Noninvasive biomarkers predict improvement in liver fibrosis after successful generic DAAs based therapy of chronic hepatitis C in Egypt

Ekram W. Abd El-Wahab (MBChB, MPH, PhD)^{a,1,*},
Hesham AK. Ayoub (MBChB, MA, MD, PhD)^{b,c,d}, Aziza A. Shorbila (MBChB, DPH, MPH)^e,
Ashraf Mikheal (MBChB, MA, MD, PhD)^f, Mohamed Fadl (MBChB, MA, MD, PhD)^f,
Amira M. Kotkat (MBChB, MPH, Dr.PH)^a

^a Department of Tropical Health, High Institute of Public Health, Alexandria University, Egypt

^b Department of Hepatology and Endemic Medicine, Military Medical Academy, Cairo, Egypt

^c Director of the Viral Hepatitis Unit, Qappary Hospital, Ministry of Health and Population, Alexandria, Egypt

^d Chief consultant of Hepatology Department, Qappary Hospital, Ministry of Health and Population, Alexandria, Egypt

^e Fellow of Tropical Health Department, High Institute of Public Health, Alexandria University, Egypt

^f Consultant of Hepatogastroenterology and Endemic Diseases, Damamhour Fever Hospital, Ministry of Health and Population, Egypt

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ABSTRACT

Background: The scale of HCV problem in Egypt prioritized the development of a national control strategy that employs the use of locally manufactured generic direct-acting antivirals (DAAs). Achieving sustained virologic response in chronic HCV patients is the key for preventing fibrosis progression and ultimately its resolution.

Objective(s): To evaluate impact of generic DAAs on liver fibrosis in chronic HCV patients using some non-invasive biomarkers (NIBMs).

Methods: A total of 280 chronic HCV patients (18 treatment experienced and 262 naive) were enrolled and received the standard of care. All patients were clinically evaluated before starting treatment and at 4, 12 and 24 weeks of treatment.

Results: The sensitivity and the specificity of the tested NIBMs for predicting cirrhosis versus liver ultrasound ranged between 75.0–79.6% and 59.4–67.2% respectively. All NIBMs showed significant linear decline during the treatment and follow up periods among responders but tended to fluctuate in values toward an increase among non-responders. In cox regression analysis, normal baseline ALT, AST, total serum cholesterol, serum albumin, WBCs, prothrombin activity and early stages of liver fibrosis predicted improvement in liver fibrosis by 1.5–2 folds. The mean probability of recovery at 1, 3, 6 months varied between the different scores and ranged from 0.059 to 0.637, 0.125–0.741, 0.538–0.951 respectively.

Conclusion: The results provide prognostic data on the improvement in liver fibrosis over the treatment course and follow up periods without the need for a liver biopsy. Early detection and management of HCV infection should be emphasized to maximize the benefits of the treatment.

1. Introduction

Chronic hepatitis C (CHC) is a leading cause of advanced liver disease and associated mortality. Successful antiviral therapy with sustained virological response (SVR) can reduce liver-related morbidity and mortality as well as the need for transplantation.^{1–3} Assessment of the stage of liver fibrosis is important for diagnosis, treatment, and follow-up both during and after completion of the treatment. Non-

invasive methods for assessing the degree of fibrosis are currently substituting liver biopsy owing to its inherent risk of complications.^{4–6} Among the non-invasive modalities, transient elastography (TE; FibroScan®) is the most accurate in assessing liver stiffness.^{7,8} However, this non-invasive method is expensive and is not widely available particularly in poor resource countries.⁸

Recently many noninvasive biomarkers (NIBMs) for assessing liver fibrosis have been developed, and are frequently used in clinical

* Corresponding author. Tropical Health Department High Institute of Public Health 165 El-Horreya Road, 21561, Alexandria, Egypt.

E-mail address: ekram.wassim@alexu.edu.eg (E.W. Abd El-Wahab).

¹ To whom Correspondence should be addressed.

practice. They have been validated in different studies, and some were found to be highly accurate in the assessment of liver fibrosis comparing to liver biopsies⁹, which have always been used as the standard reference for evaluating the accuracy of noninvasive methods. The NIBMs are ideal in assessing the stage of fibrosis in patients with CHC or chronic hepatitis B and who require longitudinal evaluation of the stage of fibrosis after achieving the SVR.^{8–12}

NIBMs for liver fibrosis comprise direct biomarkers (class 1) and indirect biomarkers (class 2). The direct markers are produced by the hepatic stellate cells and correlate with extracellular matrix turnover in fibrosis process. On the other hand, the indirect markers including the AST/ALT Ratio (AAR), AST to Platelet Ratio Index (APRI), Forns index, FIB-4 score, Lok index, cirrhosis discrimination score (CDS) and Göteborg University Cirrhosis Index (GUCI) reflect changes in liver functions and are released in case of liver inflammation.^{8,10,12–14}

Together with the suppression of HCV replication, response to antiviral therapy with interferon or ribavirin reduces liver complications in CHC^{15,16} with sufficient evidence of improvement in liver histology.^{17,18} However, little is known about the evolution of liver fibrosis in HCV patients in the new era of directly acting antivirals (DAAs) that revolutionized HCV treatment with cure rates exceeding 95% regardless the infecting genotype.¹⁹ In this study, we proceeded to evaluate post-treatment improvement in liver fibrosis in chronic HCV patients using some NIBMs in settings where TE is not available.

2. Methods

2.1. Study design and patients

A prospective real-life cohort study [one group pre-test post-test design] was conducted between July 2016 and October 2017 at some Liver Center in Alexandria and El-Behira governorates. These centers are affiliated to the Ministry of Health and Population in Egypt and provide the standard of care of CHC according to the international guidelines.^{20,21}

A minimal sample of 35 HCV patients with hepatic fibrosis was required to estimate an average reduction of 50% at fibrosis rate after having HCV treatment protocol.¹⁸ The employed equation used an alpha error = 0.05 and intervention effect size = 0.5 with an expected drop out rate = 10% that will provide a study power of 80%. The sample size was calculated using G. power software.

