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Evaluation of Liver Fibrosis by FibroScan in β-Thalassemia Children Infected with Hepatitis C Virus Before and After Ledipasvir/Sofosbuvir Therapy

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Authors' contributions

This work was carried out in collaboration among all authors. Author ALY designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors HHS, GESS and MEDAAA managed the analyses of the study. Author EESEH managed the literature searches and wrote the final version of this manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Thalassemic children develop liver fibrosis because of liver iron overload and hepatitis C virus (HCV) infection. Transient elastography (FibroScan) can be a reliable non-invasive method for evaluating liver fibrosis in thalassemic patients. Treatment with ledipasvir/sofosbuvir (LED/SOF) direct acting antiviral agents can significantly affect liver stiffness measurement (LSM) by FibroScan.

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Aims: To assess liver fibrosis by non-invasive FibroScan through LSM before and after generic LED/SOF therapy in multi-transfused β-thalassemic children infected with HCV.
Place and Duration of Study: Pediatric Hematology Unit, Tanta University Hospital, from November 2017 to May 2019.
Methodology: Fifty multi-transfused β-thalassemic treatment-naïve children (aged 12-18 years and weighing ≥35kg) with chronic HCV infection were subjected to clinical evaluation, quantitative HCV PCR assay, FibroScan examination, and calculation of APRI, FIB4 index and AST/ ALT ratio. In addition to standard therapy, generic LED/SOF (90/400 mg) treatment was given for 12 weeks' duration with follow up for further 12 weeks after end of treatment.
Results: A positive HCV PCR was changed into negative for all studied patients starting from week 4 after treatment. There was highly significant reduction in the LSM values by FibroScan in the studied patients after therapy (p-value <0.001) with median reduction of 19.4 %. The significant reduction in LSM values was particularly prominent in patients with significant (F2) and advanced (F3) liver fibrosis stages as well as cirrhotic patients (F4). There was significant reduction in the values of other non-invasive liver fibrosis markers FIB-4 index, APRI score and AST/ ALT ratio (p-value <0.001, <0.001 and 0.020 respectively) after therapy.
Conclusion: Generic LED/SOF therapy for 12 weeks' duration resulted in eradication of HCV infection that was associated with significant decrease in LSM by FibroScan particularly those with higher baseline liver fibrosis stages.

Keywords: FibroScan; liver fibrosis; liver stiffness measurement; β-Thalassemia; Hepatitis C virus; direct acting antiviral drugs; ledipasvir/sofosbuvir.

1. INTRODUCTION

Thalassemias are heterogeneous group of genetic disorders of hemoglobin synthesis [1]. In Egypt, thalassemia is considered a major health problem since 1000 children with thalassemia are born annually [2].

In thalassemia patients infected with hepatitis C virus (HCV), the development and severity of liver fibrosis are strongly related to the extent of liver iron overload as well as to the presence of chronic HCV infection [3]. It's now clearly recognized that, HCV infection is the main risk factor for liver fibrosis in transfusion-dependent thalassemia patients, while excess liver iron is a cofactor for the development of advanced fibrosis in patients with HCV infection [4].

Although, hepatic fibrogenesis has long been considered an irreversible process, it is now evident that it is a dynamic process with significant potential for reversal; unlike cirrhosis, which is irreversible. That makes identification of liver fibrosis at an early stage of great significance [5,6].

Liver biopsy has long been considered the gold standard for assessing hepatic fibrosis [3]. However, it is an invasive and painful procedure, with rare but potential life threatening complications, limiting its acceptance and repetition by the patients. Thus there is a need to develop and validate non-invasive tests that can accurately reflect the full spectrum of hepatic fibrosis, cirrhosis, and its severity in liver diseases [7]. Transient elastography (FibroScan) is a novel, rapid, and non-invasive technique which measures liver stiffness [8]. A recent study demonstrated that transient elastography (TE) is a reliable noninvasive method for diagnosing liver fibrosis in thalassemia patients regardless of the degree of iron overload [9].

2. MATERIALS AND METHODS

This study was carried out on fifty multi-transfused β-thalassemic children with superadded hepatitis C virus infection with no previous trials of HCV treatment. Children included were aged from 12-18 years and weighted ≥35 kgm. The study was carried out at the Pediatric Hematology Unit, Tanta University Hospital, after approval by the ethical committee of the Faculty of Medicine, Tanta University. The study was done over 17 months from November 2017 up to May 2019. Children recruited in the study received DAAs therapy in the form of Ledipasvir (90 mg)/Sofosbuvir (400 mg); as a daily single oral tablet for 12 weeks, then re-evaluated after another 12 weeks by PCR for HCV RNA to insure sustained viral remission (SVR).

