Hepatocellular Regeneration and Necrosis Markers in Chronic HCV Patients under Sofosbuvir-Based Regimen

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Abstract

Objectives: To evaluate noninvasive fibrosis markers, retinol binding protein 4 (RBP4), and alpha-feto protein (AFP) in foreseeing liver fibrosis in persistent hepatitis C patients and their correlations with Fibroscan.

Materials and Method: Fifty naive CHC patients and 20 healthy age and sex-matched subjects as controls were included. Blood samples and fibroscan obtained before and 3 months after treatment with DAAs for CBC, INR, and Liver function tests, RBP4, AFP and different fibrosis markers as APRI, CDS, FIB-4, GUCI, Lok index.

Results: All patients achieved SVR (12 weeks) with anchange of liver function and decrease of liver fibrosis as measured by Fibroscan. The Fib-4 score gave the best performance and diagnostic accuracy for the prediction of the presence of significant fibrosis, then (APRI and GUCI) followed by the CDC and LOK index; and lastly RBP4 as they were efficient to differentiate significant fibrosis (moderate and severe) liver scarring (F2-F3) from mild fibrosis (F0-F1). However; the accuracy of RBP4 improved with a higher fibrosis grade and thus can differentiate the presence or absence of severe (F3-F4) from mild & moderate (F1-F2) liver scarring with 93.75% for PPV and NPV but with a poor prediction of the absence of significant fibrosis (F1 versus F2–F3), while LOK score was excellent in the prediction of the absence of significant fibrosis (F1 versus F2–F3).

Conclusions: FIB-4, APRI, GUCI, CDS, and Lok index were accurate to predict the fibrosis grade in CHC patients, correlated with hepatic injury and can assess DAAs treatment success.

Keywords: HCV; markers of regeneration,necrosis, Sofosbuvir.

Introduction

HCV is a major cause of chronic hepatic disease worldwide with a significant variable prevalence, related to the investigated geographic location (**Martins et al. 2011**).

In chronic HCV patient, 30% of cases acquire decompensated liver cirrhosis within10 years(**Thomas and Seeff, 2005**). Treatment and eradication of HCV infection with DAAs can improve fibrosis and thus, reduce the disease-related morbidity and mortality (**Gene et al., 2012**). This was verified by significant regression of the parameters of transient elastography after sustained virologicalresponse (SVR) (**Bachofner et al., 2017**).

Staging and grading of hepatic fibrosisare fundamentalin CHC patients not only for monitoring liver illnessadvance, but also for treatment indication (**Castera, 2011**).Liver biopsy was the gold standard for assessing liver fibrosis grade. However; owing to its invasiveness, specialists and patients favor non-invasive tests for fibrosis staging as Fibroscan as it is a safe, rapid, but it is

expensive and may be not always available **(El-Hariri et al., 2017).**

Many non-invasive tests based on measurement of direct or indirect serum markers have been developed. Almost all of them have the ability to identify significant fibrosis and cirrhosis in particular **(Saad, 2014).**Serum liver cell necrosis markersinclude theincreasesin transaminases as aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, lactate dehydrogenase, Y-glutamyl transferase (GGT) activity, bile acids, alkaline phosphatase, and liver regeneration markers include RBP4, gamma-glutamyl transferase, AFP, and des-gamma-carboxy-prothrombin**(Kevin et al., 1999).**

Fibrosis calculated scores and indices are simple, inexpensive, noninvasive tests used in defining the degree of fibrosis, they include FIB4, APRI, CDC, GUCI and LOK Index **(Bonacini et al., 1998; Islam et al., 2005; Lok et al., 2005; Wai et al., 2003; Vallet-Pichard et al., 2007).**

Materialsand Method
Study design:A prospective, case-control, hospital based study carried out on HCV patients attending; Gastroenterology and Hepatology and Infectious Diseases Department out-patient clinic in Qena university hospital from Jan. 2018 to Dec. 2018.
Sample size:50 HCV patients and 20 healthy age and sex-matched subjects as controls.

Target population
Inclusion criteria:treatment naïve patients, age older than 18 years with HCV RNA positivity.
Exclusion criteria:
Gestation, acutehepatitis, or other forms of hepatic disorders such ashepatitis-B and HIV infection, Bilharziasis, auto-immunehepatitis, DM, drugs addiction or alcohol abuse, hepatocellular carcinoma, evidence of liver cell failure, cardiac cirrhosis or 1ry biliary cirrhosis or hereditary hepatic disorders.