The sample size was increased to compensate for the drop out during the follow-up. The sample units were consecutively selected after fulfilling inclusion criteria. The study patients comprised chronic HCV positive patients (naïve and -experienced) eligible for receiving DAAs. In total, 18 (6.4%) patients were experienced for INF and RBV regimens. The patients are referred to the center for receiving DAAs as standard care. The criteria for inclusion were: ages above 18 years, both sex, HCV-RNA positive PCR, HBsAg seronegative, complete blood picture within normal ranges and all eligible cases for treatment according to international guidelines (Child A, Child B).^{21,22} Those found ineligible for the treatment were excluded from the study [Age under 18, HBV seropositive, history of malignancy, untreated thyroid disease, active substance abuse, and hepatic decompensation [(Child C), (Albumin < 2.8 g/dl, TSB > 3 mg/dl, international normalized ratio (INR) > 1.3, platelets < 50000/cmm)], presence of ascites on Ultra sound, uncontrolled diabetes (HbA1C > 9%), current or planned pregnancy, end stage renal disease or serum creatinine greater than 1.5 mg/ml, continued pattern of alcohol abuse > 40 g/day in the last 6 month, WBC < 3000/cmm, Hemoglobin < 10 g/dl, hepatocellular carcinoma (HCC), retinopathy, HIV, and obese patients (Body Mass Index (BMI) > 35)].^{21,22}

2.2. Clinical and laboratory investigations

All enrolled patients were interviewed using structured predesigned

questionnaire to collect sociodemographic data, history of previous treatment for HCV and were subjected to complete medical examination (general and abdominal) including the estimation of the BMI and any features of decompensated cirrhosis. Baseline laboratory investigations for proper selection of eligible cases for treatment comprised: complete Blood Count (CBC), fasting blood glucose, liver function tests, total serum bilirubin (TSB), alanine Aminotransferase (ALT), aspartate Aminotransferase (AST), total serum cholesterol (TSC), serum gamma-glutamyl transferase (γGT), prothrombin time, prothrombin activity (PA), international normalized ratio (INR), serum albumin, serum creatinine, anti-nuclear antibody (ANA), thyroid stimulating hormone (TSH), free T3, free T4, alpha fetoprotein (AFP), antibilharzial Ab, HIV Ab, HBsAg and HbCAb as markers for HBV infection [Enzyme-linked immunosorbent assay (3rd generation ELISA kit DIALAB®, Austria), HCV RNA quantitation by Real-Time PCR using Cobas Ampli Prep/Cobas TaqMan HCV-RNA assay (Roche Diagnostics; Pleasanton, CA, USA) with a threshold of detection = 15 IU/ml, anti-bilharzial Ab: [indirect hemagglutination test (IHA) (Fumouze Diagnostics, France)]. Pregnancy test was done for women in child bearing age. ECG and fundus examination were done for all patients. Those found seropositive for bilharziasis received antibilharzial treatment regimen for 10 days prior to enrollment in the HCV treatment cohort.

All patients underwent abdominal ultrasound examination to check for the general condition of the liver and other abdominal organs. Liver fibrosis or cirrhosis was evaluated by ultrasound and was inferred from the calculated liver fibrosis scores namely; AAR ratio, APRI score, Forns index, FIB-4 score, lok index, and GUCI.^{8,23}

2.3. Medications and regimens of combination therapy

The anti-HCV drugs used in the current study were supplied by local manufacturers in Egypt and their effectiveness and safety were found comparable to brand drugs.^{24,25}

Since genotype 4 is predominating in Egypt,^{26–29} all enrolled patients received Sofosbuvir/Daclatasvir combination therapy; i) Patients with chronic hepatitis without cirrhosis were given SOF + DAC for 12 weeks, ii) patients with cirrhosis or relapsers after pegyinterferon/ribavirin combination therapy were given SOF + DAC + RBV for 12 weeks. The combination therapy was given as: Sofosbuvir 400 mg PO qDay, Daclatasvir 60 mg PO qDay, Ribavirin (Rebetol; Schering-Plough K.K.), was given in two divided daily oral doses adjusted to body weight (800 mg for weights < 50 kg, 1,000 mg for weights 50–65 kg, 1,200 mg for weight 65–80 kg, and 1,400 mg for weights > 80 kg).

To compensate for Hb drop caused by Ribavirin, folic acid and iron supplements were given to patients. All patients found positive for Anti-shistosomal Ab received praziquantal 60 mg/kg/day orally in 3 divided doses for 10 day before starting DAAs.

2.4. Follow up

Patients underwent follow up during the 12 weeks of treatment period and for 12 weeks after treatment. At week 4, 12 of treatment and at weeks 12 post-treatment all enrolled patients were subjected laboratory investigations, including CBC, liver function and renal function tests as well as qRT-PCR for viral load to detect early (EVR) and sustained virologic responses (SVR12).

2.5. Treatment endpoints

EVR was defined as undetectable HCV-RNA or below the lower limit of quantification at 4 weeks of initiation of treatment, while the SVR12 was defined as undetectable HCV-RNA or below the lower limit of quantification at 12 weeks after the last dose of treatment (or at 24 week of starting treatment) as endpoint.

The primary efficacy endpoint was achieving an SVR at 12 weeks after the end of therapy (EOT) defined as HCV-RNA below the assay's

lower limit of detection (LLOD). Secondary efficacy endpoints included EOT virological response, defined as HCV-RNA below the assay's LLOD measured at the EOT. Null-responder was defined as HCV RNA persistently high not reaching < LLOD throughout the treatment period, or HCV-RNA $\geq$ LLOD at EOT. Relapse was defined as confirmed HCV RNA > LLOD during any post-treatment follow-up visit in patients with HCV-RNA < LLOD at the EOT.

