

The Utility of Retinol-Binding Protein 4 in Predicting Liver Fibrosis in Chronic Hepatitis C Patients in Response to Direct-Acting Antivirals

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Background: Hepatic fibrosis grading is crucial for chronic hepatitis C (CHC) patients in monitoring liver disease progression and antiviral treatment indication. Retinol-binding protein 4 (RBP4), an adipokine secreted by adipocytes and hepatocytes, has variable levels in health and disease.

Purpose: To comparatively evaluate RBP4 serum levels in predicting liver fibrosis in CHC versus fibroscan, noninvasive fibrosis, and inflammatory indices.

Patients and Methods: Cohort study included 50 naive non-obese CHC patients and 20 age-, sex- and body mass index-matched healthy subjects. Fibroscan, RBP4, and noninvasive fibrosis as APRI, CDS, FIB-4, GUCI, Lok index indices based on serological markers, and inflammatory indices as platelet to lymphocyte ratio (PLR) and liver regeneration markers as; alpha-fetoprotein (AFP) and APRI, were evaluated in response to direct-acting antivirals (DAAs).

Results: RBP4 was significantly lower in patients than in controls ($P=0.0001$) and progressively decreased with the increase in fibrosis grade ($F0-F=41.42\pm3.08$), ($F2=39.32\pm1.43$), ($F3-F4=35.31\pm0.5$), ($P=0.0001$). Liver function, stiffness, and RBP4 significantly improved after treatment ($P=0.0001$). RBP4 negatively correlated with viral load ($r=-0.78$, $p=0.0001$), fibroscan fibrosis grade ($r=-0.52$, $p=0.0001$), AFP ($r=-0.63$, $p=0.0001$), and positively correlated with platelet ($r=0.424$, $p=0.0001$), and white cell count ($r=0.298$, $p=0.002$). RBP4 at a cutoff value <40.55 ng/mL might predict significant fibrosis (90.48% sensitivity, 62.5% specificity, AUROC=0.811, 95% CI=67.5–90.0) and at a cutoff value <35.9 ng/mL could predict advanced fibrosis (100% sensitivity, 100% specificity, AUROC =1.0, 95% CI=0.929–1).

Conclusion: RBP4 showed excellent accuracy, sensitivity, specificity, PPV, and NPV. RBP4 has a superior diagnostic performance in predicting advanced fibrosis grads in CHC patients and hence can replace expensive invasive procedures.

Keywords: APRI, FIB-4, GUCI, noninvasive fibrosis indices, platelet lymphocyte ratio, transient elastography fibroscan

Introduction

Precise staging and grading of hepatic fibrosis/cirrhosis are of critical concern in chronic hepatitis C (CHC) patients not only for monitoring the progression of liver disease but also for antiviral treatment indication.¹

Retinol-binding protein 4 (RBP4), an adipokine primarily secreted by adipocytes and hepatocytes, is the sole transporter of retinol from liver stores to peripheral tissues, so its serum estimate reflects serum retinol concentration and the

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increase in retinoic acid genesis is related to the progressive decrease in serum RBP4 levels and hepatic vitamin A storage exhaustion and hence the liver functional status, both of which accelerate liver fibrosis giving the chance for tumor development.²

Liver necrosis or active regeneration due to ongoing inflammation and/or fibrosis with an altered hepatocyte–hepatocyte interaction and loss of normal architecture produces elevation of alpha-fetoprotein (AFP).³

The benefit of extended sustained virological response (SVR) in reaction to the antiviral drug is to block the fibrogenic progression, thus escaping the progressive normal tissue destruction or the replacement of hepatic parenchyma, changing micro-environment from pro-inflammatory to anti-inflammatory restorative state, and redirect the synthesis of inflammatory molecules to a regenerative state.⁴

Little known about the effect of current direct-acting antiviral drugs (DAAs) on RBP4. This study aimed to estimate serum RBP4 as a screening tool for hepatocellular regeneration in response to Sofosbuvir-based treatment regimen in genotype 4, naïve CHC Egyptian patients, and relate the finding to the result of transient elastography (TE) – Fibroscan and non-invasive liver fibrosis marker testing in liver fibrosis grading and diagnosis of early cirrhosis.