2.1 FibroScan (Transient Elastography) Examination [8, 10, 11]

Liver stiffness measurement was performed by the FibroScan apparatus (Echosens, Paris). This apparatus is an ultrasound transforming detector

with oscillation source of medium amplitude and low frequency which measures liver stiffness by non-invasive technique. Briefly, this system is equipped with a probe consisting of an ultrasonic transducer (3.5-MHz) mounted on the axis of a vibrator. A vibration of mild amplitude and low frequency (50 Hz) is transmitted from the vibrator to the tissue by the transducer itself. This vibration induces an elastic shear wave which propagates through the tissue. While this shear wave propagates away from the probe, a series of ultrasonic acquisitions is performed. By comparison of successive ultrasonic signals, local deformations of the medium caused by the propagation of the shear wave are mapped according to time and depth to measure its velocity, which is directly related to tissue stiffness (or elastic modulus). The harder the tissue, the faster the shear wave propagates. The median value expressed in kilopascal (kPa) of 10 successful measurements with a success rate of at least 60%, was considered as representative of the liver stiffness as suggested by the provider of the instrumentation (Echosens, Paris). The values for different fibrosis stages were as follows: F0/F1 ≤ 7.2 kPa, F2 = 7.3:10.1 kPa, F3 = 10.2:14.9 kPa, and F4 (≥ 15:75 kPa) [12].

2.2 Other Non-Invasive Assessment Markers for Liver Fibrosis

Aspartate aminotransferase/ Alanine aminotransferase (AST/ ALT) ratio was calculated for all patients as a necro-inflammatory marker. A ratio of AST/ALT of more than 1 has been proposed to indicate the presence of fibrosis/cirrhosis [13].

Assessment of liver fibrosis was also done using a serological marker, Aspartate aminotransferase to platelet ratio index (APRI) which was calculated for all our patients before and after DAADs. It was calculated as follows:

> APRI = [AST level / Upper limit of normal (ULN)] / Platelet count (10^9 /l) x 100.

An APRI of ≤ 0.5 indicated no significant fibrosis, an APRI of ≥ 1.5 indicated significant fibrosis, while figures in-between were considered inconclusive [14].

We also applied another non-invasive scoring system; Fibrosis-4 score (FIB-4). Using a lower cutoff value of 1.45, and FIB-4 score <1.45 had a negative predictive value of 90% for advanced fibrosis. In contrast, a FIB-4 score >3.25 would have a 97% specificity and a positive predictive value of 65% for advanced fibrosis [15].

2.3 Statistical Analysis

The collected data were organized, tabulated and statistically analyzed using SPSS software (Statistical Package for the Social Sciences, version 13, SPSS Inc. Chicago, IL, USA). The quantitative data were expressed as means ± standard deviation, for normally distributed data, median values with Interquartile range (IQR) were used for non-normally distributed data. Comparison between means of 2 groups was done using Student's t-test for parametric data. Comparison of different parameters in patients before and after direct acting antiviral therapy was performed by Wilcoxon Signed Ranks Test due to wide variations of the patients' data. Correlation between variables was evaluated using Pearson's correlation coefficient (r). Significance was adopted at P value = 0.05 for interpretation of results of tests of significance.

3. RESULTS AND DISCUSSION

Children with chronic HCV infection are at risk for major complications, including cirrhosis, hepatocellular carcinoma, and death. The combined PEGylated interferon-α and ribavirin (PEG-IFN/ribavirin) remained the standard therapy for chronic HCV infection in children until early 2017 [16-18]. The approved PEG-IFN/ribavirin therapy for children is often held from use until adulthood because of its extensive list of potential side effects and high likelihood of causing adverse symptoms [18].

In recent years, a number of direct-acting antiviral agents (DAAs) were developed for treatment of HCV [19,20]. Recently (on April 7, 2017), the U.S. Food and Drug Administration (FDA) approved the first DAAs for children that included sofosbuvir and ledipasvir/sofosbuvir to treat HCV in children and adolescents aged 12 years and older and weighing at least 35 kilograms [21,22].