2.6. Statistical analysis

Data were collected, revised for accuracy and completeness, coded and fed to statistical software SPSS (Statistical Package for the Social Sciences) version 21.0 (IBM® Corp., Armonk, NY, USA). The results were statistically analyzed, tabulated and displayed in appropriate figures and tables. All statistical analysis was done using two tailed tests and alpha error of 0.05. Significance of the obtained results was judged at the 5% level ($p \leq 0.05$). Numbers, percent, means with standard deviation and the median were used to describe the scale and categorical data, respectively. Pearson's Chi-square test, Mont Carlo exact test and Fishers exact test were used to test for the association between the categories of two independent samples. The mean $\pm$ SD of the calculated scores were calculated at 0, 4, 12, and 24 weeks and their performance was compared between responders and non-responders. Repeated measures ANOVA was used to test the differences in means of quantitative variables measured at different time points. Cox regression analysis (or proportional hazards regression) was used to investigating the effect of several variables upon the time a specified event takes to happen; thus to determine the factors associated post treatment improvement in liver fibrosis over the course of treatment and follow up periods. Recovered defined improvement in liver fibrosis based on NIBMs scores and censored defined lack of improvement. In this context, FIB-4 was used a liver fibrosis scoring model. The model included all host related variables [Age, gender, occupation, residence, co-morbidities, past history of INF therapy] as well as the different hematological, biochemical and virologic parameters. Receiver operator characteristic curve (ROC curve) was used to assess the diagnostic accuracies of noninvasive indices for predicting cirrhosis versus liver ultrasound. Kaplan Meir test was used to assess post treatment regression in liver fibrosis using some non-invasive biomarkers. In Kaplan Meier analysis, recovery (improvement in liver fibrosis scores) was used as the event-of-interest. Seven liver fibrosis biomarker scores were tested and their performance was compared.

Extrapolation forecasting equation was used to predict the continuous progressive regression in liver fibrosis. The latter employed the use of a linear equation to predict the probability of improvement in liver fibrosis beyond our data sets.

3. Results

3.1. Socio-demographics of the enrolled HCV patients

We enrolled a total of 280 chronic HCV patients. The majority of the participants were rural residents (60.4%) and in the age group 50–75 years (66.1%) with an overall mean $\pm$ SD of 52.0 ± 10.3 years. Males and females were equally presented (50.7% vs 49.3% respectively). Other sociodemographics are detailed in (Table 1).

3.2. Medical history and physical examination of the enrolled HCV patients

Previous history of INF therapy was reported by 6.4% of the enrolled HCV patients under treatment. Almost one half (53.5%) of chronic HCV patients were free of co-morbidities, while 30.0% were diabetic, 14.3% were hypertensive, 5.7% had renal diseases and 5.4% had portal hypertension (Fig. S1). The most frequently reported symptoms throughout the course of DAAs based therapy were fatigue (88.2%), flu like symptoms (60.7%), gastrointestinal upsets (19.3%),

Table 1
Socio-demographic characteristics and personal habits of the enrolled HCV patients.

Characteristic		Total participants (n = 280)	
		No.	%
Age	20–34	17	6.1
	35–49	78	27.8
	50–73	185	66.1
Gender	Male	142	50.7
	Female	138	49.3
Residence	Urban	111	39.6
	Rural	169	60.4
Marital status	Single	13	4.6
	Married	230	82.1
	Widowed	28	10.1
	Divorced	9	3.2
Education	Illiterate	12	4.3
	Read & Write	17	6.1
	Primary Education	27	9.6
	Preparatory Education	22	7.8
	Secondary Education	129	46.1
Occupation	University Education	73	26.1
	Not Working	32	11.4
	Farmer	40	14.3
	Employee	62	22.1
	Professional	5	1.8
	HCW	9	3.2
	Housewife	82	29.3
	Clerk	43	15.4
	Student	3	1.1
	Others	4	1.4
BMI	<18 underweight	1	0.4
	18– normal weight	9	3.2
	25– overweight	69	24.6
	30+ overweight	201	71.8
Smoking	Yes	80	28.6
	No	200	71.4
Alcohol	Yes	0	0.0
	No	280	100.0
Drug abuse	Yes	1	0.4
	No	279	99.6

infections (13.2%) and skin rash (6.8%). Liver ultrasound of the enrolled HCV patient revealed liver cirrhosis (64.6%), abnormal echo pattern (25.2%), splenomegaly (9.6%), fatty fibrosis (8.9%) and coarse bright liver (6.1%) (Fig. S2). Accordingly, the presence of cirrhosis or fibrosis among HCV patients under treatment was 77.1%.

3.3. Efficacy, safety and tolerability of the administered DAAs drug regimens

The sustained virologic response rate among the HCV patients who received the standard DAA regimen was 98.6%. By week four of treatment, 87.9% had cleared the virus and became HCV-RNA negative, thus achieved EVR. All but 2 patients maintained virologic suppression while receiving therapy and recorded an EOTSVR as a treatment end-point (99.3%). Thus, EOT virologic non response at week 12 was experienced by 2 patients (0.7%). The latter maintained non response during follow up and another two patients relapsed at week 24 during follow up (Fig. 1).

All but one of the non-responders (75.0%) were naïve and the response rate among treatment experienced patients was 94.4%. All non-responders (100.0%) were cirrhotics (> F3 on FIB-4), had significantly high TSB, AFP, INR, γ GT levels and decreased platelet count and prothrombin activity thus were categorized as difficult to treat ($p < 0.001$).

In univariate logistic regression analysis, several factors were found significantly associated with non-response to HCV therapy (Table S1). SVR12 was not achieved in smokers [OR (95% CI) = 0.1 (0.01–1.3), $p = 0.04$], elevated AFP [OR (95% CI) = 1.1 (1.01–1.2) $p < 0.001$], decreased prothrombin activity [OR (95% CI) = 1.1 (1.0–1.1) $p = 0.001$],

Fig. 1. Virologic response during treatment and follow-up periods.

higher than normal INR [OR (95% CI) = 1.1 (1.0–1.2), $p < 0.001$], higher than normal TSB [OR (95% CI) = 1.1 (1.0–1.1) $p = 0.001$], low platelet count [OR (95% CI) = 1 (1–1.1) $p = 0.023$], higher than normal γ GT [OR (95% CI) = 1.04 (1.01–1.04) $p = 0.004$], being difficult to treat [OR (95% CI) = 1.1 (1.0–1.3) $p < 0.001$], having F3 fibrosis as determined by FIB-4 [$p = 0.015$]. The means of all the calculated noninvasive liver fibrosis scores were significantly higher among non-responders [$p = 0.015$]. Nonetheless, none of these variables predicted non response in the multivariate logistic regression model.