Materials and Methods

The current study is a prospective case-control study, involving 50 naïve CHC non-obese patients, who were a candidate for antiviral therapy and monitored at the out-patient clinics of Tropical Medicine and Gastroenterology department – Qena University Hospital, from January 2018 to December 2018. 20 healthy subjects, matched by age, sex, and BMI, form a control group.

The inclusion criteria were treatment naïve patients, age older than 18 years with HCV RNA positivity. The study was conducted after approval by the Qena faculty of medicine – South Valley University institutional ethics committee review board, according to the Declaration of Helsinki and its subsequent amendments, and after obtaining written informed consent from all subjects.

The virological assessment of HCV-RNA level determined three times: at the baseline (before treatment), at the end of treatment (week 12), and 12-week post-treatment, using the Cobas TaqMan HCV assay V.2.0 (Roche Diagnostics – Mannheim, Germany). The lower limit of detection (15 IU/mL). Sustained virological response (SVR12) is defined as an undetectable HCV-RNA at

12 weeks after completion of therapy. The HCV genotypes were identified by direct sequencing of the untranslated regions using RT-PCR-based assay (Ampli Sens 61 HCV-genotype-FRT PCR kit).

Treatment Regimen

Based on European Association for Study of Liver (EASL) guidelines, for treatment of HCV, patients were classified into two groups: easy-to-treat group, including (naïve patients with a serum bilirubin level ≤ 1.2 mg/dl, albumin ≥ 3.5 gm/dl, INR level ≤ 1.2 and the platelet count $\geq 150 \times 10^9/L$, their regimen was sofosbuvir 400 mg and daclatasvir 60 mg once daily for 12 weeks), and difficult-to-treat group (total bilirubin ≥ 1.2 mg/dl, albumin level ≤ 3.5 gm/dl, INR ≥ 1.2 and the platelet count $< 150 \times 10^9/L$) treated by sofosbuvir 400 mg and daclatasvir 60 mg and dose-weighted ribavirin (800–1200 mg) daily for 12 weeks.⁵

Exclusion Criteria

Pregnancy, BMI > 25 , acute exacerbation of viral hepatitis, or other forms of liver diseases such as concomitant hepatitis-B and HIV infection, schistosomal, autoimmune or alcoholic hepatitis, diabetes, drug addiction or alcohol abuse, hepatocellular carcinoma (HCC), evidence of decompensated liver cirrhosis, cardiac cirrhosis or primary biliary cirrhosis or hereditary liver condition, presence of cancer or hepatoma, patients received recent platelet transfusion and associated kidney disease.

All the patients subjected to history taking, including (age, sex, previous operation, disease duration, past history, and family history), and physical examination, abdominal ultrasonography to assess the presence of liver cirrhosis; portal vein diameter, splenic size, the existence of ascites and evaluation of cirrhosis clinical-grade using Child–Pugh score.⁶

Blood Sampling

10-mL fasting venous blood sample, collected under aseptic conditions and divided into 3 tubes; an EDTA tube for a complete blood count, a citrate tube for prothrombin time, and a plain tube for liver functions and ELISA techniques. Serum samples were obtained by centrifugation of the clotted blood at $3000 \times g$ for 10 min at room temp and allocated and kept in sterile containers at -80°C till the time of RBP4 assay.

Complete Blood Count

Using cell dyne-1800 (Abbott Diagnostics – Santa Clara, CA, USA). Platelet count (PLT) and the neutrophil and lymphocyte counts used to calculate the platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) to reflect the patient's inflammation and immune response status.

Liver Function Assessment

Alanine (ALT) and aspartate (AST) aminotransferases, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ -glutamyl transferase, albumin, total bilirubin, glucose, urea, and creatinine, using Cobas c311 (Roche Diagnostics, Mannheim, Germany). prothrombin time (PT) using STA Compact Max[®] Coagulation System (Stago-USA).

Hepatitis B (HBsAg) and HCV Antibody Testing

By an enzyme immunoassay (EIA)-Cobas e411 (Roche Diagnostics, Mannheim, Germany).

Alpha-Fetoprotein (AFP)

According to the manufacturer's instructions, by an automated immunoassay system – Tosoh Bioscience (ST AIA-PACK[™] AFP Cat. No. 0025252) (Tosoh Corporation Minato-Ku, Tokyo, Japan). The adult reference interval for serum AFP was <7 ng/mL and the coefficient of variation was $\leq 5\%$ across the linear range (1–1210 ng/mL) of measurement and the minimal detectable concentration (MDC) was 1.0 ng/mL. The normal level was defined as 0–10 ng/mL.

