapy [5, 17]. In our study SVR was achieved in about 50% of our patients. Our results are encouraging; however, our data mean that about half of our patients failed to achieve SVR. This warrants clinical trials of the developing direct-acting antiviral drugs (DAA) in patients with haemoglobinopathy.

Previous studies conducted in the central region in Iraq showed that 35% of the samples typed as genotype 4 while 50% of the recruited samples typed as genotype 1 [18]. No genotypes 2 and 3 were reported in that study. In our study, 53% of our samples typed as HCV genotype 4 followed by 23% for genotype 1, 20·9% for genotype 3 and 2·3% for genotype 2. A previous study conducted in Iran showed that the majority of chronically HCV-infected Iranian patients were infected with HCV genotype 1 (47%) followed by genotype 3 (36%) [19]. Previous studies from Saudi Arabia, Kuwait [20] and Yemen [21] found that genotype 4 was the most prevalent HCV genotype in those countries. It is probable that differences in race, routes of transmission, and different socioeconomic factors might explain this variation.

In agreement with other studies [16], RVR proved to be the strongest independent predictor of SVR. No RVR achievement is an unfavourable marker for SVR. It was previously shown that HCV genotype, pre-treatment viral load and IL-28B polymorphisms played a significant role in SVR achievement [13, 14,

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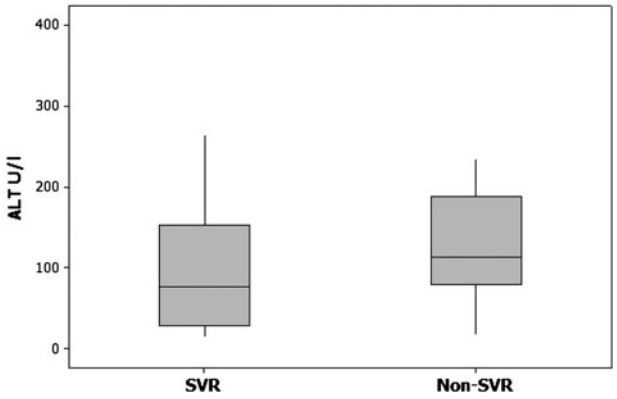

Fig. 2. Boxplot depicting serum levels of alanine transaminase (ALT) according to sustained virological response (SVR) achievement. ALT levels were measured before starting the treatment and stratified according to SVR achievement. Significant differences were found in the levels of ALT between the group of patients who achieved SVR and those who did not achieve SVR.

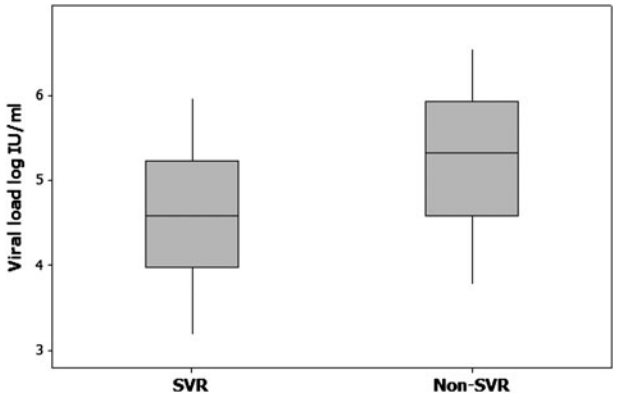

Fig. 3. Boxplot depicting viral load log according to sustained virological response (SVR) achievement. Viral load log levels were measured before starting the treatment and stratified according to SVR achievement. Significant differences were found in the levels of viral load log between the group of patients who achieved SVR and those who did not achieve SVR.

22–24]. In our study, most patients with IL-28B CC genotype and those infected with HCV genotype 3 achieved SVR. In agreement with previous studies [16], we found a significant association between ALT or viral load levels and SVR achievement while no association was found between AST levels and SVR achievement. However, when multivariate analysis was performed, RVR was the only factor that showed association with SVR achievement. This might be due to the small sample size used in this study. Until other studies are performed with larger sample sizes, these results can be used to counsel patients on the likelihood of achieving SVR. As the new DDAs are expensive and not available for all patients, these predictive factors can be used as parameters for selecting patients for these new regimens. No association was found between gender, ABO blood group or ultrasound findings and SVR achievement.

Table 4. *The relationship between response achievement (SVR) and the study independent variables by multivariate regression analysis*

Variable	S.E.	*P* value	OR	95% CI
ALT	0·0113	0·8500	1·0021	0·9803–1·0245
AST	0·0078	0·1205	0·9880	0·9730–1·0032
Viral load	0·5887	0·1074	0·3876	0·1222–1·2288
IL-28	1·2409	0·1653	0·1788	0·0157–2·0350
RVR	0·8814	0·0002	27·4128	4·8719–154·242
Ultrasound findings	1·1610	0·1921	0·2199	0·0226–2·1409
Genotype	1·1755	0·2691	1·034	0·7210–2·7316

OR, Odds ratio; CI, confidence interval; ALT, alanine transaminase; AST, aspartate aminotransferase; RVR, rapid virological response; SVR, sustained virological response.

To conclude, little is known about the response rate to PEG-INF treatment of HCV in Iraq. This study elucidated whether early changes in HCV RNA levels during treatment with PEG-IFN could be used to accurately predict treatment response and identify the factors that influence response rate to antiviral therapy. Therapy is very expensive in Iraq and not all patients can afford it, therefore early discontinuation of treatment in non-responders could avoid the expense and inconvenience of continuing unnecessary treatment allowing recourse to other alternatives such as DAA.

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DECLARATION OF INTEREST

None.

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