3.4. Hematological, biochemical and virological changes over the course of treatment

There was a significant and linear improvement in all the laboratory findings over the treatment course and during the follow up periods among the responders comparing to non-responders as revealed by the repeated measures ANOVA test. In overall, the effect size attributed to the treatment response was significantly higher comparing to the effect of the time course (Table S2 and Fig. S3).

3.5. Diagnostic accuracies of noninvasive indices for predicting cirrhosis versus liver ultrasound

Fig. 2 illustrates the diagnostic accuracies of noninvasive indices for predicting cirrhosis versus liver ultrasound. The area under the ROC curve (AUROC) for the liver fibrosis score showed that the best performance was for FIB-4 (AUROC = 0.791; CI = 73.4%–84.8%) followed by king's score (AUCROC = 0.786; CI = 72.7%–84.5%), mFIB-4 (AUROC = 0.771; CI = 70.9%–83.8%), Forns index (AUROC = 0.771; CI = 71.1%–83.1%), GUCI (AUCROC = 0.766; CI = 70.5%–82.6%), Lok index (AUROC = 0.762; CI = 69.9%–82.5%), and APRI score (AUROC = 0.760; CI = 69.9%–82.1%). On the other hand, the performance of AAR was not accurate in detecting the state of fibrosis (AUROC = 0.567; CI = 48.9%–65.4%). The sensitivity and the specificity of the evaluated liver fibrosis scores ranged between 75.0–79.6% and 59.4–67.2% respectively. Assuming that the ultrasound is a reliable and valid instrument for diagnosing liver fibrosis/cirrhosis, the inter-rater reliability as measured by Cohen's Kappa (κ) test for all the calculated liver fibrosis scores in comparison to liver ultrasound was fair (κ values ranged between 0.261 and 0.355 if excluding the AAR and the AARPRI)³⁰ for detecting liver fibrosis/cirrhosis ($p < 0.001$).

3.6. Assessment of post treatment regression in liver fibrosis using some NIBMs

Apart from the AAR, all non-invasive liver fibrosis scores showed significant linear decline during treatment and follow up periods among

responders ($p < 0.05$). On the other hand, the majority of these scores tended to fluctuate in values toward an increase in a non-linear pattern among non-responders. In overall, the effect size in liver fibrosis scores attributed to the treatment response was significantly higher comparing to the effect of the time course. The largest effect size was demonstrated in a linear pattern by the King's score (20.5%), GUCI (18.7%), APRI (13.1%), FIB-4 (9.0%), and Forns index (4.4%) ($p < 0.05$) (Table 2 and Fig. 3).

3.7. Factors associated with post treatment improvement in liver fibrosis

In Cox regression analysis, normal ALT [OR 95% CI = 1.7 (1.14–2.5)], serum albumin [OR 95% CI = 1.9 (1.1–3.4)], WBCs [OR 95% CI = 1.12 (1.02–1.2)], prothrombin activity [OR 95% CI = 1.5 (1.02–2.2)] and F0-F1 in FIB-4 liver fibrosis scoring [OR 95% CI = 1.9 (1.13–3.2)] predicted 1.5–2 folds improvement in liver fibrosis ($p < 0.05$). The increase in AST, TSC, Forns index and CDS by one unit was associated with decreased probability for fibrosis regression by about 8%, 6%, 41.5% and 16.6% respectively ($p < 0.05$). On the other hand, the increase in γ GT, King's and CDS by one unit increased the probability for fibrosis regression by about 7% and 9% respectively ($p < 0.05$) (Table 3).

3.8. Prediction of continuous progressive regression in liver fibrosis overtime

Kaplan Meier analysis revealed that the probability of recovery among the treatment non-responders was consistently zero as demonstrated by all the tested scores. The number of people who recovered among responders varied between the different scores and ranged from 100 to 183; being lowest for the Lok Index (100) and highest for the APRI and GUCI score (183). In total, 34 subjects did not recovery until the end of the study. The probability of recovery at 1, 3, 6 months varied between the different scores and ranged from 0.059 to 0.637, 0.125–0.741, 0.538–0.951 respectively. APRI, FIB-4, GUCI and King's scores demonstrated statistically significant differences for the Log Rank, Breslow and Tarone-Ware tests of equality of recovery among responders and non-responders ($p < 0.001$) (Table 4 and Figs. 4–5).

The employed extrapolation forecasting test predicted continuous progressive regression in liver fibrosis (Fig. 6).

4. Discussion

In 2014, generic DAAs regimens were successfully introduced as a part of the national CHC treatment program in Egypt. Eligible patients receive the standard of care according to the approved treatment recommendations.²¹ SVR and improvement of liver fibrosis are ultimate goals for DAAs based therapy.³¹ Gradual resolution of liver fibrosis occurs after alleviating the necroinflammation and damage in sustained viral responders. However, this requires accurate and serial longterm evaluation.^{31–35}

A total of 280 chronic HCV patients were enrolled in the study. Their ages ranged between 20 and 73 as complying with eligibility criteria for treatment. However, the majority of the participants were in the age group 50–73 with an overall mean age of 52 years. This is consistent with the higher HCV infection rates among older population in Egypt. In fact HCV infected patient in this age group must have acquired HCV infection through blood transfusion before implementing the screening of blood and blood products in blood banks for blood borne viruses in 1992 or during the era of parenteral anti-schistosomal therapy.³⁶ The latter was led in large-scale campaigns (between 1950s and 1980s) by the Egyptian Ministry of Health for controlling schistosomiasis and was the main driver of the HCV epidemic in Egypt.³⁶

In accordance with El-Zanaty and co-workers³⁷ most of the participants were from rural side where the prevalence is greatest for Schistosomiasis and HCV infection. Population characteristics were typical to those of rural communities in Egypt where farming is the

Fig. 2. AUROC summary of performance characteristics of tested noninvasive biomarkers for the diagnosis of cirrhosis among the enrolled HCV patients.

principal occupation and illiteracy is prevailing.^{36,37} Almost one half were free of co-morbidities. The most frequently reported chronic diseases were DM, hypertension and renal disease and portal hypertension. In fact, these chronic diseases are prevailing in the general population worldwide and in Egypt.^{37,38} Moreover, the majority (78.5%) of those having co-morbidities were in the age group 50–75 years ($p < 0.001$).