Retinol-Binding Protein 4 (RBP4)

According to the manufacturer's directions, using ELISA (Kit Cat. No: E0929h-EIAab[®] Science Co. Ltd. Wuhan – China). The MDC was 0.051 ng/mL and the detection range was 0.156–10 ng/mL.

Liver Stiffness Measurement

Using Transient Elastography (TE) Fibroscan (Echosens, FS-502 touch device, Paris, France). The results expressed in kilopascals (kPa), with normal values, range from 2.5 to 7.0 kPa. Patients were classified into subgroups based on the optimal cutoff value of liver stiffness ranges 2 to 7 kPa = F0-F1 (no liver scarring – mild liver scarring), 8 to 9 kPa = F2 (moderate liver scarring), 9 to 14 kPa = F3 (severe liver scarring), 14 kPa or higher = F4 (advanced liver

scarring or cirrhosis).⁷ The calculated computed scores and indices according to published or patented formulas APRI, Cirrhosis discriminate score (CDS), FIB-4, GUCI, and Lok index.^{8–12}

Statistical Analyses

All calculations performed using the Statistical Package for Social Sciences (SPSS) version 20 software for Windows (IBM Corporation, Armonk, NY, USA). The data tested for normality using the Kolmogorov–Smirnov test and for homogeneity variances before further statistical analysis. Symmetrically distributed continuous variables presented as mean and standard deviation (mean, SD). The skewed continuous variables presented as median and range. Categorical variables presented as number (NO.) and percentage (%). Quantitative data were analyzed using the Mann–Whitney *U*-Test and Kruskal–Wallis test and the χ^2 probability test for the categorical data. Spearman correlation coefficients used to study the correlation between different parametric variables. Receiver operating characteristic (ROC) curves constructed to determine the optimal cutoff value to each biomarker. The accuracy and the diagnostic performance of noninvasive fibrosis tests compared with the area under the receiver operating curves (AUROC) and their corresponding 95% confidence intervals (CI) and the probability of a true positive (sensitivity) and a true negative (specificity), positive predictive value (PPV), negative predictive value (NPV), calculated. The diagnostic accuracy and AUROC calculated based on TE Fibroscan performance as a reference. All calculation was tow-tailed, and P-value <0.5 considered significant.

Results

Demographic data: 50 CHC patients (28 males and 22 females), with a mean age of (55.66 ± 10.49) years, BMI $(22.27 \pm 2.10 \text{ kg/m}^2)$. 45 patients received (SOF/DAC) therapy and five patients received (SOF/DAC/RBV) therapy. The HCV RNA median viral load was (929,350.00 IU/mL) with a mean of $(1,449,308.18 \pm 1,114,712.26)$, 58% ($\geq 800,000$ IU/mL) and 42% ($<800,000$ IU/mL). All patients achieved both rapid virological response and SVR. Twenty healthy subjects selected as a control group with a mean age of (54.60 ± 3.56) years, (11 males and 9 females) and BMI of $(22.74 \pm 2.13 \text{ kg/m}^2)$.

Laboratory investigation and TE fibroscan findings in CHC patients before and after treatment compared to controls: all parameters showed significant improvement after

therapy with a reduction of liver stiffness as assessed by TE fibroscan and improvement of liver synthetic function and reduction of liver enzymes (Table 1).

Laboratory investigation and noninvasive fibrosis indices in CHC patients concerning Fibroscan fibrosis grade (Table 2).

Correlations in CHC patients before therapy: RBP4 showed a positive association with the platelets, WBC, neutrophil, lymphocyte counts, and a negative link with fibrosis grade, viral load,

AFP, the five non-invasive fibrosis indices (Table 3).

To differentiate no or mild fibrosis (F0–F1) from significant and advanced liver fibrosis (F2–F3–F4): the diagnostic performance of the Fib-4 score is the most superior followed by GUCI, APRI, and LOK index with an excellent performance, followed by the good performance of CDC, RBP4, and PLR. (Table 4 and Figure 1).

