Non-invasive Evaluation of Liver Fibrosis Changes in Patients with Chronic Hepatitis C after Directly Acting Antiviral Drugs

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Background and study aims: Liver biopsy is the standard method to assess hepatic fibrosis, but it is unpractical as it is invasive and not agreed by most patients. So, many non-invasive methods for evaluating liver fibrosis have been used including fibrosis-4 (Fib-4) score. We aimed to evaluate hepatic fibrosis changes in patients with chronic hepatitis C after directly acting antiviral therapy.

Patients and Methods: 162 chronic hepatitis C patients were given directly acting anti-viral drugs (DAAs): sofosbuvir (400 mg/d) plus daclatasvir (60 mg/d), with or without Ribavirin (600 mg/d) for 12 weeks. Liver fibrosis was assessed before and 48 weeks after end of treatment (EOT) through evaluation of: 1- Serum tissue inhibitor of metalloproteinases-1 (TIMP-1), 2- Liver fibrosis score (Fib-4 score), 3- Liver stiffness measurement (LSM) using Shear Wave Elastography (SWE).

Results: sustained virological response was achieved in 95.1% of cases at 48 weeks after EOT. Liver fibrosis was improved at 48 weeks after EOT as evidenced by significant decline in serum level of TIMP-1, significant improvement in Fib-4 score, and significant decline in LSM values using SWE. Multivariate regression analysis showed that higher baseline ALT level and higher baseline liver fibrosis score were predictive of liver stiffness.

Conclusion:DAAs therapy showed not only high success rate in HCV eradication (95.1%), but also lead to significant improvement in liver fibrosis .

INTRODUCTION

Infection with Hepatitis C virus (HCV) is a great health problem worldwide. Egypt is the country with the highest incidence of HCV infection **[1]**, but the rate is declining after the introduction of mass treatment with directly acting antiviral drugs (DAAs). Chronic hepatitis C (CHC) infection is known to be a leading cause to liver cirrhosis, hepatoma and liver-related mortality **[2]**. Hepatic fibrosis is a reversible process related to hepatic necro-inflammation and represents a balance between extracellular matrix production and degradation **[3]**. The extent and degree of hepatic fibrosis are predictors of the disease progression and help in selecting patients who need antiviral treatment, the duration of therapy, and the choice of treatment regimen **[4]**. Liver biopsy is the standard method to assess liver fibrosis but it carries many disadvantages, such as its invasiveness, small sample size, and sampling variations. Also, liver fibrosis is an active process that can't be assessed by single biopsy. Therefore, other non-invasive methods are used to assess liver fibrosis including fibrosis-4 (Fib-4) score, Lok score and Forns Index score **[5]**. On the other hand, there are many serum markers for assessing liver fibrosis including extracellular matrix proteins such as matrix metalloproteinases (MMPs), MMP inhibitors e.g. tissue inhibitors of matrix metalloproteinases (TIMPs), as well as fragments of procollagen II **[6]**. Shear wave elastography (SWE) is a new ultrasound-based method designed to assess liver stiffness alternative to liver biopsy. This SWE is non-invasive and can easily and

accurately evaluate the degree of liver fibrosis. In addition, it allows repeated evaluation of liver fibrosis in patients with chronic liver disease **[7]**. We aimed to evaluate the effect of DAAs therapy on liver fibrosis stages in CHC patients using shear wave elastography, serum level of TIMP-1, and Fib-4 score as noninvasive methods.

PATIENTS AND METHODS

This is a prospective study in which **214** consecutively enrolled patients with CHC who attended the HCV clinic of Endemic Medicine Department, during November 2018 through October 2019. Only **162** patients met the inclusion criteria: Naïve patients with chronic HCV infection (based on HCV antibody and HCV RNA) who were candidates for antiviral therapy according to the HCV treatment protocol issued by the Egyptian National Committee for Control of Viral Hepatitis (NCCVH). **52** cases were excluded due to HBV co-infection (n=8), diabetes mellitus/hypertension (n=12), patients with ascites/jaundice (n=6), HCC (n=2), renal impairment (Serum creatinine > 2.5 mg/dl) (n=2), refusal to share in the study (n=9) and patients lost during follow-up (n=13). Every patient was subjected to thorough history taking and clinical examination**,** laboratory investigations at baseline and 48 weeks after EOT {including complete blood count, ALT, AST and GGT, serum albumin, serum bilirubin, INR, serum cholesterol level, blood urea and serum creatinine, random blood sugar, real time PCR for HCV RNA (lower limit of detection 15 IU/m)**,** HBsAg, HIV antibodies**,** serum Alpha-fetoprotein}; then the patient started the treatment protocol: sofosbuvir (400 mg tablet once daily) + daclatasvir (60 mg tablet once daily), with or without ribavirin (600mg daily) for 12 weeks. Abdominal ultrasound was performed to all patients at 0 week and 48 weeks after EOT to assess the liver, spleen, gall bladder, portal vein, ascites, and others. Hepatic fibrosis was assessed through three levels:

A- Assessment of serum level of Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) at baseline and 48-week after EOT.

B- liver fibrosis (Fib-4) score was measured and reported at baseline and 48-week after EOT:

FiB-4 = [age (years) ×AST (IU/L)]/ [platelet count (10^9/L) × square root ALT (IU/L) **[8]**.

C- Liver stiffness measurements (LSM) were conducted using two-dimensional shear wave elastography (2D-SWE) using ultrasonography device (Aplio 500 system, Toshiba, Tokyo, Japan) at the start and 48 weeks after EOT. Results were computerized like fibro scan: F0, F1, F2, F3 and F4. The examination was performed in the right lobe of the liver through intercostal spaces, after fasting for minimum of 6 hours while the patient lying supine with the right arm in maximal abduction and breathing normally, with breath holding for few seconds during the acquisition. Five measurements were recorded in the kilopascal (kpa) unit, and their mean was recorded as the valid measurement. The used cut-off values were correlated to METAVIR fibrosis scoring system as follows: **F0** <8 kpa (no fibrosis), **F1** ≥8 kpa (fibrosis exists with expansion of portal zones), **F2** ≥ 9.2 kpa (fibrosis exists with expansion of most portal zones and occasional bridging), **F3** ≥14.6 kpa (fibrosis exists with expansion of most portal zones and marked bridging), and **F4** ≥24.5 kpa (presence of cirrhosis) **[9].**

Statistical analysis: Analysis of data was performed using the statistical package for the scientific studies (SPSS). Quantitative variables were presented as mean & SD. Comparison was carried out using paired sample t-test. While qualitative variables were presented as number & percent. Comparison was carried out using Chi-squared. P value < 0.05 was recorded as significant.

RESULTS:

This study included **162** chronic hepatitis C patients who were candidates for antiviral therapy. Their mean age was 48.7±14.7 years. They were 88 males and 74 females. The mean BMI was 24.3 ± 2.8 kg/m^2 (**Table 1)**. They received sofosbuvir and daclatasvir (154 patients) while the other 8 patients received sofosbuvir, daclatasvir and ribavirin (triple therapy). Sustained virological response (SVR) at 48 weeks after EOT was achieved in 95.1% of patients. Eight patients (4.9%) showed relapse during the follow up period**.** We found significant reduction in serum ALT, AST, GGT and bilirubin levels at 48 weeks after EOT (P-value < 0.001, < 0.001, 0.003, 0.026,

respectively). Platelets count significantly increased (P-value < 0.003), while hemoglobin significantly decreased 48 weeks after EOT (p-value < 0.007). Serum cholesterol significantly increased 48 weeks after EOT (P-value < 0.001) **(Table 2).** The serum level of TIMP-1 significantly declined from baseline to 48 weeks after EOT (895.9 ± 85.1 **Vs** 682.8 ± 72.7, respectively, P-value < 0.001). Also, a significant improvement in Fib-4 score from baseline to 48 weeks after EOT (1.8 ± 1.4 **Vs** 1.1 ± 0.8, respectively, P-value < 0.001), **(Table 2)**.

Figure1 showed a decrease in the mean LSM from baseline to 48 weeks after EOT through all fibrosis stages, but it was more evident among patients with higher baseline values: F4, F3, F2, F1 and F0 (32.62; 18.87; 10.85; 8.59, and 6.58 **to** 28.5, 14.9, 9.6, 8.1, and 6.55, respectively).