Based on liver ultrasound, the majority of the enrolled HCV patient had some degrees of liver fibrosis or cirrhosis. In fact, most of liver disease centers in Egypt relay on liver ultrasound for providing diagnostic and prognostic information as well as detecting complications such as HCC and portal hypertension. However, it does not provide sufficient clues for diagnosing liver fibrosis and is only sensitive in 60% of the cases.^{39,40} So, patients in the present cohort with detected abnormal echo pattern or coarse bright liver may have a degree of fibrosis which cannot be judged by the conventional ultrasound. Moreover, it is difficult in some cases to differentiate liver fibrosis from fatty liver which is a common condition among the Egyptian population particularly HCV patients.^{41,42} Further, MR elastography (MRE) appears to be the most reliable method for grading liver fibrosis.⁴³ TE and MRE have comparable accuracy for detecting significant fibrosis.⁴⁴ However, these modalities are not used in Egypt for the evaluation of HCV patients before treatment or during their follow up.

In concordance with results from previous clinical trials and real life experiences, all but 4 (1.4%) of the enrolled patient became HCV-RNA

negative by 12 weeks of treatment with an overall response rate (SVR12) of 98.6% for the administered DAAs regimens.^{45,46} The treatment was well tolerated and none of the self-reported drug adverse reactions forced any of the study participants to discontinue the treatment course.^{45–48} Interestingly, all experienced patient but one achieved SVR despite that the majority (88.93%) had cirrhosis but only 22.0% of them were classified as difficult to treat. This agrees with Nagaty et al.,⁴⁶ but contradict Elsharkawy et al. as they reported that treatment-naïve patients had higher SVR rates than the treatment-experienced patients.⁴⁹

Prediabetics are less likely to respond to treatment comparing to diabetics suggesting insulin resistance as main player.^{50,51} However, in the current real life cohort, all diabetics responded well to HCV therapy and all non-responders were non diabetics and had normal baseline FBS, PPBG as well as normal HbA1C. In fact, diabetics should have glycemic control under control of antidiabetic therapy that was found to improve the response to antiviral treatment.⁵² On the other hand, glucose abnormalities appear to have little impact on treatment response in DAAs treated patients.^{53,54} High insulin in insulin resistance may inhibit the ability of interferon α to block HCV replication due to the activation of PI3K by insulin, thus leading to inhibition of STAT-1, which is involved in the interferon α pathway.⁵⁵ The impact of diabetes mellitus and insulin resistance on HCV infection and treatment response needs to be further evaluated with the new DAAs.

The overall performance of the liver fibrosis indices in predicting

Table 2
Assessment of liver fibrosis score over time among HCV patients under treatment using repeated measures ANOVA.

