Safety and Efficacy of Direct Acting Antivirals for Hepatitis C Virus Infection in Thalassemic Patients and Its Effect on Transfusion Requirements

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ABSTRACT

Background: The prevalence of hepatitis C virus (HCV) infection among thalassemic patients in Egypt ranged between 24 and 37%. Direct acting antivirals (DAAs) have revolutionized the standard of care for treatment of hepatitis in hemoglobinopathies. **Objective:** The aim of this study was to evaluate the safety, efficacy, and tolerability of ribavirin (RBV) free DAAs in thalassemic patients and its effect on transfusion requirements.

Patients and Methods: In this study, 200 adult chronic hepatitis C (CHC) patients were enrolled. They were further divided into two groups. Group (I) included 150 HCV-thalassemic patients and group (II) included 50 HCV only patients. Sustained virological response (SVR) was assessed by reverse transcriptase polymerase chain reaction (RT-PCR) for HCV-RNA 12 weeks post-treatment. Any treatment related adverse events were reported.

Results: SVR was achieved in 89.33% in group (I) and in 92% in group (II). Among group (I), there was significant improvement in mean hemoglobin level after treatment. Moreover, mean ALT, AST, total and indirect bilirubin levels dropped significantly after treatment (P<0.001). There was decrease in blood transfusion requirements after treatment. There was no need to change or modify the dose of iron chelating agents. Apart from four patients in group (Ib) who developed hepatic fulmination and hepatocellular carcinoma requiring cessation of treatment, minor side effects were reported that were managed conservatively. **Conclusion:** RBV-free-DAAs are effective and well tolerated among HCV-thalassemic patients.

Keywords: HCV, Thalassemic patients, Direct acting antivirals, Hepatitis C Virus, Sofosbuvir, Daclatasvir.

INTRODUCTION

Thalassemia is one of the most common inherited genetic blood disorders which results from reduced rate of synthesis of alpha or beta globin chains leading to destruction of red cells in the bone marrow (ineffective erythropoiesis) and peripheral circulation (hemolysis) [(1)]. β-thalassemia is more common in Mediterranean region while α-thalassemia is more common in the Far East [(2)]. β-thalassemia constitutes a major health problem in Egypt with an estimated carrier rate of 9-10% [(3)].

Survival of thalassemic patients depend on regular blood transfusion which lead to further complications including iron overload and transfusion-transmitted infections (TTIs) such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) [(4)]. HCV is a major health problem in transfusion-dependent thalassemic patients, along with iron overload it represents a risk factor for the development of liver fibrosis, cirrhosis, decompensation, and hepatocellular carcinoma [(5, 6)]. The prevalence of HCV infection among thalassemic patients in Egypt ranged between 24 and 37% [(3)].

Aside from effective chelation treatment, eradication of HCV is the sole approach to stop the progression of liver disease, since achieving sustained virological response (SVR) has been found to reduce liver-related mortality and the need for liver transplantation [(7)].

Standard antiviral therapy with pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) has been the standard of care for HCV infection for many years [(8)]. However, IFN modest efficacy and increased RBV induced hematological adverse effects in thalassemic patients has deferred the use of this combination thus failing to halt the progression to cirrhosis [(9)].

IFN-free regimens with direct acting antivirals (DAAs) have been approved for HCV treatment with impressive cure rates (> 90%) and mild adverse events [(10)]. According to international guidelines, thalassemic patients should be treated with these new regimens preferably those without RBV [(11)].

However, limited data exist regarding real life experiences on the safety and efficacy of DAAs in this special population. Therefore, this study was designed to evaluate the safety and efficacy of RBV-free DAAs in adult thalassemic Egyptian patients with HCV infection.

PATIENTS AND METHODS

This prospective observational study included a total of 200 adult chronic hepatitis C (CHC) patients who were eligible for therapy, attending at Viral Hepatitis Treatment and Research Centre, Ain Shams University, Cairo, Egypt. This study was conducted between September 2017 to September 2019.

