

Liver Fibrosis Assessment in Cases of Chronic Hepatitis C after Direct Acting Antivirals Therapy using Aspartate Aminotransferase to Platelet Ratio Index and Transient Elastography

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Background and study aim: The treatment of chronic hepatitis C infection (CHC) has been revolutionized by using novel direct-acting antivirals (DAAs). Transient elastography (TE) and aspartate aminotransferase to platelet ratio index (APRI) are simple and convenient diagnostic tools for the assessment of liver fibrosis. This study was designed to evaluate liver fibrosis by using TE and APRI before and one year after a full course of DAAs therapy in cases with CHC infection.

Patients and methods: TE and APRI were measured before treatment and one year after a full course of DAAs therapy in a prospective study of 82 CHC cases. All candidates with CHC infection were genotype 4. TE was measured by Fibroscan and liver stiffness measurement (LSM) was considered reliable if 10 successful LSM had been obtained with a

success rate (SR) $\geq 60\%$ and interquartile range (IQR) $<30\%$.

Results: The current study was conducted on 82 subjects of CHC. The median value of liver stiffness measurement was markedly decreased from 10.6 to 6 kPa after 12 months of completion of DAAs therapy ($p < 0.05$). Significant reduction of fibrosis stages had occurred in 14/16 (87.50%) of patients with F2 stage, 14/16 (87.5%) of patients with F3 stage, and 26/34 (76.5%) of patients with F4 stage ($p < 0.001$). The Median APRI value was markedly decreased from 1.12 to 0.42 after 12 months of completion of DAAs therapy ($p < 0.001$).

Conclusion: Liver fibrosis evaluated by TE and APRI markedly decreased in patients with CHC infection after DAAs therapy reflecting regression of liver pathology.

INTRODUCTION

Hepatitis C virus (HCV) is considered a challenge for all hepatologists because more than 180 million individuals had been infected all over the world. Chronic hepatitis C infection (CHC) is a significant aetiology of causing liver cirrhosis. Additionally, long term infection with HCV may cause decompensated liver disease, hepatocellular carcinoma (HCC) and even high incidence mortality rate in hepatic cases [1].

Recently, direct-acting antivirals (DAAs) are considered the cornerstone for the management of HCV infection. These drugs have many advantages including a higher success rate (more than 90%) than

interferon-based therapy regarding sustained virological response (SVR). In addition, novel DAAs drugs used only for a short duration (12 or 24 weeks) and have fewer adverse effects. An important question regarding the reversibility of liver fibrosis following SVR after DAAs therapy had been raised. Several recent studies have shown short term partial regression of fibrosis stages after the achievement of SVR using DAAs therapy in patients with CHC infection. Moreover, improvement of liver histology had been noted regarding the long-term effects of antiviral therapy which was reported in recently published studies [2-5].

For a long time, Liver biopsy was the main method for the follow-up of liver fibrosis regression after effective antiviral therapy. This may explain the scarcity of published studies in this aspect as most of the patients refuse multiple liver biopsies especially after complete eradication of HCV infection [6-7].

Transient elastography (TE) is an ultrasound-based elastography method and convenient tool for the evaluation of liver fibrosis through liver stiffness (LS) measurement. TE used several years ago in clinical practice and the recent guidelines of EASL recommended it for the assessment of liver fibrosis in chronic liver diseases [8-10].

TE is a simple, easy, cheap, painless and non-invasive method for LS measurement compared to the liver biopsy procedure. Several published papers have shown the value of TE in assessing fibrosis in CHC and cirrhosis. The follow up of CHC cases regarding liver fibrosis assessment became much easier by using TE because of the good performance of this method [11-14].

Another common tool for liver fibrosis assessment is a combination of biochemical markers such as aspartate aminotransferase to platelet ratio index (APRI). Several studies demonstrated that APRI was significantly associated with liver fibrosis stage in chronic liver diseases. Theoretically, the platelet counts usually decreased in the case of progression of liver fibrosis. The consequences of advanced fibrosis as portal hypertension and splenomegaly increase platelet sequestration and destruction [15-18]. The present study was conducted to assess the treatment response of CHC cases through liver fibrosis assessment before and 12 months after direct-acting antivirals treatment using TE and APRI.