The study protocol was approved by the local institutional ethics committee and all patients gave their written consent prior to the study.

Methodology
All of the patients and controls were subjected to10 ml fasting venous blood sampling,obtained under aseptic conditions into 3 tubes; tube for complete blood count, tube for prothrombin time, and plain tubes for liver functions and ELISA assays.Serumwere gained by centrifugation of the clotted blood at 3000 x g for 10 min at room temp, and allocated and kept at - 80°C till the time of RBP4 assay.

Full blood count: using cell dyne-1800 (Abbott Diagnostics - SantaClara-California-USA).

Liver function assessment:ALT (0–41 U/L), and AST, (0–38 U/L), alkaline phosphatase (ALP), lactate dehydrogenase (LDH)], γ-glutamyl transferase, albumin, total bilirubin, glucose (70–100 mg/ dl), urea (10–50 mg/dl), and creatinine (0.50–1.2 mg/dl), using Cobas c311 (Roche Diagnostics, Mannheim-Germany). Prothrombin time (PT) using STA Compact Max® Coagulation System (Stago-USA).

Hepatitis serumtesting for hepatitis B (sAg) and HCV antibody detected by an enzyme immunoassay (EIA) Cobas e411 (Roche Diagnostics, Mannheim- Germany).

Alphafetoprotein (AFP): assayedbased on the manufacturer's directives, by an automated immunoassay-Tosoh Bioscience(ST.AIA-PACK™ AFP Cat. No; 0025252) (Tosoh Corporation Minato-ku-Tokyo- Japan).The adult reference range(< 7.0 ng/ml) and the coefficient of variation was ≤ 5% across the linear range (1-1210 ng/ml) of measurement and the minimal measurable concentration (MDC) was 1.0 ng/ml. The standard level was(0-10 ng/ml).

Serum retinol-binding protein 4 (RBP4):assayed according to the manufacturer's guidelinesusing ELISA Kit Cat. No.E0929h-EIAab® Science Co. Ltd. Wuhan-China. The MDC 0.0510 ng/ml and the finding range was 0.156-10.0ng/ml.

Liver fibrisisextent:by Transient Elastography (TE) Fibroscan (Echosens, FS-502 touch device, Paris, France).normal values(2.5 to 7.0 kPa). subjectsdivided into subgroups accoedingto the optimal cutoff value of liver fibrosis ranges 2.0 to 7.0kPa = F0-F1 (no/mildhepatic scarring), 8.0 to 9.0kPa = F2 (Moderate hepatic scarring), 9.0 to 14.0kPa = F3 (Severe hepatic scarring), 14 kPa or higher = F4 (advanced hepatic scarring or cirrhosis)**.**The calculated computed scores were made according to published formulas APRI (**Wai et al., 2003**), Cirrhosis discriminate score (CDS) (**Bonacini et al., 1998**), FIB-4(**Vallet-Pichard et al., 2007**), GUCI (**Islam et al., 2005**), and Lok index (**Lok et al., 2005**).

Statistical analyses: All analyses were performed with the IBM SPSS 20.0 software.The data were tested for normality using the Anderson Darling test and for homogeneity variances prior to further statistical analysis. Categorical variables were described by number and percent (N, %), where continuous variables described by mean and standard deviation (Mean, SD). Chi-square used for comparison between categorical variables, whereas independent samples t test and paired t test used to compare between continuous variables. Receiver operating characteristic (ROC) curves were constructed to determine the optimal cutoff values for each bio-marker. The accuracy and the diagnostic performance of noninvasive fibrosis tests were compared with the area under the receiver operating curve (AUROC) and their corresponding 95% confidence intervals (CI) and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), were calculated. The diagnostic accuracy and AUROC were calculated based on TE

Fibroscan as a reference. All calculation is two-tailed, $p < 0.05$ was considered statistically significant. Pearson correlation was used to detect the association between variables.

Results

Demographic data: 50 chronic HCV patients (28 males and 22 females) with a mean age (55.66±10.49 years), BMI (25.27±2.1). All patients were Child-Pugh score A Mean ±SD (5.12±0.33),where45 patients received (SOF/DAC) therapy and 5 patients received (SOF/DAC/RBV) therapy. The mean PCR HCV viral load was (1449308.18±1114712.26). All treated patientsachievedSVR. The control group hada mean age of 54.60± 3.56 years (11 were males and 9 females) and their BMI was 25.74± 3.45 kg/m^2.