Patients were further categorized into 2 groups:

Group I included 150 patients with CHC and thalassemia, either transfusion dependent thalassemia (TDT), or non-transfusion dependent thalassemia (NTDT). Whereas **Group II** included 50 patients with CHC only. Patients with co-infection with HBV or HIV, hepatocellular carcinoma (HCC), and pregnant women were excluded from the study.

Ethical Consideration:

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All participants signed an informed written consent prior to inclusion in the study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. The study was approved by the Ethical Review Board of Ain Sham University.

The study population was subjected to a well-designed data sheet covering the following topics: demographic data, treatment status (naïve or experienced), comorbidities, blood transfusion requirements. HCV-thalassemic patients receive iron chelation therapy as shown in (Table 1). Deferiprone is an oral preparation given at an initial dose of 75 mg/kg/day and a maximum dose of 99 mg/kg/day. Deferasirox is another oral preparation given at a dose of 20 mg/kg/day and a maximum dose of 40 mg/kg/day. Deferoxamine is given intravenously at a dose of 40-50 mg/kg/day over 8-12 hours for 5-7 days/week. Transfusion dependent patients were transfused to keep a pre-transfusion hemoglobin level of 8 g/dl.

Table (1): Comparison between Group Ia and group Ib as regard iron chelating agents.

Type of Chelation	Group I (n=150)					Chi-Square	
	Group Ia		Group Ib		Total		
	N	%		%	N (%)	X^2	P-value
No chelator	2	2.78	14	17.95	16 (10.6%)	9.747	0.021*
Deferiprone	32	44.44	26	33.33	58 (38.6%)		
Deferoxamine	6	8.33	8	10.26	14 (9.3%)		
Deferasirox	32	44.44	30	38.46	62 (41.3%)		
Total	72	100.00	78	100.00	150 (100%)		

All patients underwent baseline investigations including complete blood count (CBC), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum total and direct bilirubin, serum albumin, international normalization ratio (INR), serum creatinine, alpha-fetoprotein, transferrin saturation, serum ferritin, and pelvi-abdominal ultrasound. HCV viral load was determined by real-time polymerase chain reaction (RT-PCR) assay. Moreover, fibrosis 4 index (FIB-4) was calculated as a non-invasive assessment for liver fibrosis by the following equation=age (years)×AST (IU/L)/ [Platelet count (10^9 /L) ×$ALT^{1/2}$ (IU/L)]), where the result of FIB-4 score was classified into: <1.45: Low risk of advanced liver fibrosis, 1.45-3.25: Intermediate risk of advanced liver fibrosis, and >3.25: High risk of advanced liver fibrosis.

All thalassemic patients received sofosbuvir (SOF) 400 mg and daclatasvir (60 mg). The treatment duration ranged from 12 to 24 weeks according to patients' categorization according to National Committee for Control of Viral Hepatitis (NCCVH). Patients were categorized as easy to treat if treatment naïve, total bilirubin < 1.2mg/dl, serum albumin > 3.5g/dl, INR < 1.2, platelet count > 150,000/mm^3 and hard to treat if meeting any of the following criteria: treatment experienced, total bilirubin ≥ 1.2 mg/dl, serum albumin ≤ 3.5g/dl, INR ≥ 1.2 or platelet count ≤ 150,000/mm^3.

Patients underwent a 4-weekly follow-up CBC, ALT, AST, serum total and direct bilirubin, INR, and serum creatinine. At each clinical visit blood transfusion requirements (unit/month) were recorded and compared with previous requirements before starting treatment, and any treatment related adverse events were reported. The effectiveness of the treatment was assessed based upon the achievement of SVR twelve weeks after cessation of the treatment. The patient's quality of life such as daily activities, social life and work performance was evaluated by Eastern Cooperative Oncology group (ECOG) performance status[12] (Table 2).