PATIENTS AND METHODS

This was a cohort prospective study conducted at Mansoura university hospital in the period between May 2016 and July 2018. The study included 82 patients previously diagnosed with CHC based on detectable HCV RNA by the PCR method and all of them were infected by HCV genotype 4. All patients underwent DAAs therapy (sofosbuvir-based therapy) over a period of 3 months and underwent regular clinical evaluation during the study period and all of them achieved SVR. patients who missed their

follow up have been excluded. Cases of HCC, decompensated cirrhosis, coinfection with HBV and HIV, pregnancy, other co-existing aetiology of chronic liver diseases and unreliable TE readings were also excluded.

In all subjects, successful TE using fibroscan and calculation of APRI values were done at baseline before therapy and 12 months after completion of management. Informed consent was secured from all candidates involved in this study.

Clinical and laboratory assessment:

The following parameters and details were collected regarding each subject in this study after full clinical examination: age, sex, BMI, liver chemistry tests including ALT and AST. In addition, renal function tests, CBC including platelets count. HCV RNA was detected by the quantitative PCR method. Tests for hepatitis B surface antigen and HIV antibodies were performed using the enzyme immunoassays method. The parameters including AST, platelet count, and HCV RNA were repeated one year after DAAs therapy. All subjects in this study were screened for HCC by diagnostic tools including abdominal ultrasound and alpha-fetoprotein tumour marker tests.

TE assessment:

Liver stiffness measurements (LSM) were performed using FibroScan® device (EchoSens, Paris, France), which has the ability to incorporate an ultrasound transducer probe mounted on the axis of a vibrator. The eligible cases were assessed at the baseline before treatment and one year after DAAs therapy by the same operator.

LSM using fibroscan was done through the right lobe of the liver based on the methodology described in the recent guidelines. The depth of measurements was between 25 and 65 mm and the measured values of LSM were represented in kilopascals (kPa). The successful TE results were defined as median values of 10 valid LSM readings with a success rate (SR) $\geq 60\%$ and interquartile range (IQR) $<30\%$. The probes used in this study were Both M and XL probes. The stages of fibrosis were classified according to LSM readings by fibroscan as follows: F0 stage (≤ 5 kPa), F1 stage ($> 5-6$ kPa), F2 stage ($> 6-9$ kPa), F3 stage ($> 9-12$ kPa), F4 stage (≥ 12 kPa) [19-21].

Calculation of APRI:

APRI values were measured as [(AST/ AST ULN) x100] / platelet count ($10^9/L$). The reference range of AST was (5- 45) IU/mL in men and (5- 35) IU/mL in women. The cut-off value regarding APRI readings to diagnose considerable liver fibrosis ($F \geq 2$) was (0.5) [22].

Ethical approval was secured from IRB (Institutional Research Board) of Mansoura faculty of medicine before the start of the study.

Data collection and Statistical analysis:

Data analysis and interpretation were done by program SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). Categorical data were presented as numbers and percentages. Continuous data were presented as mean and standard deviation or as median and range when appropriate. The comparison between continuous variables was performed by using Mann–Whitney tests or paired t-test. Additionally, the comparison between categorical variables was performed by using the chi-square test. P-value < 0.05 was considered statistically significant.

RESULTS

A total of 82 CHC cases were involved in this research. Males were (70.7%) of the studied

subjects. The mean age was 51.2 ± 9.7 years and the mean BMI was 28.3 ± 4.4 . (table1).

