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Hyaluronic Acid Level for assessment of Liver Fibrosis in β-Thalassemia Children Treated for Hepatitis C Virus by Direct Acting Antiviral Drugs

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Abstract: *Background:* Direct acting antiviral drugs (DAAs) (Ledipasvir/Sofosbuvir) can contribute to improvement and prevent progression of liver fibrosis in children with β- thalassemia infected with hepatitis C virus (HCV). *Objective:* The aim of this study was to assess liver fibrosis by measuring serum hyaluronic acid (HA) in β-Thalassemia children infected with HCV before and after DAAs (Ledipasvir/Sofosbuvir) therapy. *Patients and Methods:* 50 children with β- thalassemia, who had chronic HCV infection, aged 12 -18 years, were treated by DAAs for 12 weeks. They were evaluated before and 12 weeks after the end of treatment by PCR for HCV. Liver fibrosis was evaluated before and after treatment by serum HA and Aspartate aminotransferase to platelet ratio index (APRI). *Results:* Assessment of liver fibrosis with APRI before and after treatment with DAAs demonstrated a statistically significant reduction in the number of patients with severe fibrosis, and redistribution of cases into the less severe classes (P.*Value* 0.039). There was significant reduction in fibrosis-4 index and serum HA after treatment in comparison to their pretreatment values (P.*Value* <0.001). *Conclusion:* HA was reduced together with improved APRI after treatment of HCV infection in children with thalassemia using DAAs, which indicates improvement of liver fibrosis.

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Introduction

Thalassemias are a heterogeneous group of genetic disorders of hemoglobin (Hb) synthesis, considered as the most common monogenic disorder in the world (1). In Egypt; Thalassemia is a major health problem since its incidence is estimated as 1000 children/1.5 million live birth per year (2).

Thalassemic patients can develop liver fibrosis because of iron liver overload and hepatitis C virus (HCV) infection. Studies have reported that the development and the severity of liver fibrosis are strongly related to; not only the extent of liver iron overload, but also to the presence of chronic HCV infection (3). HCV infection is even considered as the main risk factor for liver fibrosis in transfusion-dependent thalassemic patients, whereas excess liver iron is now clearly recognized as a cofactor for the development of advanced fibrosis in patients with HCV infection (4).

Although, hepatic fibrogenesis has long been thought to be an irreversible process, it is now evident that it is a dynamic process with significant potential for reversal; unlike cirrhosis, which is irreversible. That's why, treatment of HCV infection and identification of liver fibrosis at an early stage would be of great significance (5).

The combined pegylated interferon-α and ribavirin (PEG-IFN/ribavirin) remained the standard therapy for Chronic HCV infection in children until 2016. But it was often held from use until adulthood because of its extensive list of potential side effects and high likelihood of causing adverse symptoms (6-8).

Recently (on April 7, 2017), the U.S. Food and Drug Administration (FDA) approved the first direct-acting antiviral agents (DAAs) for children that included sofosbuvir and sofosbuvir/ledipasvir to treat

HCV in children and adolescents aged 12 years and older (9).

Liver biopsy has long been considered as the gold standard for assessing hepatic fibrosis. However, it is an invasive procedure that may lead to serious complications, limiting its acceptance and repetition in many patients. In addition, the accuracy of liver biopsy may be questioned because of sampling errors and inter-observer variability, which may lead to under or over staging of fibrosis or cirrhosis (10). That created a need to develop and validate non-invasive tests which can accurately reflect the full spectrum of hepatic fibrosis, cirrhosis, and its severity in liver diseases (11).

The Measurement of hepatic fibrosis helps to stage the severity of disease, and also allows serial determination of disease progression. Further, the fibrosis progression rate is an important predictor of the time to develop cirrhosis. Moreover, with growing evidence that fibrosis is reversible; methods will need to assess both progression and regression accurately (12).

Hyaluronic acid (HA) is a high molecular weight glycosaminoglycan which is an essential component of extracellular matrix in almost every tissue in the body (13). In the liver, HA is mostly synthesized by the hepatic stellate cells and removed via sinusoidal cell adhesion molecules (14). This mechanism is impaired in fibrosis, leading to a rise in serum levels of HA. Therefore, serum HA is considered a marker that appears early before pathological changes occur (15).

In the current study, we evaluated serum hyaluronic acid (HA) as a marker for hepatic fibrosis as it has been reported that there was significant association between the level of serum HA and the extent of liver fibrosis, indicating that higher levels of HA were highly associated with advanced stages of liver fibrosis. The fact that HA has close relationship with stellate cells and that the activation of stellate cells is crucial for developing hepatic fibrosis led to the hypothesis that serum levels of HA could be significantly associated with fibrosis. Serum HA is considered as a marker that appears early even before advanced pathological changes occur (16).