There is still insufficient research on whether DAAs therapy can improve liver fibrosis in thalassemic patients with chronic HCV especially in children. The results of recent studies suggested that early improvement of liver stiffness starts during the administration of DAAs in adult patients, and this effect was particularly pronounced in patients with progressive liver fibrosis [23].

Quantitative HCV PCR was positive in all patients as a prerequisite for enrollment before LED/SOF treatment with a median value of 450960.5 IU/ml and was negative (below detection level; 15 IU/ml) after 4 weeks of therapy initiation and remained negative at 12 weeks at the end of treatment (EOT), as well as at 12 weeks after the EOT in all treated children. These results indicated that all patients had achieved rapid virologic response (RVR) at 4 weeks of therapy, kept a negative serum HCV RNA levels at 12 weeks at the EOT, and achieved sustained SVR at 12 weeks after the EOT (SVR12) (Table 1).

Balistreri et al., (2017) found that 98% of 100 HCV-infected adolescent patients reached SVR at 12 weeks (SVR12) after receiving a combination tablet of LED/SOF (90/400 mg) once daily for 12 weeks in a multicenter, open-label study [24]. Similar results were obtained by Yakoot et al., (2018) who showed that SOF/DCV combined therapy could be a safe and effective treatment in adolescent patients 12 to 18- years old with chronic HCV genotype 4 infection [25]. Moreover, these results agreed with Nagral et al., (2019) in their prospective single-arm study of 18 thalassemic adolescents with CHC who received sofosbuvir based generic DAAs. All of whom achieved SVR at 3 months after treatment with DAAs [26]. Likewise, El-Shabrawi et al., (2018), reported that all patients achieved very rapid virologic response (vRVR) at week 2 of treatment, still had negative serum HCV RNA levels at week 12 at the EOT and maintained SVR at week 12 post treatments in a pilot study on a total of 20 chronic HCV infected children, aged from 6- to 12- years and treated with a fixed LED/SOF combination in half the adult dose (45/200 mg) once daily for 12 weeks [27].

The post-treatment FibroScan liver stiffness values were reduced in comparison with pre-treatment values in all of the individualized studied patients and the median post-treatment percentage of reduction was 19.4 kPa. As regard FibroScan fibrosis stages, many post-treatment FibroScan fibrosis stages (in 30 cases: 15 cases in F2, 11 cases in F3, and 4 cases in F4) were reduced to the preceding stage in comparison with pre-treatment stages in many individualized patients. This means that 15 cases in F2 were transformed from stage F2 before therapy to stage F1 after therapy, and 11 cases in F3 were transformed from stage F3 before therapy to stage F2 after therapy, whereas 4 cases in F4 were transformed from stage F4 before therapy to stage F3 after therapy. Nevertheless, some post-treatment FibroScan fibrosis stages (in 20 cases) remained in the same fibrosis stage (14 cases in F1, 4 cases in F2, and 2 cases in F4) in whom the reduction in post-treatment Fibro Scan liver stiffness values was not enough to transfer the patients into a lower fibrosis stage. As a result of this post-treatment redistribution of the fibrosis stages, the post-treatment number of cases in F1 was changed to 29, the number of cases in F2 was changed to 15, the number of cases in F3 was changed to 4 and the number of cases in F4 was changed to 2 (Table 2).

Bachofner et al., (2017) found that the median decrease in liver stiffness (LS) values over 30% was observed in the group achieving SVR in a large cohort study of 392 CHC patients undergoing DAAs treatment [28]. Also, Elsharkawy et al., (2017) found that LS values were significantly lower in patients with SVR12 in a retrospective study that included 337 chronic HCV adult Egyptian patients with genotype 4 mainly who were treated with sofosbuvir-based treatment regimen [29]. Furthermore, Tada et al., (2017) found that LS decreased over time, and there were significant differences between baseline and EOT and between EOT and SVR24 in chronic HCV patients who received daclatasvir and asunaprevir therapy and achieved SVR [17].