Liver Fibrosis Score	Treatment Response	Pretreatment			On treatment			Posttreatment			Within Subject Effects			Between Subject Effects		
		Day 0		Mean ± SD	Week 8		Mean ± SD	Week 12		Mean ± SD	Week 24		Effect of Time (T) versus Response (T x R)	F ^a	p	Effect Size (Partial Eta Squared) ^c
		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD				
AAR	Yes	1.06 ± 0.37	1.11 ± 0.36	1.11 ± 0.36	1.11 ± 0.40	1.11 ± 0.32	1.11 ± 0.32	1.11 ± 0.32	1.11 ± 0.32	1.11 ± 0.32	1.11 ± 0.32	1.11 ± 0.32	T	1.49	0.216	0.016
	No	1.74 ± 1.0	1.95 ± 1.2	1.7 ± 0.837	1.7 ± 0.837	1.5 ± 0.7	1.5 ± 0.7	1.5 ± 0.7	1.5 ± 0.7	1.5 ± 0.7	1.5 ± 0.7	1.5 ± 0.7	T x R	1.45	0.227	0.016
APRI	Yes	1.2 ± 1.42	0.65 ± 0.8	0.53 ± 0.53	0.53 ± 0.53	0.42 ± 0.33	0.42 ± 0.33	0.42 ± 0.33	0.42 ± 0.33	0.42 ± 0.33	0.42 ± 0.33	0.42 ± 0.33	T	9.6	0.0001	0.095
	No	1.7 ± 0.35	1.6 ± 0.5	1.7 ± 0.45	1.7 ± 0.45	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	T x R	13.9	0.0001	0.131
AAPRI	Yes	1.17 ± 0.94	1.17 ± 0.7	1.14 ± 0.74	1.14 ± 0.74	1.10 ± 0.70	1.10 ± 0.70	1.10 ± 0.70	1.10 ± 0.70	1.10 ± 0.70	1.10 ± 0.70	1.10 ± 0.70	T	0.18	0.91	0.002
	No	3.0 ± 1.0	2.9 ± 1.4	2.8 ± 1.3	2.8 ± 1.3	3.1 ± 1.3	3.1 ± 1.3	3.1 ± 1.3	3.1 ± 1.3	3.1 ± 1.3	3.1 ± 1.3	3.1 ± 1.3	T x R	0.402	0.752	0.004
Forns index	Yes	5.9 ± 2.0	5.9 ± 1.9	5.9 ± 1.9	5.9 ± 1.9	5.7 ± 1.8	5.7 ± 1.8	5.7 ± 1.8	5.7 ± 1.8	5.7 ± 1.8	5.7 ± 1.8	5.7 ± 1.8	T	2.3	0.08	0.024
	No	9.4 ± 0.6	8.5 ± 0.5	8.7 ± 0.4	8.7 ± 0.4	9.7 ± 0.7	9.7 ± 0.7	9.7 ± 0.7	9.7 ± 0.7	9.7 ± 0.7	9.7 ± 0.7	9.7 ± 0.7	T x R	4.3	0.006	0.044
FIB-4	Yes	3.1 ± 2.9	2.3 ± 2.0	2.1 ± 1.6	2.1 ± 1.6	1.8 ± 1.2	1.8 ± 1.2	1.8 ± 1.2	1.8 ± 1.2	1.8 ± 1.2	1.8 ± 1.2	1.8 ± 1.2	T	4.7	0.0001	0.049
	No	6.1 ± 2.1	5.7 ± 2.1	5.8 ± 2.1	5.8 ± 2.1	8.0 ± 2.7	8.0 ± 2.7	8.0 ± 2.7	8.0 ± 2.7	8.0 ± 2.7	8.0 ± 2.7	8.0 ± 2.7	T x R	9.1	0.0001	0.09
mFIB4	Yes	4.2 ± 3.6	4.1 ± 3.1	4.0 ± 2.9	4.0 ± 2.9	3.8 ± 2.4	3.8 ± 2.4	3.8 ± 2.4	3.8 ± 2.4	3.8 ± 2.4	3.8 ± 2.4	3.8 ± 2.4	T	0.16	0.921	0.002
	No	10.6 ± 4.4	10.4 ± 5.5	10.0 ± 5.0	10.0 ± 5.0	10.9 ± 5.0	10.9 ± 5.0	10.9 ± 5.0	10.9 ± 5.0	10.9 ± 5.0	10.9 ± 5.0	10.9 ± 5.0	T x R	0.49	0.687	0.005
Lok Index	Yes	0.53 ± 24	0.54 ± 0.24	0.54 ± 0.25	0.54 ± 0.25	0.48 ± 0.24	0.48 ± 0.24	0.48 ± 0.24	0.48 ± 0.24	0.48 ± 0.24	0.48 ± 0.24	0.48 ± 0.24	T	0.045	0.987	0.004
	No	0.95 ± 0.02	0.94 ± 0.1	0.94 ± 0.1	0.94 ± 0.1	0.98 ± 0.02	0.98 ± 0.02	0.98 ± 0.02	0.98 ± 0.02	0.98 ± 0.02	0.98 ± 0.02	0.98 ± 0.02	T x R	0.53	0.66	0.006
Göteborg University Cirrhosis Index (GUCI)	Yes	1.47 ± 2.1	0.83 ± 1.3	0.7 ± 0.78	0.7 ± 0.78	0.50 ± 0.42	0.50 ± 0.42	0.50 ± 0.42	0.50 ± 0.42	0.50 ± 0.42	0.50 ± 0.42	0.50 ± 0.42	T	16.0	0.0001	0.148
	No	2.3 ± 0.34	2.1 ± 0.5	2.3 ± 0.44	2.3 ± 0.44	4.4 ± 0.65	4.4 ± 0.65	4.4 ± 0.65	4.4 ± 0.65	4.4 ± 0.65	4.4 ± 0.65	4.4 ± 0.65	T x R	21.1	0.0001	0.187
Cirrhosis Discrimination Score (CDS)	Yes	5.33 ± 1.51	5.67 ± 1.52	5.61 ± 1.65	5.61 ± 1.65	5.60 ± 1.71	5.60 ± 1.71	5.60 ± 1.71	5.60 ± 1.71	5.60 ± 1.71	5.60 ± 1.71	5.60 ± 1.71	T	1.4	0.253	0.015
	No	8.0 ± 1.2	7.5 ± 1.7	6.8 ± 2.0	6.8 ± 2.0	6.8 ± 1.50	6.8 ± 1.50	6.8 ± 1.50	6.8 ± 1.50	6.8 ± 1.50	6.8 ± 1.50	6.8 ± 1.50	T x R	2.8	0.042	0.029
King's Score	Yes	31.4 ± 44.2	17.6 ± 26.1	14.0 ± 16.0	14.0 ± 16.0	10.1 ± 9.1	10.1 ± 9.1	10.1 ± 9.1	10.1 ± 9.1	10.1 ± 9.1	10.1 ± 9.1	10.1 ± 9.1	T	17.5	0.0001	0.16
	No	48.4 ± 10.6	43.3 ± 8.6	47.5 ± 7.2	47.5 ± 7.2	92.5 ± 16.4	92.5 ± 16.4	92.5 ± 16.4	92.5 ± 16.4	92.5 ± 16.4	92.5 ± 16.4	92.5 ± 16.4	T x R	23.7	0.0001	0.205

p < 0.05 is significant.

^a F value based on Wilks' Lambda multivariate test when Mauchly's test is significant.

^b significant quadratic effect was considered in highlighted cells when linear effect was insignificant.

^c large effect if the value of partial Eta squared > 0.14.

Fig. 3. Liver fibrosis score over time among HCV patients under treatment.

liver fibrosis/cirrhosis versus ultrasound was fair as the AUROC did not exceed 8 in any of the tested scores. Moreover, comparing the performance versus ultrasound as a gold standard is not a good choice owing to its suboptimal accuracy in detecting liver fibrosis^{39,40} and thus we got fair agreement between the two instruments in diagnosing liver

fibrosis/cirrhosis. In fact, routine clinical ultrasound is not a sensitive predictor of early fibrosis in chronic viral hepatitis. Surface nodularity is the most sensitive sonographic feature for the detection of significant fibrosis and routine clinical ultrasound is the most useful for the detection of cirrhosis. This means that a significant number of patients

Table 3

Cox regression [Backward Wald method] for factors affection the improvement in liver fibrosis.

Step 61	Variable	B	SE	Wald	df	Sig. <i>p</i> value	Exp(B)	95.0% CI for Exp(B)	
								LB	UB
	Normal ALT	.518	.195	7.029	1	.008	1.679	1.145	2.462
	Elevated AST	-.008	.004	4.689	1	.030	.992	.985	.999
	Normal Albumin	.645	.303	4.535	1	.033	1.906	1.053	3.449
	Normal Prothrombin Activity	.397	.194	4.203	1	.040	1.488	1.018	2.175
	Normal WBCs	.116	.045	6.725	1	.010	1.123	1.029	1.227
	Elevated total serum cholesterol	-.006	.003	5.175	1	.023	.994	.988	.999
	Elevated γ GT	.007	.003	5.473	1	.019	1.007	1.001	1.012
	Forns Index	-.415	.150	7.680	1	.006	.660	.492	.886
	FIB4 (F0-F1)	.646	.264	5.977	1	.014	1.908	1.137	3.204
	Cirrhosis Discrimination Score (CDS)	-.166	.077	4.596	1	.032	.847	.728	.986
	King's score	.009	.004	5.491	1	.019	1.009	1.002	1.017

p < 0.05 is significant.**Table 4**

Kaplan Meier analysis for the probability of improvement in liver fibrosis over the treatment course and follow up periods using different liver fibrosis biomarkers.