Liver Fibrosis assessment before treatment:

Based on LSM using fibroscan, the readings ranged from 4.1 to 61.5 kPa. The median value of liver stiffness measurement by fibroscan before treatment was 10.6 kPa. Significant liver fibrosis ($\geq F2$) was detected in 66 (80.4%) patients. The median range of APRI was 1.12 for patients at baseline before DAAs therapy. (Table2-4)

Liver fibrosis assessment after treatment:

The median APRI value was reduced from 1.12 at the baseline before treatment to 0.42 (range, 0.12-2.00) after 12 months of DAAs therapy ($p < 0.001$). The median value of liver stiffness measurement after one year of DAAs therapy was reduced from 10.6 KPa before treatment to 6 KPa after 12 months of DAAs therapy ($p < 0.05$). Significant reduction of fibrosis stages had occurred in 14/16 (87.50%) patients with F2 stage, 14/16 (87.5%) of patients with F3 stage, and 26/34 (76.5%) of patients with F4 stage ($p < 0.001$) (Table 3). All patients with liver fibrosis stages (F2 or F3) did not show the progression of liver fibrosis stages after the full course of DAAs therapy.

Table (1): Characteristics of the subjects in this study (n=82).

Parameters	Values
Male gender, n (%)	58 (70.7)
Age (years), Mean \pm SD	51.2 \pm 9.7
BMI (kg/m ²), Mean \pm SD	28.3 \pm 4.4
Platelet count (10^9), Mean \pm SD	192.2 \pm 72.2
AST (IU/L), median	50
ALT (IU/L), median	46
- ALT normal value, n (%)	20 (25.3)
-ALT (1-2) fold, n (%)	24 (30.3)
-ALT (>2) fold, n (%)	38 (44.4)

Table (2): Fibrosis stages by TE before and after DAAs therapy in patients with CHC(n=82).

Fibrosis stage	Before therapy N (%)	After therapy N (%)
F0	0	16 (19.51%)
F1	16(19.51 %)	26 (31.70)
F2	16(19.51%)	18 (21.95%)
F3	16(19.51%)	14 (17.07%)
F4	34(41.46%)	8 (9.75%)

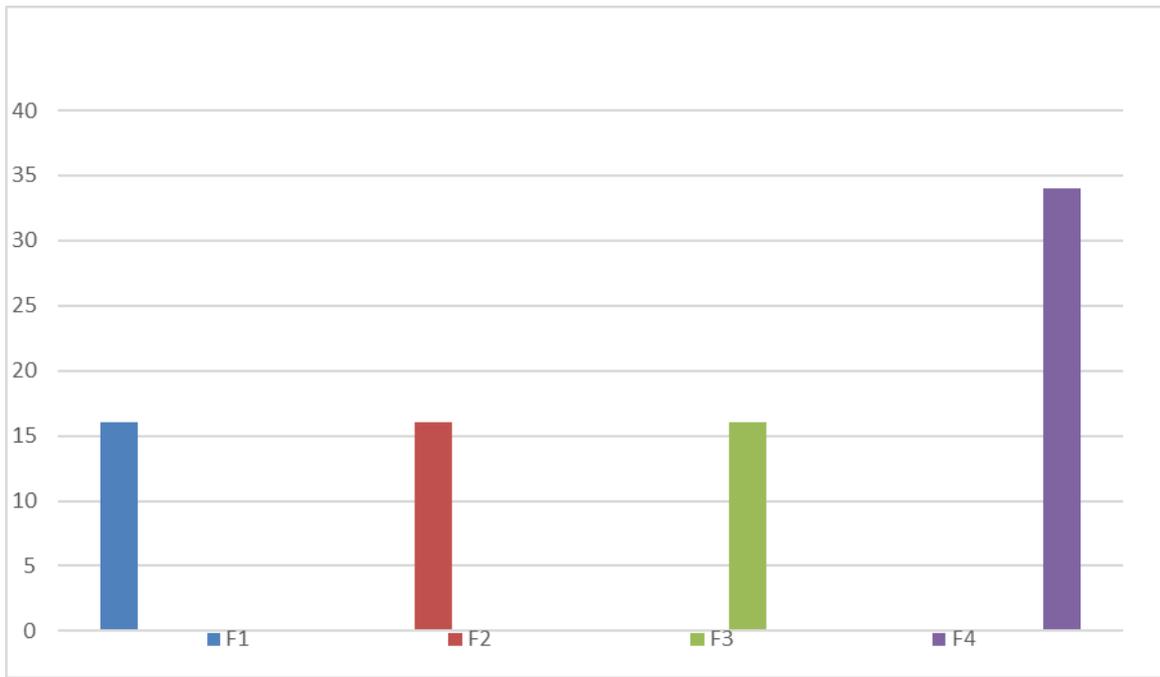


Figure (1): Fibrosis stages by TE before DAAs therapy in patients with CHC(n=82).