As shown in **table 3**, we found significant decline in mean level of liver stiffness measured by SWE from baseline to 48 weeks after EOT; [13.8 ± 8.1 **Vs** 9.5 ± 6.2 kPa, respectively, P-value < 0.001]. Patients with less LSM (F0 and F1) were increased significantly from 56 to 116 patients while those with higher LSM (F2, F3, F4) was decreased from 106 to 46 patients after 48 weeks of therapy (P <0.001). This means that there were 46 patients that fail to improve regarding LSM. Multivariate regression analysis showed that only high baseline ALT level and high baseline LSM (F3 and F4) were the independently predictors of failure of LS improvement (**Table 4).**

Table (1): Baseline characteristics of 162 patients with chronic hepatitis C

Variable	Descriptive statistics
Age (years): Mean ±SD/ (range)	48.7±14.7 / (24-73)
Sex: Males/ Females	88 (54.3%) / 74 (45.7%)
BMI (kg/m^2): Mean ±SD (range)	24.3 ± 2.8 (20-32)
Smoking: Yes / No	54 (33.3%) / 108 (66.7%)

BMI: body mass index SVR: sustained virological response

Table (2): Laboratory data, serum TIMP-1 and Fib-4 score values of all patients at baseline and 48 weeks after EOT.

	Baseline Mean ±SD	48-weeks after EOT Mean ±SD	p-value
ALT (U/L)	42.2 ± 18.4	21.1 ± 6.1	<0.001
AST (U/L)	44.2 ±22.7	23.6 ± 12.9	<0.001
GGT (U/L)	54.6 ± 21.9	39.7 ± 31.2	0.003
Total bilirubin(mg/dL)	.71 ± 0.25	.64 ± 0.21	0.026
Albumin (g/dL)	4.3 ± 0.51	4.2 ± 0.37	0.510
INR (N= ≤ 1.2)	1.06 ± 0.09	1.05 ± 0.07	0.533
Hemoglobin (g/dL)	13.4 ± 1.56	12.3 ± 1.13	0.007
Platelets (X 10^3/ml)	212.5 ± 61.6	231.8 ± 54.2	0.003
Cholesterol (mg/dl)	151.2 ± 39.1	228.6 ± 68.6	<0.001
Serum TIMP-1	895.9 ± 85.1	682.8 ± 72.7	<0.001
Mean FIB-4 score:	1.8 ± 1.4	1.1 ± 0.8	<0.001
F0 - F1: < 1.4: No (%)	78 (48.1%)	118 (72.8%)	<0.001
F2: 1.4 - 3.25: No (%)	54 (33.3%)	36 (22.3%)	0.001
F3 - F4: >3.25: No (%)	30 (18.5%)	8 (4.9%)	<0.001

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: Gamma-glutamyl Transferase; INR: international normalized ratio, TIMP= Tissue inhibitor of metalloproteinase. FIB-4: fibrosis. EOT= end of treatment. Quantitative data expressed as mean & SD (analyzed by paired sample t-test). Qualitative data expressed as Number & percent (analyzed by Chi-square test).

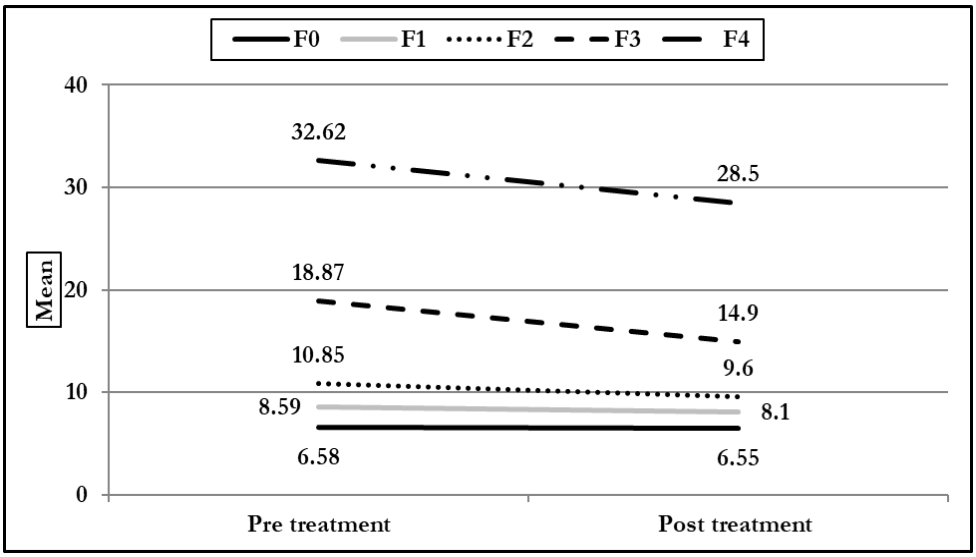

Figure (1): showing changes in the mean liver stiffness values at baseline and 48- weeks post treatment