Patients And Methods

This study was carried out on β-thalassemic children with chronic HCV infection at the Hematology Unit, Pediatric Department, Tanta University Hospital, after approval by the ethical committee of the Faculty of Medicine, Tanta University. HCV infection was initially diagnosed by serological detection of HCV-Ab then confirmed by polymerase chain reaction (PCR) for HCV RNA in the serum. We recorded 72 children with positive HCV, 18 of them were excluded as they were less than 12 years old, which is the age of FDA approval for the use of DAAs in children, and two children were excluded due to HBV co-infection. The remaining 52 children were recruited in the study.

They 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).

Hyaluronic acid was used as a marker for assessment of hepatic fibrosis before and 12 weeks after the end of treatment using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Another serological marker, Aspartate aminotransferase to platelet ratio index (APRI) was calculated for all our patients before and after DAADs. It was calculated as:

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 (17).

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 (18).

Results

Of the 52 patients who started the study, two children dropped out; one of them had negative PCR for HCV by the 8th week but did not complete treatment for the rest of the 12 weeks' protocol, and the other one stopped treatment on the second week of therapy and did not show for subsequent doses. The remaining 50 children, completed the study and received treatment for a duration of 12 weeks and were reassessed again 12 weeks after the end of treatment. The mean age of patients who completed this study was 13.38 years (range: 12-18 years), 29 males and 21 females.

A positive HCV PCR at baseline was a prerequisite in 100% of patients, and was repeated for reevaluation after 12 weeks at the end of treatment (EOT), as well as at 12 weeks later. HCV PCR became negative in 100% of patients with SVR.

In pretreatment assessment with APRI, 12 patients had a score < 0.5 (mild fibrosis), 32 patients had a score of 0.5 – 1.5 (moderate fibrosis) and 6 patients had a score > 1.5 (severe fibrosis), whereas

after treatment 15 patients had a score < 0.5 (mild fibrosis), 35 patients had a score of 0.5 – 1.5 (moderate fibrosis) and none of them had a score > 1.5 (severe fibrosis). This demonstrates a statistically significant reduction in the number of patients with severe fibrosis, and redistribution of larger number of cases into the less severe classes of fibroses (mild and moderate classes) (P.*Value* 0.039) ***(Table 1).*** The overall APRI values after treatment compared to those before treatment with DAAs, also demonstrated a statistically significant reduction (P.*Value* <0.001) ***(Table 2).*** There was significant reduction in fibrosis-4 index after treatment in comparison to their pretreatment values (P. *Value* <0.001) ***(Table 3).*** Treatment with DAAs led also to significant reduction of serum hyaluronic acid in HCV infected patients in comparison with its pretreatment values for the same patients (P.*Value* <0.001) ***(Table 4).***

When we compared APRI values to serum hyaluronic, there was positive significant correlation as regard both pre-treatment and post-treatment value (P.*Value* <0.001) ***(Figure 1 & 2 respectively).***

Table (1) Number of patients with different fibrosis grades by Aspartate transaminase platelet ratio index (APRI) before and after treatment with direct acting antiviral drugs.

APRI	Number of patients		Chi-Square	
	Pre-treatment	Post-treatment	X^2	P.*Value*
< 0.5 APRI	12 (24 %)	15 (30 %)	6.468	0.039*
0.5-1.5 APRI	32 (64 %)	35 (70 %)		
> 1.5 APRI	6 (12 %)	0		

Table (2) Comparison of aspartate transaminase platelets ratio index (APRI) values before and after treatment with direct acting antiviral drugs.

Time	APRI		Differences	Paired T-Test	
	Range	Mean ± SD	Mean ± SD	t.test	P.*Value*
Pre-treatment	0.220 - 1.988	0.909 ± 0.459	-0.256 ± 0.251	-7.199	<0.001*
Post-treatment	0.200 - 1.181	0.653 ± 0.264			

Table (3) Comparison of FIB-4 score before and after treatment with direct acting antiviral drugs.

Time	FIB-4 score		Differences		Paired T-Test	
	Range	Mean ± SD	Mean ± SD		t.test	P.Value
Pretreatment	0.12 - 0.98	0.502 ± 0.236	0.076	0.124	4.320	<0.001*
Post treatment	0.11 - 1	0.426 ± 0.194				

Table (4) Comparison of hyaluronic acid before and after treatment with direct acting antiviral Drugs.