Table (2): ECOG performance status[12].

ECOG Performance Status	
Grade	**ECOG score**
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead.

Statistical analysis

Statistical analysis of the present study was conducted, using the mean, standard deviation (SD), student t-test, Chi-square, linear correlation coefficient, and Analysis of variance [ANOVA] tests by SPSS V17. Student t-test was used to compare between two groups in quantitative data. Linear correlation coefficient was used for detection of correlation between two quantitative variables in one group. ANOVA test was used for comparison among different times in the same group in quantitative data. P value < 0.05 was considered significant.

RESULTS

Study population:

A total of 200 CHC patients were enrolled in this study. They were divided into 2 groups. Group I included 150 patients with CHC and thalassemia, and Group II included 50 patients with CHC only. Each group was further classified into 2 subgroups: easy to treat (a) and difficult to treat (b).

Group I included 72 (48%) HCV-thalassemic patients easy to treat (Ia), and 78 (52%) HCV-thalassemic patients difficult to treat (Ib). Group II included 35 (70%) HCV patients easy to treat (IIa), and 15 (30%) HCV patients difficult to treat (IIb). All patients completed the treatment course except 4 patients in group (Ib). The rest of the patients' demographic, clinical characteristics, and laboratory investigations are summarized in (Table 3).

Table (3): Baseline demographic, clinical characteristics, and laboratory investigations of the enrolled patients.

	Group I (n=150)		**Group II (n=50)**	
	Ia (n=72)	**Ib (n=78)**	**IIa (n=35)**	**IIb (n=15)**
Age (years), mean±SD	29.75±8.21	33.56±7.89	37.97±13.51	40.8±12.73
Gender, n (%)				
• **Male**	44 (61.11)	42 (53.85)	24 (68.57)	11 (73.33)
• **Female**	28 (38.89)	36 (46.15)	11 (31.43)	4 (26.67)
Weight (kg), mean±SD	71.22±5.64	73.2±6.47	76.17±9.31	79.07±8.05
Treatment status, n (%)				
• **Naïve**	72 (100)	68 (87.18)	35 (100)	15 (100)
• **Experienced**	0 (0)	10 (12.83)	0 (0)	0 (0)
Comorbidities, n (%)				
• **Free**	56 (77.78)	58 (74.36)	29 (82.86)	10 (66.67)
• **DM**	6 (8.33)	12 (15.38)	4 (11.43)	2 (13.33)
• **HTN**	10 (13.89)	0 (0)	1 (2.86)	0 (0)
• **DM±HTN**	0 (0)	8 (10.26)	1 (2.86)	3 (20)
ECOG performance, n (%)				
• **0**	0 (0)	0 (0)	29 (82.86)	11 (73.33)
• **1**	66 (91.67)	58 (74.36)	6 (17.14)	3 (20)
• **2**	6 (8.33)	20 (25.64)	0 (0)	1 (6.67)
Ferritin (ng/ml), mean±SD	1403.556±1014.915	1389.256±635.481		

Prior to DAAs administration, liver fibrosis was assessed for all patients by non-invasive FIB-4 score. Risk of advanced liver fibrosis according to FIB-4 interpretation is shown in (Table 4).

Table (4): Risk of advanced fibrosis according to FIB-4 interpretation in all studied group of patients.

Risk of Advanced Fibrosis (FIB4 Interpretation)		**Groups**								**Chi-Square**	
		Group Ia		**Group Ib**		**Group IIa**		**Group IIb**			
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**X^2**	**P-value**
Before treatment	**Low**	59	81.94	53	67.95	31	88.57	12	80.00	7.410	0.060
	Intermediate	13	18.06	25	32.05	4	11.43	3	20.00		
After treatment	**Low**	66	91.67	53	67.95	29	82.86	8	53.33	22.512	0.001*
	Intermediate	6	8.33	21	26.92	6	17.14	7	46.67		
	Stopped	0	0.00	4	5.13	0	0.00	0	0.00		