Table 1. Results of quantitative HCV PCR at different intervals

Different intervals	Quantitative HCV PCR (IU/ml)
Initial HCV PCR before therapy	Range : 116470 – 4785644
	Median (IQR): 450960.5(466861)
4 weeks of therapy initiation	Negative
12 weeks at the EOT	Negative
12 weeks after the EOT (SVR12)	Negative

HCV PCR, Hepatitis C virus polymerase chain reaction; IQR, Interquartile range; EOT, end of treatment; SVR, sustained virologic response

Table 2. Pre-treatment and post-treatment liver stiffness values and FibroScan fibrosis stages

Case number	Pre-treatment		Post-treatment		
	FibroScan Fibrosis stage	LS value (kPa)	FibroScan fibrosis stage	LS value (kPa)	% Reduction of LS value
Patients who were F1 before treatment					
1	F1	6.8	F1	5.5	19.1
2	F1	5.4	F1	4.5	16.6
3	F1	5.8	F1	4.6	20.6
4	F1	6.1	F1	4.8	21.3
5	F1	6.8	F1	5.7	16.1
6	F1	6	F1	5.1	15
7	F1	4.1	F1	3.7	9.7
8	F1	7.1	F1	6.04	14.9
9	F1	6.5	F1	5.2	20
10	F1	6.4	F1	5.4	15.6
11	F1	5.2	F1	4.4	15.3
12	F1	4.4	F1	3.9	11.3
13	F1	6.7	F1	5.6	16.4
14	F1	5.3	F1	4.3	18.8
Patients who were F2 before treatment					
15	F2	7.4	F1	6	18.9
16	F2	9.1	F1	7.1	14.2
17	F2	9.7	F2	7.7	20.6
18	F2	8.7	F1	6.9	20.6
19	F2	7.8	F1	6.2	20.5
20	F2	7.9	F1	6.3	20.2
21	F2	8.5	F1	7.2	15.2
22	F2	7.9	F1	6.7	15.1
23	F2	7.8	F1	4.9	37.1
24	F2	9.2	F1	6.9	25
25	F2	10	F2	8.2	18
26	F2	9.5	F2	8.1	14.7
27	F2	8.3	F1	6.7	19.2
28	F2	7.3	F1	5.8	20.5
29	F2	8.3	F1	7.1	14.4
30	F2	8.9	F1	7.2	19.1
31	F2	9.6	F2	7.4	22.9
32	F2	8.4	F1	6.7	20.2
33	F2	7.9	F1	6.7	15.1
Patients who were F3 before treatment					
34	F3	10.6	F2	9	15
35	F3	11.7	F2	9.4	19.6
36	F3	11.4	F2	9.1	20.1
37	F3	10.5	F2	8.4	20
38	F3	11	F2	8.2	25.4
39	F3	11.6	F2	9.3	19.8
40	F3	10.2	F2	8.1	20.5
41	F3	13.2	F2	9.9	25
42	F3	11.6	F2	9.3	19.8
43	F3	10.4	F2	8.4	19.2
44	F3	12.6	F2	10.1	19.8

Case number	Pre-treatment		Post-treatment		
	FibroScan Fibrosis stage	LS value (kPa)	FibroScan fibrosis stage	LS value (kPa)	% Reduction of LS value
Patients who were F4 before treatment					
45	F4	17.3	F3	13.8	20.2
46	F4	20.4	F3	13.1	35.7
47	F4	49.6	F4	42.2	14.9
48	F4	48	F4	27	43.7
49	F4	15.1	F3	12.2	19.2
50	F4	18.6	F3	13.9	25.2
Range		4.1-49.6		3.7-42.2	9.7-43.7
Mean±SD		10.772±8.537		8.413±6.106	19.706±5.983
Median(IQR)		8.6(4.3)		7 (3.45)	19.4 (5.25)

LS, liver stiffness; IQR, Interquartile range

Likewise, Mohammed and Omar, (2019) found that LS by FibroScan was significantly reduced from baseline along the follow-up periods (EOT, 6-months and 12-months post-treatment) after DAAs treatments in 228 adult Egyptian patients with chronic HCV infection [30]. Singh et al., (2018) in a systematic review and meta-analysis study, found that the magnitude of decline in LS is incremental over time after completion of therapy, increasing progressively from 2.4 kPa at EOT to 4.1kPa at 12 months and beyond (median decrease, 28.2%) in patients who achieved SVR [31].

Comparison of values of liver enzyme; AST, aspartate amino transferase and ALT, alanine amino transferase, between patients before and after LED/SOF treatment showed that there was highly significant reduction of their values after LED/SOF therapy (p-value <0.001) in comparison to pre-treatment values (Table 3).