Time	Hyaluronic acid (ng/ml)		Differences		Paired T-Test	
	Range	Mean ± SD	Mean ± SD		t.test	P.Value
Pretreatment	31.4 - 226.6	97.762 ± 41.792	-32.05	31.56	-7.181	<0.001*
Post treatment	20.6 - 148	65.71 ± 28.43				

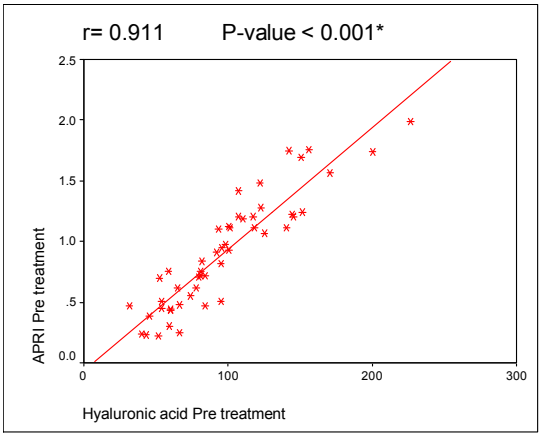

Figure (1) correlation between APRI values and serum hyaluronic acid in patients before treatment with direct acting antiviral drugs.

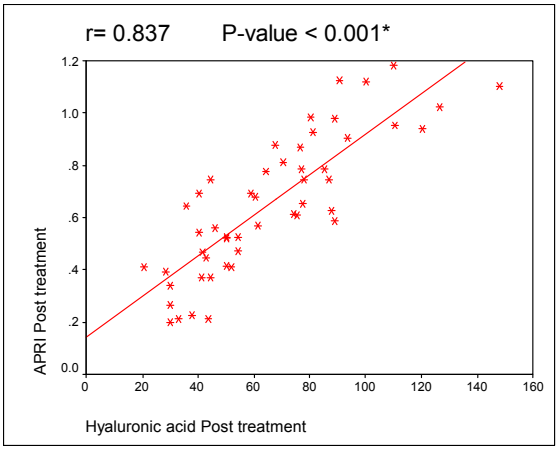

Figure (2) correlation between aspartate platelets ratio index value and serum hyaluronic acid in patients after treatment with direct acting antiviral drugs.

Discussion

The significant decrease in APRI values after treatment with DAAs was similar to the previous findings of Elsharkawy et al, who stated that there was significant difference in APRI values after treatment with DAAs when they performed retrospective study including 337 chronic HCV Egyptian patients with genotype 4 mainly who received Sofosbuvir - based treatment regimen (19).

In this study there was significant decrease in frequency of patients with severe degree of liver fibrosis after treatment with DAAs in comparison to pretreatment values. Also, there was significant increase in frequency of patients with mild and moderate degree of liver fibrosis after treatment with DAA in comparison to pretreatment values using APRI values. Similar results were obtained by **Shousha et al** who performed a cohort study that included 155 CHC Egyptian patients (20).

However, these values should be interpreted cautiously as these findings could be affected by the increased aminotransferase activity induced by exacerbation of inflammation and progression of liver fibrosis as a result of HCV-infection in the patients and increased platelets count due to splenectomy and thalassemia itself.

Treatment with DAAs was associated with significant decrease in serum hyaluronic acid in thalassemia patients infected with HCV, this is in agreement with **Miyaki et al** who performed a study on thirty patients treated with daclatasvir and asunaprevir for 24 weeks, and 26 patients achieved SVR, liver function parameters, serum alanine aminotransferase (ALT) and albumin levels and liver fibrosis markers, hyaluronic acid and type IV collagen were measured before and after completion of the treatment in SVR and non - SVR patients and showed significant decrease in hyaluronic acid after end of treatment in comparison to pretreatment levels (21).

It has been reported that the combination of HA and aspartate transaminase to platelets ratio index (APRI) in patients with chronic HCV infection allows for the non-invasive identification of patients with cirrhosis with higher accuracy (22,23). Thus, it was recommended that HA can be used as a useful biomarker to monitor progression of liver disease and risk of complications in patients with chronic viral hepatitis (24), which makes it a reliable surrogate marker in distinguishing the clinically relevant stages of fibrosis (25).

Conclusion

Since serum HA levels were positively correlated to APRI, which is a well-established indicator of liver fibrosis, HA can be considered to have a good diagnostic accuracy as a noninvasive assessment of fibrosis. Moreover, the measurement of serum HA is a non- invasive, accurate and cost-effective method for diagnosis of liver fibrosis in patients with β-thalassemia infected with hepatitis C virus.

Disclosure

All patients were enrolled after obtaining an informed consent from one of their parents. The study was registered as a clinical trial on Clinicaltrials.gov. (ClinicalTrials.gov Identifier: NCT03961828) as this was the first study to treat chronic HCV infection in children with thalassemia using DAAs.

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