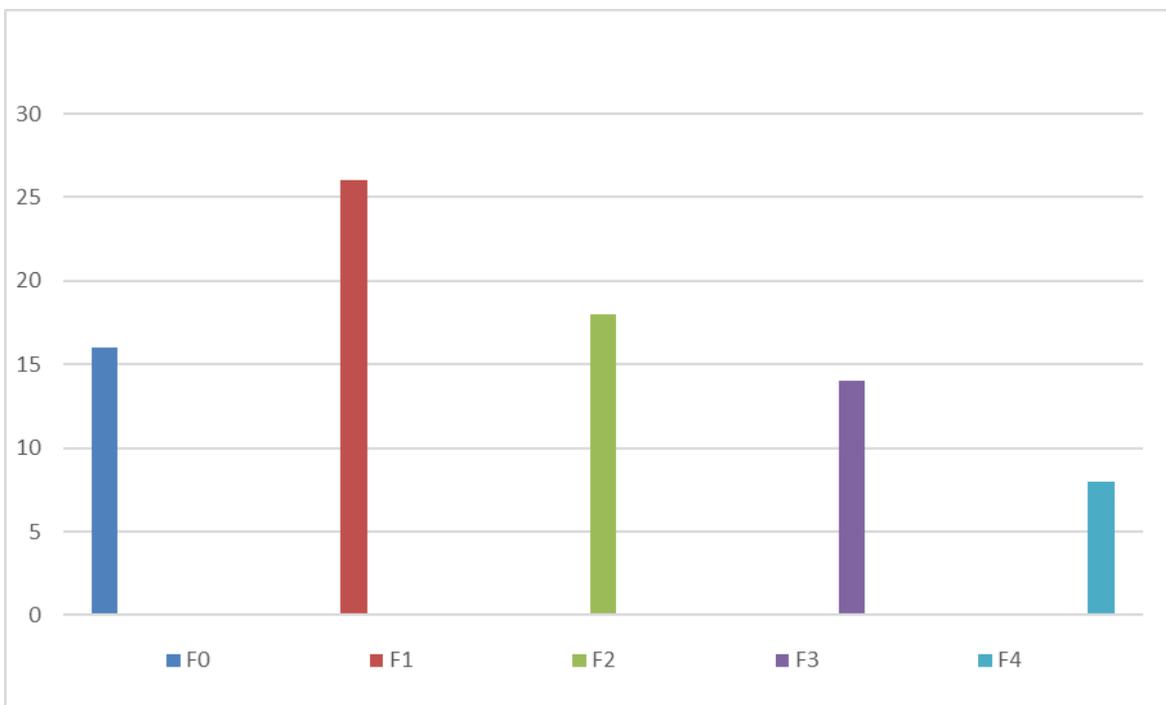


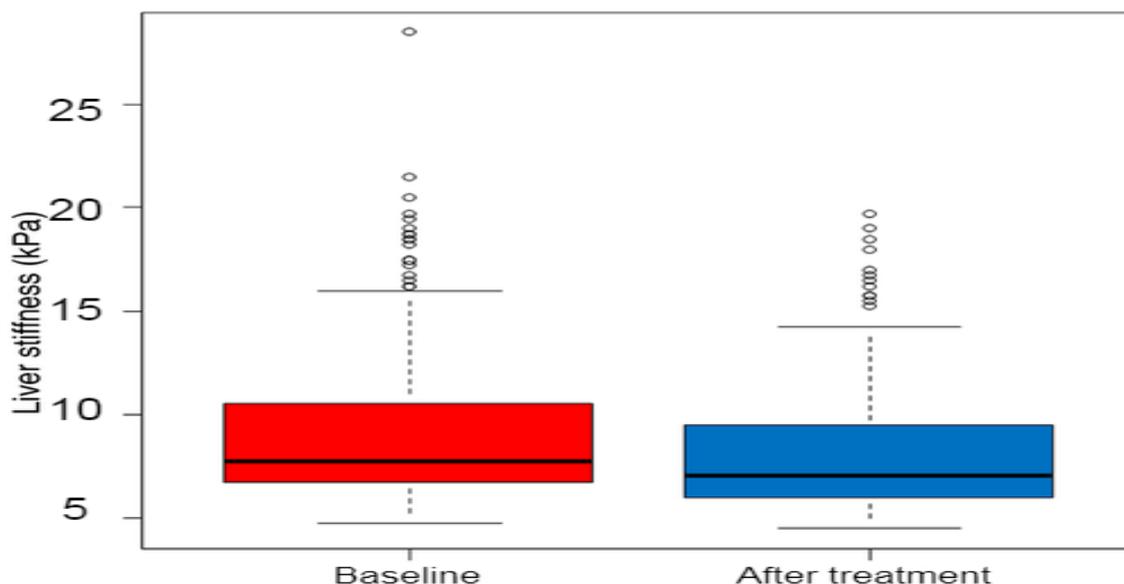
Figure (2): Fibrosis stages by TE after DAAs therapy in patients with CHC(n=82).

Table (3). Comparison between liver fibrosis stages by TE before and after DAAs therapy.

Fibrosis stage after therapy, n (%) (n=82).	Fibrosis stage before treatment by TE, n (%) (n=82).				P-value
	(F1)	(F2)	(F3)	(F4)	
F0 (≤ 5 kPa)	10(62.50)	6 (38.50)	0	0	p<0.001
F1 (> 5-6 kPa)	4 (25.00)	8 (49.00)	6 (37.50)	8 (23.53)	
F2 (> 6-9 kPa)	2 (12.50)	2 (12.50)	8 (50.00)	6 (17.60)	
F3 (> 9-12 kPa)	0	0	2 (12.50)	12 (35.40)	
F4 (≥ 12 kPa)	0	0	0	8 (23.50)	

Table (4). APRI before and after DAAs therapy in patients with CHC (n=82).

Fibrosis stage	APRI before treatment(n=82)	APRI after treatment(n=82)	p-value
F0-F1(≤ 0.5), n(%)	16 (19.5)	48 (58.5)	P<0.001
F2-F4(>0.5), n(%)	66 (80.5)	34 (41.5)	

**Figure (3):** Liver stiffness by TE before and one year after DAAs therapy(n=82).

DISCUSSION

Non-invasive tools for assessment of liver fibrosis as LSM by fibroscan and biochemical parameters such as APRI have been accepted by the patients in comparison to the liver biopsy procedure. These tools are painless, inexpensive, and repetitive. Several published studies have been done for liver fibrosis assessment in CHC patients before and after interferon-based treatment and most of them showed a significant reduction of LSM which reflects regression of liver fibrosis [23-24].

All new guidelines recommend DAAs therapy for all patients with CHC infection irrespective of liver fibrosis stage. Moreover, the world health organization (WHO) supported the drive for complete eradication of HCV by 2030 [25].

In the present research, the median APRI value was reduced from 1.12 at the baseline before treatment to 0.42 (range, 0.12-2.00) after 12 months of DAAs therapy (p<0.001). Additionally, the median value of liver stiffness measurement by fibroscan was reduced from 10.6 before treatment to 6 kPa after one year of DAAs therapy. This was consistent with a recently published study that had been performed

by Kohla et al. [26] who evaluated LSM in 165 CHC patients after complete eradication of HCV and concluded early liver fibrosis regression at the end of treatment (EOT) after successful HCV eradication with DAAs therapy. The mean LSM in their study at EOT, week 24 and week 36 were (7.01 ± 3.59 KPa), (6.18 ± 3.39 kPa) and (5.74 ± 3.21 kPa) respectively in comparison to LSM before treatment (8.49 ± 0.83 kPa) (P-value <0.001).