Parameters of efficacy, and virological response:

180 out of 200 patients achieved SVR (90%) twelve weeks after the end of treatment. Among the HCV-thalassemic group of patients, all easy to treat group (Ia) achieved SVR (100%), while 62 (79.49%) patients of the difficult to treat group (Ib) achieved SVR, and 4 (5.13%) patients didn't complete the course of treatment. Out of the 50 HCV patients, 33 (94.29%) patients of the easy to treat group (IIa) achieved SVR, and 13 (86.67%) patients of the difficult to treat group (IIb) achieved SVR.

Among the HCV-thalassemic group of patients, both easy and difficult to treat groups (Ia, and Ib) showed significant improvement in the pretransfusion mean hemoglobin level after treatment (P<0.001 and P=0.002, respectively). Moreover, mean ALT, AST, total, and indirect bilirubin dropped significantly at the end of treatment compared to baseline levels (P<0.001); (Table 5).

Table (5): Comparison between different laboratory parameters before treatment and at end of treatment (EOT) in Group Ia and Ib.

	Group I (n=150)					
	Group Ia			Group Ib		
	Baseline	EOT	P value	Baseline	EOT	P value
TLC (10^9/L)	8.35±5.6	9.91±7.49	0.026*	11.49±7.01	10.84±5.52	0.738
Hb (g/dl)	8.33±0.83	8.78±0.73	<0.001*	7.84±0.76	8.17±0.75	0.002*
PLT (10^9/L)	306.08±191.79	299.78±180.61	0.294	366.31±265.47	350.14±275.29	0.226
ALT (IU/L)	73.28±18.37	53.87±11.38	<0.001*	73.26±24.71	50.03±15.28	<0.001*
AST (IU/L)	72.31±18.92	53.83±12.69	<0.001*	73.13±24.7	48.6±14.68	<0.001*
T. Bilirubin (mg/dl)	3.38±1.1	3.04±0.95	<0.001*	3.27±1.18	2.85±1.16	<0.001*
I. Bilirubin (mg/dl)	2.64±0.97	2.46±0.91	0.002*	2.48±1.08	2.28±1.1	0.003*
Albumin (g/dl)	3.9±0.32	3.9±0.33	0.74	3.65±0.36	3.78±0.41	0.037*
INR	1.06±0.07	1.12±0.12	<0.001*	1.26±0.12	1.25±0.13	0.07

On the other hand, the hemoglobin level didn't show significant difference between baseline and post-treatment levels in group IIa and IIb (P=0.49 and P=0.103, respectively). Similarly, the mean ALT level didn't change significantly after treatment from baseline levels. However, there was significant difference in mean AST, total, and indirect bilirubin levels; (Table 6).

Table (6): Comparison between different laboratory parameters before treatment and at EOT in Group IIa and IIb.

	Group II (n=50)					
	Group IIa			Group IIb		
	Baseline	EOT	P value	Baseline	EOT	P value
TLC (10^9/L)	6.54±2.7	6.51±2.05	0.962	7.61±2.02	5.77±2.31	0.041*
Hb (g/dl)	12.28±1.85	12.44±1.46	0.49	7.75±0.57	8.03±0.55	0.103
PLT (10^9/L)	261.97±91.62	257.2±81.27	0.456	177.8±88.64	209.53±103.01	0.359
ALT (IU/L)	36.17±15.18	35.2±10.26	0.469	44.6±19.37	37.33±14.73	0.311
AST (IU/L)	33.23±11.27	35.69±11.17	0.05*	46.27±18.93	34.87±12.11	0.027*
T. Bilirubin (mg/dl)	0.86±0.2	0.99±0.17	<0.001*	0.77±0.24	0.88±0.18	0.074
I. Bilirubin (mg/dl)	0.39±0.19	0.52±0.19	0.001*	0.29±0.18	0.47±0.15	0.005*
Albumin (g/dl)	3.92±0.34	3.87±0.23	0.47	3.83±0.48	3.61±0.22	0.098
INR	0.99±0.08	1±0.08	0.497	1.06±0.17	1.05±0.15	0.66