Comparison of values of all fibrosis parameters (LSM by FibroScan, APRI, FIB4 index and AST/ALT ratio) between patients before and after LED/SOF treatment showed that there was highly significant reduction of values of most of liver fibrosis parameters (LSM by FibroScan, APRI, FIB-4 score, AST/ALT ratio) after LED/SOF therapy (p-value <0.001, <0.001, <0.001 and 0.020 respectively) in comparison to pre-treatment values (Table 4).

Correlations between LSM values by FibroScan and each of other fibrosis parameters (APRI, FIB-4 score and AST/ALT ratio) among the studied patients showed that there were statistically significant positive correlations between LS values by FibroScan and each of APRI (p-value 0.049), FIB-4 score (p-value 0.018), and AST/ALT ratio (p-value 0.038). (Table 5).

It has been reported that achievement of SVR after DAAs therapy is associated with significant fibrosis regression estimated by the reliable non-invasive markers (LSM by FibroScan, APRI score, and FIB-4 index) regardless of the baseline fibrosis stage in both compensated and decompensated CLD. But, the initial improvements in LS by FibroScan, APRI score, and FIB-4 index may merely reflect necro-inflammatory resolution rather than true fibrosis regression. This might lead to overestimation of fibrosis regression. It is suspected that the improvement in liver biology, portal hypertension, and liver architecture might develop with longer follow-up periods [32].

Table 3. Comparison of values of Liver enzymes before and after LED/SOF therapy among the studied patients

Liver enzymes		Wilcoxon signed ranks test			
		Range	Median	IQR	P-value
ALT (U/L)	Before LED/SOF	45 - 196	121.00	43.50	**<0.001***
	After LED/SOF	26 - 120	94.00	23.50	
AST (U/L)	Before LED/SOF	54 - 190	136.00	53.50	**<0.001***

AST, aspartate amino transferase,
ALT, alanine amino transferase;
IQR, Interquartile range;
LED/SOF, ledipasvir/sofosbuvir

Table 4. Comparison of values of all fibrosis parameters before and after LED/SOF therapy among the studied patients

Fibrosis parameters		Wilcoxon signed ranks test			
		Range	Median	IQR	P-value
LSM value (kPa)	Before LED/SOF	4.1 - 49.6	8.70	4.25	<0.001*
	After LED/SOF	3.7 - 42.2	7.10	3.40	
APRI Score	Before LED/SOF	0.22 - 1.988	0.91	0.70	<0.001*
	After LED/SOF	0.2- 1.181	0.65	0.42	
FIB-4 Score	Before LED/SOF	0.12 - 0.98	0.50	0.41	<0.001*
	After LED/SOF	0.11 - 1	0.41	0.20	
AST/ALT Ratio	Before LED/SOF	0.45 - 2	1.08	0.36	0.020*
	After LED/SOF	0.44 - 3	1.10	0.45	

LSM, Liver stiffness measurement;
IQR, Interquartile range;
LED/SOF, ledipasvir/sofosbuvir; APRI, aspartate amino transferase to Platelet Ratio Index;
AST/ALT ratio, aspartate amino transferase to alanine amino transferase ratio

Table 5. Correlations between liver stiffness values by FibroScan and each of other fibrosis parameters (APRI, FIB-4 score and AST/ALT ratio) among the studied patients

Correlations between LS values by FibroScan and other fibrosis parameters		
Other fibrosis parameters	LS values by FibroScan (kPa)	
	R	P-value
APRI	0.280	0.049*
FIB-4 score	0.334	0.018*
AST/ALT ratio	0.295	0.038*

LS, Liver stiffness; AST/ALT ratio, aspartate amino transferase to alanine amino transferase ratio;
APRI, aspartate amino transferase to Platelet Ratio Index

4. CONCLUSION

LED/SOF therapy for 12 weeks' duration resulted in eradication of HCV infection that was associated with significant decrease in LSM by FibroScan particularly those with higher baseline liver fibrosis stages.

CONSENT

A written informed consent form, provided by the ethical committee of the Faculty of Medicine, Tanta university, was filled by a legal guardian (mostly one of the parents) for each patient, explaining the detailed steps of the study with all the possible side effects of the used drugs. The consent form also included a consent for the use of clinical data for publication without revealing any personal data.

ETHICAL APPROVAL

The study protocol was first approved by the ethical committee of the Faculty of Medicine, Tanta university, September 2017, before starting patients' enrollment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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