Laboratory data: compared to the control group,patients had higherliver enzymes, INR, AFP, and fibrosis scores (CDS, Lok index, Fib-4 score, GUCI and APRI), and lower albumin, total protein, RBP4, hemoglobin and platelet count. All parameters showedenhancement after therapy (improvement of liver function and reduction of liver enzymesand fibrosis by Fibroscan), **Table1**.

Correlations with fibroscan result in CHC patients: TE fibroscan results are positively correlated with fibrosis scores and indices (CDS, Lok index, Fib-4 score, GUCI and APRI), INR, MPV, AST and total bilirubin. And negatively correlated with RBP4, Hb, RBCs count, platelet count, albumin, total protein, Eosinophiles percentage and absolute count, and lymphocyte absolute count, **Table 2**.

Table 1: laboratory findings in patients and controls:

Parameter	Cases (n=50)		Control (n=20)	P1	P2	P3
	Pre	Post				
Fibroscan result kPa	9.15±2.65	7.96±2.31	-	001	-	-
CDS	5.1±1.78	4.54±1.39	2.65±0.88	065	001	001
LOK INDEX	0.44±0.22	0.33±0.18	0.15±0.07	003	001	001
GUCI	1.03±1.1	0.43±0.28	0.12±0.05	001	001	118
APRI	0.93±0.97	0.4±0.24	0.12±0.05	001	001	101
FIB-4	2.64±1.84	1.58±0.84	0.68±0.18	001	001	011
Albumin (g/dl)	3.85±0.48	3.95±0.42	4.13±0.16	240	015	120
Total Protein (g/dl)	7.27±0.59	7.47±0.5	8.06±0.34	052	001	001
ALT (U/L)	9.48±48.28	3.1±15.44	15.15±6.52	001	001	041
AST (U/L)	1.78±56.46	34.92±17.3	15.4±5.58	001	001	056
T. BIL (mg/dl)	0.95±0.31	0.73±0.27	0.57±0.13	001	001	022
ALP (U/L)	10.3±42.93	96±33.49	48.45±8.54	046	001	001
GGT (U/L)	2.28±12.18	25.3±10.7	15.35±5.84	002	001	001
LDH (U/L)	57.96±33.89	48.02±28.33	16.45±13.54	001	001	001
AFP (ng/mL)	8.52±2.87	4.89±2.2	2.05±0.62	001	001	001
RBP4 (mg/l)	88.34±2.71	5.83±2.12	55.17±4.54	001	001	001
INR	1.08±0.14	1.04±0.08	1.01±0.01	036	008	273
PLT (10^3/mm^3)	97.24±80.19	35.4±73.84	28.65±59.24	012	001	001
WBC (10^3/mm^3)	5.88±1.85	7.15±1.98	6.52±2.38	002	231	230

P1: Pre VS Post; P2: Pre VS Control; P3: Post VS Control

To differentiate mild fibrosis (F0-1) from significant fibrosis (F2-F3-F4):ROC curve showed that Fib-4 score is more superior subsequently APRI and GUCI. As the accuracy of Fib-4 produced (AUROC 0.985, 95% CI 83.8-99.4). And the accuracy of APRI and GUCI both produced (AUROC of 0.948, 95% CI 77.4 - 97.3). The accuracy of CDC produced (AUROC 0.893, 95% CI 87.4 - 99.9). The accuracy of LOK index produced (AUROC 0.921, 95% CI 91.6 - 100). The accuracy of RBP4 produced (AUROC 0.811, 95% CI 77.4 - 97.3). For definitive diagnosis of significant fibrosis (i.e. 100% specificity and 100% PPV), using APRI, Fib-4 and GUCI at the cutoff value of 0.35, 1.17, and 0.37 respectively, **Figure 1.**

To differentiate (F0-1-2) versus severe fibrosis (F3-4):the diagnostic performance of FIB4 is the most superior (AUROC 0.940, 95% CI 69.8 - 99.8). Followed by RBP4 (AUROC 0.93, 93.75% CI 69.8-99.8), followed by GUCI and LOK index both produced (AUROC 0.935 and 0.882 respectively, 95% CI 47.6 – 92.7), **Figure 2.**

Table 2: Correlation with fibroscan result:

	Fibroscan result kPa	
	R	**P**
Age	0.172	0.232
BMI	0.038	0.793
CDS	0.850[**]	0.001
LOK INDEX	0.837[**]	0.001
GUCI	0.595[**]	0.001
APRI	0.560[**]	0.001
FIB-4	0.765[**]	0.001
Albumin (g/dl)	-0.336[*]	0.017
Total Protein (g/dl)	-0.457[**]	0.001
ALT (U/L)	0.245	0.086
AST (U/L)	0.388[**]	0.005
T. BILT (mg/dl)	0.323[*]	0.022
ALP (U/L)	0.177	0.218
GGT (U/L)	0.218	0.129

	Fibroscan result kPa	
	R	**P**
LDH (U/L)	-0.009	0.948
AFP (ng/mL)	0.187	0.192
RBP4 (mg/l)	-0.777[**]	0.001
PCR (IU/ml)	0.053	0.715
INR	0.416[**]	0.003
PLT (10^3 /mm^3)	-0.745-[**]	0.001
MPV	0.353[*]	0.012
WBC (10^3 /mm^3)	-0.138	0.338

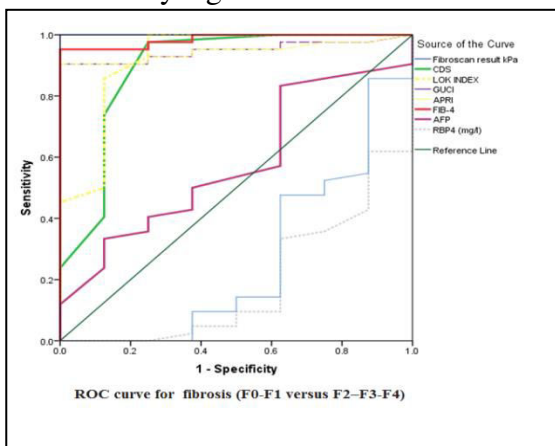*Statistically significant correlation <0.05

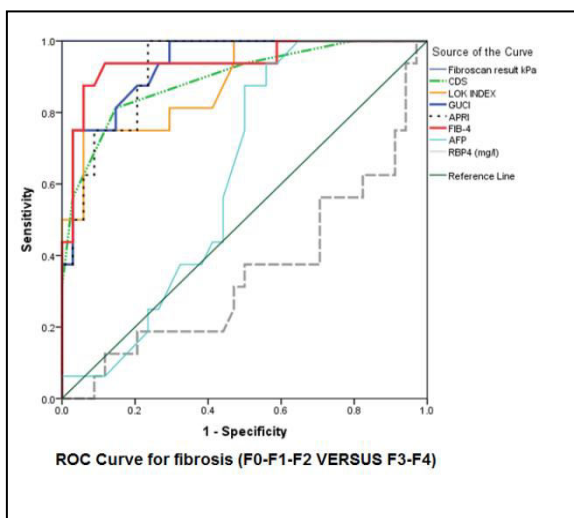**Figure 1: ROC curve for significant fibrosis(F0- F1 versus F2–F3-F4)**

Figure 2:ROC curve for significant fibrosis (F0-F1-F2 versus F3-F4)

Discussion

Eradication of HCV infection is avital medical seek in Egypt so as to avoid progression of liver fibrosis and later cirrhosis and its complications, including liver cell failure and portal hypertension **(Suo and Zhao, 2013)**.

Over the past several years, all oral DAAs agents have replaced IFN for the treatment of HCV.The advantages of DAAs improve tolerability, safety and utility and patients with advanced cirrhosis who were not candidates for antiviral therapy before could now be treated by DAAs **(Elkassas et al., 2017)**.

In this study; wenoted a significantincrease in liver enzyme levels and fall in the hepatic synthetic function (decreased serum albumin, RBP4, and platelet count and increased INR). This was in line with **(Allam et al., 2015).**

The patients had an elevated AFP compared to controls, with a noteworthydecline after treatment and SVR achievement, reflecting regression in disease activity. This was in line with **(Allam et al., 2015 and Deterding et al., 2015).**

Also in this study, we found a significant recession in fibroscan value in all patients both at the end of treatment and 6 months post-treatment from 9.15±2.65 kPa to 7.96±2.31 kPa. This finding goes in line with **(Bachofner et al., 2017)**whofound that patients with SVR showed marked improvement in the median transient elastography (TE) values from 12.65 kPa to 8.55kPa after DAA therapy.