The non-invasive assessment of liver fibrosis pre- and post-treatment showed significant improvement in FIB-4 score in group Ia and Ib (0.979±0.469 vs 0.893±0.39, 1.257±0.757 vs 1.046±0.639 respectively) but not in group IIa and IIb (P=0.521 and P=0.086 respectively) (Table 7).

Table (7): Comparison between FIB4-score before treatment and at end of treatment in the studied population.

FIB4 score		Groups				ANOVA	
		HCV thalassemic Easy to treat (Ia)	HCV thalassemic Difficult to treat (Ib)	HCV Easy to treat (IIa)	HCV Difficult to treat (IIb)	F	P-value
Before treatment	Range	0.22-2.31	0.2-3.1	0.3-2.21	0.26-1.9	3.848	0.010*
	Mean ±SD	0.979±0.469	1.257±0.757	0.912±0.520	1.004±0.458		
After treatment	Range	0.12-1.85	0.1-2.38	0.3-1.93	0.35-2.48	3.167	0.026*
	Mean ±SD	0.893±0.390	1.046±0.639	0.947±0.462	1.329±0.683		
Differences		0.086±0.213	0.172±0.426	-0.035±0.316	-0.325±0.680		
Paired Test		0.001*	0.001*	0.521	0.086		

The frequency of blood transfusion was assessed by monthly follow-up of transfusion requirements (unit/month). There was significant statistical difference between both groups throughout the course of treatment regarding the decrease in required transfusion unit to maintain a pretransfusion hemoglobin of 8 gm/dl as shown in (Table 8).

Table (8): Comparison between HCV thalassemic easy and difficult to treat group of patients regarding transfusion requirements.

Transfusion Assessment (Unit/month)		HCV thalassemic				Chi-Square	
		Easy (Ia)		Difficult (Ib)			
		N	%	N	%	X^2	P-value
Start of Treatment	0 Unit	0	0.00	2	2.56	10.234	0.037*
	0.5 unit	0	0.00	6	7.69		
	1 unit	24	33.33	18	23.08		
	2 units	36	50.00	44	56.41		
	3 units	12	16.67	8	10.26		
End of treatment	0 unit	12	16.67	8	10.25	16.872	0.001*
	1 unit	38	52.78	28	37.89		
	2 units	18	25	38	48.71		
	3 units	4	5.56	0	0		

Safety:

All adverse events experienced during treatment were noted. Apart from minor adverse events e.g., fatigue, and headache (Table 9), four patients in group Ib experienced major adverse events necessitating discontinuation of treatment. Two patients developed liver cell fulmination and the other two patients discovered HCC by triphasic pelviabdominal CT during treatment.

Table (9): Comparison between the four studied groups regarding the safety of the treatment.

Minor side effects	Groups								Chi-Square	
	HCV thalassemic easy to treat (Ia)		HCV thalassemic difficult to treat (Ib)		HCV easy to treat (IIa)		HCV difficult to treat (IIb)			
	N	%	N	%	N	%	N	%	X^2	P-value
Free	60	83.33	62	79.49	31	88.57	10	66.67	31.6	0.064
Fatigue	4	5.56	2	2.56	1	2.86	1	6.67		
Headache	2	2.78	4	5.13	1	2.86	1	6.67		
Diarrhea	4	5.56	8	10.26	1	2.86	1	6.67		
Dizziness	2	2.78	0	0.00	0	0.00	0	0.00		
Nausea	0	0.00	2	2.56	0	0.00	0	0.00		
Distention	0	0.00	0	0.00	1	2.86	1	6.67		
Constipation	0	0.00	0	0.00	0	0.00	1	6.67		
Total	72	100.00	78	100.00	35	100.00	15	100.00		

DISCUSSION

Patients with hemoglobinopathies are at a greater risk of contracting HCV, especially if they were transfused before donor screening systems were implemented. Although advances in blood transfusion policy and antiviral medication in conjunction with iron chelation therapy, HCV-related liver disorders continue to be a significant cause of death in these individuals [(13)].