Mean= mean liver stiffness value

Table (3): Liver stiffness measurements using SWE at baseline and 48-weeks after EOT

LS measured using SWE	Baseline	48 weeks after EOT	p-value
LSM: Mean ±SD (Kpa)	**13.8 ± 8.1**	**9.5 ± 6.2**	< 0.001
F0 <8 Kpa: No (%)	18 (11.1%)	66 (40.8%)	
F1 ≥8 Kpa: No (%)	38 (23.5%)	50 (30.9%)	< 0.001
F2 ≥ 9.2 Kpa: No (%)	52 (32.1%)	20 (12.3%)	
F3 ≥14.6 Kpa: No (%)	36 (22.2%)	18 (11.1%)	< 0.003
F4 ≥24.5 Kpa: No (%)	18 (11.1%)	8 (4.9%)	

LS= liver stiffness. SWE= shear wave elastography. EOT= end of treatment. Quantitative data displayed as mean & SD, (analyzed by paired sample t-test), qualitative data displayed as Number & percent (analyzed by Chi-Square test).

Table (4): multivariate regression analysis of 46 patients who failed to achieve improvement in liver stiffness

	OR	CI (95%)	P value
Age >50 Years	0.88	.77 - 3.1	<0.3
Male sex	1.34	2.41 - 4.36	<0.6
Base line ALT level	1.07	1.02-4.13	<0.01
TIMP-1 level	1.02	0.99 - 1.81	<0.4
Baseline LSM (F3 & F4)	1.9	1.22-3.31	0.001

OR= Odds ratio I= confidence interval)

DISCUSSION

Treatment of CHC with directly-acting antiviral drugs (DAAs) results in reduced risk of HCC and liver cell failure. This is may be due to the fibrosis regression after viral eradication **[10]**. In this study we assessed hepatic fibrosis changes in 162 patients with CHC by non-invasive methods. All patients had been received HCV treatment sofosbuvir/ Daclatasvir, with or without Ribavirin for 12 weeks. All of them were observed from the start of treatment till 48 Weeks after the end of treatment. The mean age of patients was 48.7±14.7, and this relatively late age of presentation may be due to most of patients are not aware of the risk of the disease and neglect to seek medical care and assessment. The SVR in this study was 95.1%, regardless the sex or age of the patients. This is consistent with Omar *et al*, ***2018,*** who reported that CHC patients who received daclatasvir plus sofosbuvir, with or without ribavirin, showed an overall response rate of 95.1% **[11]**. Also, *El*

*Kassas et al, **2018*** studied CHC patients who received seven different DAAs regimens, with an overall response rate of 95.5% regardless of the regimen used **[12]**. In our study, there was significant decline in ALT, AST and GGT and a significant increase in the platelets count from baseline to 48 weeks of EOT. Serum bilirubin level, which was already in the normal range before therapy, significantly decreased 48 weeks after EOT. This agreed this *Saleh et al **2020***, who studied 80 CHC patients who received a sofosbuvir/daclatasvir/± ribavirin treatment and showed significant decrease in the levels of ALT and AST and significant increase in the platelets count after SVR-12 **[13]**. Also, *El Kassas et al, **2018***, described an improvement of liver function tests in chronic HCV patients following DAAs therapy **[13]**. Another study by *El-Kholy et al, **2020***, reported an improvement in platelet level in patients with HCV-associated thrombocytopenia after 24 weeks SVR of sofosbuvir and daclatasvir therapy **[14]**. The reduction in AST and ALT levels may be attributed to improvement in necro-inflammation and improvement of liver functions. The reduction in serum bilirubin level observed in our study added an evidence for the improvement in liver function after antiviral therapy. Also, the increased in platelet count from baseline to 48 weeks after EOT is probably due to HCV viral eradication.