The results of our work showed significant reduction of fibrosis stages had occurred in 14/16 (87.50%) patients with F2 stage, 14/16 (87.5%) of patients with F3 stage, and 26/34 (76.5%) of patients with F4 stage ($p < 0.001$). Moreover, sixteen patients of F1 stage had a notable regression of fibrosis after DAAs therapy and converted into F0 stage. All patients with liver fibrosis stages (F2 or F3) did not show a progression of liver fibrosis stages after a complete course of DAAs therapy.

The previous results were in line with Knop et al. [27] who prospectively assessed dynamics of liver stiffness by TE in 260 patients with CHC receiving DAAs treatment and marked improvement of LSM had been noticed between the baseline result (median 8.6 kPa) and week 24 (median 7.9 KPa) ($P < 0.0001$) as well as between the EOT result (median 8.4 kPa) and week 24 ($P < 0.0001$). Elsharkawy et al. [9] also reported a significant reduction regarding the values of TE, FIB-4, and APRI in cases with CHC infection after the achievement of SVR following a Sofosbuvir-based interferon-free therapy (Sofosbuvir-based IFT).

The data in the current study was concordant with Laursen et al. [28] who investigated DAAs therapy effects on liver inflammation and fibrosis in 71 CHC patients with advanced liver disease before, during, and 12 months after successful management using sofosbuvir-based IFT. Their study showed a significant decrease in LSM by 20% at the end of treatment (17.8 vs 14.3 kPa) and early resolution of liver inflammation had been suggested. Additionally, another 15% decrease in LSM 12 months after treatment suggesting liver fibrosis regression.

Comparison between values of LSM, FIB-4 and APRI in 265 patients receiving either pegylated interferon or DAAs before and 24 weeks after treatment was done by Chen et al. [29]. They reported significant decrease in values of the three parameters from baseline to 24 weeks after

treatment in 219 patients achieved SVR. and referred this decrease to resolution of necroinflammation. Same explanation can stand for our results.

Our results were in accordance with Bachofner et al. [8] who evaluated 392 patients with CHC before and 18 months after IFT therapy. They observed a significant reduction of the median values of TE measurements (from 12.65 to 8.55 kPa) after treatment ($p < 0.001$). Additionally, the median measurements of APRI and FIB-4 values were obviously reduced after treatment.

Our results also agree with Sporea et al. [30] who studied 225 patients with compensated HCV genotype 1b related cirrhosis received IFT for 12 weeks. They concluded that LSM values were lower at End Of Therapy (EOT) in 60%, 75% of patients at EOT and 12 weeks after EOT respectively.

A meta-analysis of 24 studies performed by Singh et al. [31], showed a decrease in LSM for patients who achieved SVR by 2.4 kPa, 3.1 kPa, 3.2 kPa and 4.1 kPa at EOT, 1-6 months after therapy, 6-12 months after therapy and 12 months or more after therapy respectively. Moreover, the decrease in LSM was markedly greater in patients treated with DAAs therapy rather than with interferon-based therapy [31].

CONCLUSION

The liver fibrosis, evaluated by TE and APRI, was significantly decreased after 12 months of completion of direct antiviral therapy in patients with CHC. It supports the idea of liver fibrosis reversibility after treatment of the underlying aetiology of chronic liver disease.

Abbreviations

Aspartate aminotransferase platelet ratio index (APRI), chronic hepatitis C (CHC), body mass index (BMI), direct-acting antivirals (DAAs), fibrosis 4 (FIB-4), End of treatment (EOT), interferon-free treatment (IFT), Kilopascals (kPa), hepatitis C virus (HCV), hepatitis B virus (HBV), interquartile range (IQR), liver stiffness measurements (LSM), liver stiffness (LS), Transient Elastography (TE), sustained viral response (SVR), success rate (SR)

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