The treatment of HCV thalassemic patients generated controversy. The two-drug treatment regimen with Peg-IFN/RBV has led to RBV-induced hemolytic anemia in many patients with thalassemia [(14)]. On the other hand, it appears that omitting RBV from the regimen and/or the use of lower doses of RBV decreases SVR [(15)].

The recent advent of DAAs opened new venues in the treatment of CHC regarding high SVR rates and better compliance due to their oral route of administration and the rarity of significant side effects. The recent EASL treatment recommendations on hepatitis C suggested that patients with haemoglobinopathies should be treated with IFN-free regimen without ribavirin [(16)].

In this study, the efficacy, safety, and tolerability of the new DAAs were studied among 200 CHC patients who were classified into 2 groups; group (I) included 150 HCV-thalassemic patients and group (II) included 50 HCV patients. Each group was divided into 2 subgroups; easy to treat (a) and difficult to treat (b) according to NCCVH protocol.

The current study showed a high SVR of 90%, which agrees with other studies done on this special population of patients by **Ahmed *et al.*** [(17)] **and Mehta *et al.*** [(18)] showing SVR of 96% and 100% respectively.

Moreover, an Italian study conducted on thirty-four HCV patients with different

hemoglobinopathies including thalassemia major, intermedia, and sickle cell disease. 94% achieved SVR. Two thalassemia major patients with severe comorbidities and treated for 24 weeks, were non-responders with relapse after 1 month. One of them had hepatocellular carcinoma, and the other had severe restrictive cardiomyopathy [19].

This study showed that there was a statistically significant improvement in the mean hemoglobin level after treatment in HCV-thalassemic patients (Ia and Ib). In contrast, other studies showed that there was no change in the hemoglobin level pre-and post-treatment[20-22].

Our results showed significant reduction in the mean ALT level at the end of treatment in thalassemic patients (P<0.001) which parallel the drop in HCV RNA. This was similar to conclusions reported by different studies [21-23].

In the current study, blood transfusion requirements (unit/month) were calculated among the HCV-thalassemic patients before, during and after treatment. There was significant statistical difference between subgroups (Ia) and (Ib) before (P=0.037) and after (P=0.001) treatment. Moreover, there was decrease in the transfusion requirements among patients in both subgroups after treatment. These results were supported by **Mehta *et al.*[18] and Maffei *et al.* [19]** as they stated that there was no change in blood transfusion requirements after treatment of HCV-thalassemic patients**.** In contrast, **Ghoneem *et al.*[22],** reported twelve patients with increased blood transfusion requirements.

The iron chelating agents were studied in the present study among HCV-thalassemic patients to observe any interaction between them and DAAs and to record the need to change or modify their dose during treatment course. There wasn't any need to change or modify the dose of chelating agents nor any side effects or any interaction between them and DAAs drugs during treatment course. This comes in accordance with other studies in which there was no significant difference in requirements of iron chelators during treatment course [18-20].

Most patients experienced minor adverse events that didn't require cessation of treatment and were managed conservatively agreeing with other studies conducted on thalassemic patients[17-19, 21-22]. Regarding major adverse events, two patients in group (Ib) suffered from hepatic fulmination and another two patients suffered from HCC requiring cessation of treatment.

CONCLUSION

RBV-free-DAAs are well tolerated among HCV-thalassemic patients and can reduce the need for blood transfusion with no need to change or modify the dose of iron chelating agents nor any side effects or interaction between them and DAAs drugs during treatment course.

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