The prognosis and management of chronic liver diseases largely depends on the extent and the progress of liver fibrosis. Histopathological check of a liver specimen obtained by percutaneous biopsy has traditionally been considered as the gold standard for evaluating hepatic fibrosis **(Bravo et al., 2001)**. However, liver biopsy is an invasive procedure, had poor patient acceptance and also carries a significant, although small risk of life-threatening complications**(Cadranel et al., 2000)**. So to overcome liver biopsy limits, several non-invasive blood tests developed to assess hepatic fibrosis. In this study, we investigated five non-invasive indices that can reliably predict the histological stage of liver fibrosis/cirrhosis (APRI, CDS, Fib-4, GUCI, Lok index) **(Hsieh et al., 2012)**

The comparison of the five noninvasive fibrosis indices and scores showed that, with the rising severity of liver fibrosis (evaluated by TE Fibroscan); their values increased significantly together with the increase of serum AFP and the decrease of serum albumin, RBP4, INR and platelet count. This suggests that they are a good estimator of hepatic fibrosis and can all effectively evaluate accurately different stages of liver fibrosis in CHC patients and could be used to decrease the number of liver biopsies. This was in line with (**Amorim et al., 2012; Baranova et al., 2011;Derbala et al., 2015; Khan et al., 2008; Li et al., 2014; Snyder et al., 2006).**

In this study, the viral load correlated positively with TE Fibroscan and AFP and correlated negatively with RBP4, while RBP4 showed a positive correlation with platelet count, and a negative correlation with fibrosis stage, viral load, AFP, and the five non-invasive fibrosis indices (APRI, CDS, Fib-4, GUCI, Lok index). This was in agreement with the findings of (**Derbala et al.**, **2015; Li et al., 2014).**

In this study, in CHC, the Fib-4 score (as estimated by ROC curve) gave the greatest performance and investigative accuracy inthe prediction of significant fibrosis, then (APRI and GUCI) followed by the CDC and LOK index; and lastly RBP4 as they were efficient to differentiate significant fibrosis (moderate and severe) liver scarring (F2-F3) from mild fibrosis (F0-F1). This was in line with (**Bonacini et al., 1998; Islam et al., 2005; Lok et al., 2005; Wai et al., 2003; Vallet-Pichard et al., 2007).** However; the accuracy of RBP4 was improved with a higher grade of fibrosis and thus can differentiate the presence or absence of severe liver scarring (F3-F4) from mild & moderate (F1-F2) liver scarring with 93.75% for PPV and NPV but with a poor prediction of the absence of significant fibrosis (F1 versus F2–F3). Furthermore, LOK score was excellent in the prediction of the absence of significant fibrosis (F1 versus F2–F3). AFP showed a positive correlation with fibrosis stage, viral load, four non-invasive fibrosis indices (APRI, Fib-4, GUCI and Lok index), and had a negative correlation with RBP4. In contrast, **Tai et al.**

(2009) found that AFP levels were positively correlated with the age, ALT, fibrosis grade, and were negatively correlated with platelet count. However, in our study, it has been found to have poor judgment power to differentiate the different stages of liver fibrosis.

In this study, CHC patient had a significant decline in the mean RBP4 after treatment. This was in line with (**Kataria et al., 2016**). RBP4 correlatedpositively with platelet count and inversely correlated with the disease severity and fibrosis score, viral load, AFP, the five non-invasive fibrosis indices (APRI, CDS, Fib-4, GUCI, and Lok index), asthe serum level of retinol depends on the hepatic synthesis and excretion of RBP4 that, in turn, decreases with increased liver damage (**Peres et al., 2011).**

For the diagnosis of significant fibrosis, TE and serum biomarkers from routine blood tests were equivalent. So to raise the diagnostic accuracy, a mixture of TE and serological markers of the non-invasive fibrosis indices was recommended by using an algorithm starting with a screening test using noninvasive fibrosis indices and restricting TE or liver biopsy in patients classified as F0–F1 by noninvasive serological tests, (**Boursier et al., 2009; Castera et al. 2005; Poynard et al., 2008; Sebastiani et al., 2006).**

The limitations of this study included the small number of patients and the exclusion of patients with concomitant liver disease, thus potentially limiting the generality of our results to other populations.

Conclusion
In CHC patients, serological tests for fibrosis indices have a good accurate diagnostic value that can predict the occurrence or absence of significant fibrosis and cirrhosis. They are cost-free,available, easy to calculate and reproducible indices. The use of the combination of these tests may avoid the need for invasive or expensive tests as liver biopsy and fibroscan. Serum RBP4 is a good marker for the prediction of definite and severe fibrosis and its rise after successful

HCV eradication is considered a marker of stoppage and reduction of inflammation.

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