Liver Fibrosis Biomarker	Treatment Response	Case summary	No of Events n (%)	Censored n(%)	Event Time (months)	No. of Events (recovery)	No. at Risk (to recovery)	Probability of recovering	Test of equality of recovery for levels of treatment response		
									Log Rank (Mantel- Cox)	Breslow (Generalized Wilcoxon)	Tarone-Ware
									P value		
APRI Score	Responders	n = 183	174 (95.1)	9 (4.9)	1	87	96	0.475	<0.0001	.001	<0.0001
					3	33	63	0.656			
					6	54	9	0.951			
	Non- Responders	n = 4	0 (0.0)	4 (100.0)	1	0	4	0.0			
					3	0	4	0.0			
					6	0	4	0.0			
Forns Index	Responders	n = 193	116 (60.1)	77 (39.9)	1	51	142	0.264	.060	.068	.062
					3	33	109	0.435			
					6	32	77	0.601			
	Non- Responders	n = 4	0 (0.0)	4 (100.0)	1	0	4	0.0			
					3	0	4	0.0			
					6	0	4	0.0			
FIB-4	Responders	n = 192	165 (85.6)	27 (14.1)	1	58	134	0.302	.001	.005	.002
					3	38	96	0.500			
					6	69	27	0.859			
	Non- Responders	n = 4	0 (0.0)	4 (100.0)	1	0	4	0.0			
					3	0	4	0.0			
					6	0	4	0.0			
mFIB-4	Responders	n = 192	107 (55.7)	85 (44.3)	1	13	179	0.068	.131	.138	.134
					3	11	168	0.125			
					6	83	85	0.557			
	Non- Responders	n = 4	0 (0.0)	4 (100.0)	1	0	4	0.0			
					3	0	4	0.0			
					6	0	4	0.0			
Lok Index	Responders	n = 186	100 (53.8)	86 (46.2)	1	11	175	0.059	.057	.063	.060
					3	32	143	0.231			
					6	57	86	0.538			
	Non- Responders	n = 4	0 (0.0)	4 (100.0)	1	0	4	0.0			
					3	0	4	0.0			
					6	0	4	0.0			
GUCI	Responders	n = 195	183 (93.8)	12 (6.2)	1	100	95	0.513	<0.0001	.001	<0.0001
					3	31	64	0.672			
					6	52	12	0.938			
	Non- Responders	n = 4	0 (0.0)	4 (100.0)	1	0	4	0.0			
					3	0	4	0.0			
					6	0	4	0.0			
King's Score	Responders	n = 193	180 (93.3)	13 (6.7)	1	123	70	0.637	<0.0001	<0.0001	<0.0001
					3	20	50	0.741			
					6	37	13	0.933			
	Non- Responders	n = 4	0 (0.0)	4 (100.0)	1	0	4	0.0			
					3	0	4	0.0			
					6	0	4	0.0			

p < 0.05 is significant.

Fig. 4. The equality of recovery (improvement in liver fibrosis) over the treatment and follow-up periods among responders and non-responders.

Fig. 5. The mean probability of recovery (improvement in liver fibrosis) over the treatment and follow-up periods among responders and non-responders.

Fig. 6. Prediction of the continuous progressive regression in liver fibrosis overtime.

with fibrosis could be missed from the diagnosis which is an important factor affecting the efficacy of chronic hepatitis treatment.³⁹ Fibrosis can be detected and staged with reasonable accuracy using TE and ARFI. These have high sensitivities and specificities to diagnosis from significant fibrosis to liver cirrhosis and can be used to predict prognosis.^{56,57} However, when the performance of NIBMs was compared to TE, ARFI or liver biopsy, comparable sensitivity and specificity values to our study results were obtained. Moreover, NIBMs are less accurate

in detecting intermediate stages of fibrosis than cirrhosis with a mean standardized AUROC for diagnosis of fibrosis/cirrhosis of 0.76–0.89 and the sensitivities and specificities ranged between 50 and 90%.^{8,10,13,58–65}

Resolution of established liver fibrosis might take several years to occur.^{66,67} Earlier findings from animal models of chronic liver disease alongside a growing evidence from clinical studies evaluated early changes in dynamic fibrosis-related parameters during DAA based therapy using the enhanced liver fibrosis (ELF) panel, combined with liver stiffness measurement.^{31,68–70} In the present real-life cohort, we employed the use of some NIBMs for liver fibrosis which showed significant reduction reaching >90% (53.8–95.1%) 12 weeks after EOT compared to baseline values. These scores are affected by the increased platelets count as well as the reduction of AST, ALT, and γ GT levels. Sustained responders in the present cohort showed significant reduction in these scores comparing to relapsers denoting improvement in necroinflammation and liver fibrosis after successful DAAs based antiviral therapy. Early studies comparing INF based regimens reported a 62% improvement in liver fibrosis after achieving SVR in paired liver biopsies.⁵⁸ In fact, improvement of liver stiffness measurements occurs by the EOT regardless the treatment outcome which can reflect the temporary reduction in viral replication. This remains controversial as this early changes may represent an actual improvement in liver fibrosis or a result of resolved necroinflammatory activity following antiviral treatment. However, other studies stated that the inflammatory activity did not contribute to liver stiffness.^{68,71} The median of the liver stiffness measurement was observed to decrease by more than 60% of pre-treatment values in patients with advanced fibrosis or cirrhosis.⁷² Various studies reported different degrees of improvement in liver fibrosis as assessed by different modalities and the median time for improvement (Table 5).

With the previously used INF and ribavirin, several studies reported factors associated with decline in liver stiffness measurements after antiviral therapy as baseline fibrosis stage, baseline activity, SVR, BMI, age, and viral load.⁶⁷ With the recent use of DAAs, this issue is still under research. However it seems that age, viral load, BMI, and baseline activity will no longer have a role. In the present study, normal baseline ALT, AST, TSC, serum albumin, WBCs, prothrombin activity and early stages of liver fibrosis as revealed by liver fibrosis scoring (FIB-4, Forns, and CDS) predicted improvement in liver fibrosis by 1.5–2 folds.