In the present study we assessed serum tissue inhibitor of metalloproteinase-1 level (TIMP-1) as a marker of liver fibrosis at baseline and 48 weeks after EOT; and found significant decline in its level (P value = <0.001) reflecting improvement in liver fibrosis. This result agreed with *Sebastian et al, **2016,*** and *Thalia, et al, **2020,*** who investigated the effects of a sofosbuvir-based antiviral therapy for CHC on early liver fibrosis regression and found that TIMP-1 level was significantly reduced in CHC patients from baseline to 12 weeks after DAA therapy ($P < 0.001$) **[15,16]**. It was reported that the decrease in TIMP level associated with fibrosis regression is probably due to apoptosis-mediated clearance of activated stellate cells **[17]**. In our study we also, assessed FIB-4 score as an indirect method of liver fibrosis assessment and found significant improvement in FIB-4 score after 48 weeks of EOT compared to baseline (P-value < 0.001). This result agreed with *Haseltine et al, **2015,*** who reported that CHC patients treated with a telaprevir-based regimen showed improvement in FIB-4 Score at 24 weeks post-treatment **[18]**. Also, *Bachofner et al, **2017,*** reported a significant decrease in LS measurements and other fibrosis scores such as FIB-4 after 12 weeks of EOT **[19]**. Similarly, *Elsharkawy et al, **2017***, reported improvement in liver stiffness but the improvement was variable among the different noninvasive scores used **[20]**. The improvement in these fibrosis scores is probably due to the reduction of AST and ALT levels and to the improvement in platelets levels, reflecting significant regression in hepatic fibrosis and necro-inflammation following DAAs therapy. In our study, the mean liver stiffness values using SWE for all patients were significantly declined from baseline to 48 weeks after EOT (13.8 ±8.1 and 9.5 ±6.2 kPa, respectively, P-value < 0.001) regardless treatment outcome. This means that the reduction in viral replication leads to improvement in necroinflammatory changes that lead to improvement of LSM that points to an improvement in hepatic fibrosis after antiviral treatment. This result is consistent with *Mohammed and Omar**,*** **2019,** who found significant reduction in LS measured by Fibro Scan from baseline to EOT, 24-weeks and 48-weeks after DAAs therapy in CHC patients **[21]**. Similarly, Lee SH, **2020,** who studied 68 patients with chronic HCV infection and reported that SWE findings showed significant improvement in LSM after 12-week SVR **[22]**. Also, *Serkan et al, **2020***, and *Ezzelregal, **2020,*** found a significant regression in the LSM of CHC patients receiving DAA therapy using SWE that carried out at the start of treatment, EOT, and 12 weeks after EOT **[23,24]**. On the other hand, *Ekaterinea et al, **2017,*** evaluated the effect of DAA therapy on liver fibrosis regression measured by transient elastography in CHC patients and reported decline in the median liver stiffness at week 24 after treatment compared with baseline but despite decreased LS, more than half of the cirrhotic patients with LS remained cirrhotic at week 24 following treatment **[25]**. But this may be explained by the fact that cirrhotic process is an irreversible process and starting DAAs early before reaching the cirrhotic stage is our target to improve liver fibrosis regression

Limitation of our study: one of the limitations is the lack of simultaneous liver biopsies for comparison between the improvement in liver

stiffness by SWE and the histological improvement. Larger scale of patients and longer duration of follow up are warranted to detect the effects of DAAs therapy on the dynamics of hepatic fibrosis.

CONCLUSION

12-week of DAAs therapy showed not only high success rate in HCV eradication (95.1%), but also lead to significant improvement in liver fibrosis manifested in: decline in TIMP-1, improvement in Fib-4 score and improvement in liver stiffness measured with 2D-SWE. DAAs therapy resulted in significant improvement in parameters of liver fibrosis supporting the fact that early diagnosis and treatment of chronic HCV infection before advanced fibrosis and permanent liver damage is necessary to get the maximum benefits from antiviral therapy.

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Conflict of interest: All authors guarantee no conflict of interest .

Ethical considerations: This study was approved by the Research Ethics Committee of Minia University Faculty of Medicine. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice. Informed consent was obtained from each participant.

RESEARCH HIGHLIGHTS

1. In this study: 162 chronic hepatitis C patients were given directly acting anti-viral drugs (DAAs): sofosbuvir (400 mg/daily) plus daclatasvir (60 mg/daily), with or without Ribavirin (600 mg/daily) for 12 weeks.
2. Liver fibrosis was assessed before and 48 weeks after end of treatment (EOT) through evaluation of: 1- Serum tissue inhibitor of metalloproteinases-1, 2- Fib-4 score, 3- Liver stiffness measurement using shear wave elastography (SWE).
3. Liver fibrosis was improved at 48 weeks after EOT as evidenced by significant decline in serum level of TIMP-1, significant improvement in Fib-4 score, and also significant decline in LSM values using SWE.
4. Sustained virological response was achieved in 95.1% of patients after 48 weeks EOT.

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