SVR has an established role in improvement in liver fibrosis status.⁷³ Poynard et al. identified in a multivariate logistic regression analysis factors other than SVR that were associated with likely fibrosis regression/reversal after treatment. These included younger age, low BMI, higher platelet count, absence of portal hypertension and mild virus activity, supporting a better chance for early cirrhosis to regress

Table 5
Studies assessing change in liver stiffness in patients with chronic HCV.

Study	Publication Year	No. of patients	Treatment regimen	Assessment method used	% improvement in liver fibrosis	Median time to improvement
Poynard et al. ⁸²	2013	933	INF based regimens	FibroTest™ and TE	49%	10 Years
Crissen et al. ^{63,83}	2015	100	INF or DAAs based regimens	TE, APRI scores	60%	2.5–3 years
Akhtar et al. ³⁴ (Meta-analysis)	2015	443	INF or DAAs based regimens	liver biopsy after more than	53%	36 months post treatment
Bruno et al. ⁸⁴	2016	100	DAAs based regimens	APRI scores and Fib-4 index	36.7% and 67.2%	6 Months
Chekuri et al. ³¹	2016	100	INF or DAAs based regimens	TE	40%	24 weeks and 1.67 year (median); 100 at 24 weeks and 56 at >1 year
Bernuth et al. ⁷⁰	2016	32	DAAs based regimens	Enhanced liver fibrosis (ELF) scores and TE	38%	3 months post treatment
Bachofner et al. ⁸⁵	2017	392	DAAs based regimens	TE, APRI scores and Fib-4 index	60.9% and 29.1%	18 months post treatment
Mehrez et al. ⁸⁶	2017	50	DAAs based regimens	APRI scores and Fib-4 index	66% and 36.8%	3 months post treatment
Elsharkawy et al. ³⁵	2017	337	DAAs based regimens	TE, APRI scores and Fib-4 index	77%	3 months post treatment
Dolmazashvili et al. ⁷³	2017	304	DAAs based regimens	TE	65%	6 Months
Singh et al. ⁸⁷ (Meta-analysis)	2018	2934	INF or DAAs based regimens	TE	47%	EOT, 1–6, 6–12, > 12 months post-treatment
Rayes et al. ⁸⁸	2018	18	DAAs based regimens	TE and Controlled Attenuation Parameter (CAP)	30%	2 Years
Pan et al. ⁸⁹	2018	84	DAAs based regimens	TE	64%	1 Year
Current study	2019	280	DAAs based regimens	Ultrasound, AAR, APRI score, Forns index, Fib-4 score, Iok index, GUCI, CDS, King's Score, AARPRI	53.8–95.1%	3 months post treatment

than established cirrhosis.¹⁷

The presence of co-morbidities, hepatic steatosis or risky behaviors such as smoking and alcohol consumption are likely determinants to limit liver fibrosis regression in sustained responders⁶⁷ although this was not evident in the present study. Such analysis of underlying risk factors would be helpful during the follow-up of SVR patients to exclude potential post-SVR HCV specific mechanisms that account for fibrosis progression.

HCV can alter glucose metabolism leading to insulin resistance⁷⁴ which has a strong influence on SVR^{75,76} and accelerated fibrogenesis.^{77,78} This comes in line with the current study, as the FBG, PPBG and HbA1C showed linear improvement over the course of DAAs treatment and during the 3 months of follow up.

The employed extrapolation forecasting test in the present cohort predicted continuous progressive regression in liver fibrosis over time. Prior studies showed that fibrosis markers and liver histology improves significantly post-SVR.^{79,80} On the other hand, Chekuri et al., found that minimal further reduction in liver-stiffness can occur in the interval from SVR24 to the end of follow-up, suggesting that fibrosis regresses slowly after achieving SVR when it regresses at all.³¹

Treatment regimens for HCV are rapidly changing, and there is a great need for further studies on the effect of each regimen on liver fibrosis. Fibrosis regression has been shown to decrease the risk of HCC and mortality,⁸¹ but many patients are offered HCV treatment after fibrosis has been established. Since in the present study fibrosis was found in many patients, early diagnosis and treatment before advanced fibrosis and permanent liver damage has been established is warranted to maximize the benefits from antiviral treatment.

Collectively, the rapid improvements in HCV therapies will greatly enhance the likelihood of SVR among all patient groups with HCV infection, including those traditionally considered 'hard to treat'. Sofosbuvir-based therapy causes a clinically significant improvement in hepatic fibrosis as measured non-invasive liver fibrosis biomarkers even in cirrhotic patients. Our results indicate that non-invasive fibrosis scores were fairly accurate noninvasive blood tests to predict the presence or absence of significant fibrosis. This enabled us to provide prognostic data regarding the improvement in liver fibrosis over the treatment course and follow up periods without the need for a liver biopsy. Nevertheless, adequate follow-up is warranted to evaluate impact of SVR on fibrosis since the impact of HCV infection could stay many years and result in advanced liver diseases.

4.1. Limitation of the study

Both direct and indirect variables are utilized to possibly predict liver fibrosis. However, all have limitations in accuracy indices as a perfect test. Accordingly, indirect NIBMs are crude indices to reflect real changes in liver fibrosis. Most of these indices rely on necro-inflammatory markers which obviously improve much with eradication of the virus. Thus, the decreased values of these NIBMs are reflecting improvement in the necro-inflammatory state rather than a real regression of liver fibrosis which may occur simultaneously but at a different rate. Moreover, the follow up duration was too short to infer a change in liver fibrosis state.

Research involving human participants/or animals

The study was approved by the institutional review board and the ethics committee of the High Institute of Public Health affiliated to Alexandria University, Egypt. The research was conducted in accordance with the international ethical guidelines of the Declaration of Helsinki (2013) and the International Conference on Harmonization Guidelines for Good Clinical Practice. Data sheets were coded to ensure anonymity and confidentiality of patient's data. This article does not contain any studies with animals performed by any of the authors.

Informed consent

Informed written consent was obtained from all individual participants enrolled after explaining the aim and concerns of the study.

Availability of data

All data are fully available without restriction.

Declaration Conflict of interest

None to Declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cegh.2020.04.011>.

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