To differentiate significant liver fibrosis (F0 - F1 - F2) versus advanced liver fibrosis (F3 - F4): the diagnostic performance of RBP4 is the most superior; likewise, fibroscan, with an outstanding performance at a cutoff value <35.9 ng/mL, with 100% sensitivity, 100% specificity, 100% PPV, 100% NPV, AUROC =1.0, 95% CI=0.929–1), followed by Fib-4 score, GUCI, and APRI showed excellent performance, and very good performance for LOK index and CDC, and good performance for PLR (Table 4 and Figure 2).

Discussion

The liver performs a critical role in regulating and suppressing inflammation by controlling both local and systemic inflammatory reactions. In CHC, the inflammatory response is linked to the progression of extra-hepatic and

Table 1 A Laboratory Investigation and Fibroscan Findings in CHC Patients and Healthy Control

Parameter	Pre-Treatment	After Treatment	Control	P-value*
Fibroscan KPa*	8.8(3.2–13.6)	7.7(2.8–11.83)	–	0.0017
Fibroscan**	No(%)	No(%)	–	0.00001
F0–I	8(16%)	34(68%)		
F2	26(52%)	1(2%)		
F3–4	16(32%)	15(30%)		
Total protein (g/dl)	7.2(6–8.3)	7.4(6.4–8.3)	8(6.9–8.5)	0.00001
Albumin (g/dl)	3.8(2.8–5)	4(3.2–5)	4.1(3.8–4.4)	0.0015
PT-INR	1.02(1–1.8)	1(1–1.5)	1(1–1.05)	0.0224
Total bilirubin (mg/dl)	0.9(0.46–1.72)	0.7(0.2–1.25)	0.56(0.25–0.75)	0.00001
AST (U/L)	46.5(16–291)	34(10–109)	14(10–30)	0.00001
ALT (U/L)	45(10–228)	30(10–58)	13.5(8–32)	0.00001
GGT (U/L)	34.5(15–58)	26.5(11–51)	13.5(9–31)	0.00001
ALP (U/L)	119.5(46,191)	103(40–160)	49.5 (36–62)	0.00001
LDH (U/L)	175.5(110–250)	153(97–190)	116(100–155)	0.00001
AFP (ng/mL)	8.5(3.7–23)	5(2–15)	1.8(1.30–3.30)	0.00001
RBP4 (mg/l)	38.4(34.4–47.1)	45.5(40.4–51.3)	55.57(47.50–62.30)	0.00001
APRI	0.59(0.18–5.18)	0.35(0.11–1.22)	0.1(0.07–0.26)	0.00001
CDS	5(1–9)	4(2–8)	2.5(1–4)	0.00001
Fib-4 score	2.13(0.28–8.17)	1.35(0.05–4.2)	0.65(0.44–1.19)	0.00001
GUCI	0.62(0.18–6.22)	0.35(0.11–1.4)	0.11(0.07–0.26)	0.00001
Lok index	0.4(0.1–1)	0.27(0.06–0.96)	0.16(0.06–0.29)	0.00001
Platelets (10 ⁹ /L)	182.5(60–415)	350.5(121–411)	332(236–411)	0.00001
WBCs (10 ⁹ /L)	5600(3200–11,020)	6850(4500–14,070)	5460(4250–10,900)	0.005
#Neutrophil count	2742(961–7163)	3660(1440–10,975)	3537(2233–7725)	0.0097
#Lymphocyte count	2130(735–5220)	2567(1140–4800)	1703(796–3509)	0.0002
#Monocyte count	321(105–746)	333(84–846)	254(87–763)	0.2208
NLR	1.27(0.44–5.33)	1.35(0.5–4.70)	2.14(0.64–4.76)	0.0012
PLR	81.04(26.89–204.44)	85.59(38.65–310.75)	198.88(70.96–474.87)	0.00001

Notes: *Kruskal–Wallis test; *Man Mann–Whitney U-Test; **Chi-Square; n (%): number (percentage); #Absolute. Bold: $P < 0.05$

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate amino-transferase; AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GGT, gamma-glutamyl transpeptidase; GUCI, Göteborg University Cirrhosis Index; INR, international normalized ratio; LDH, kPa: kilopascal; lactate dehydrogenase; PT-INR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; RBP4, Retinol-binding protein 4; WBCs, white blood cell.

Table 2 The Laboratory Investigation and Noninvasive Fibrosis Indices in CHC Patients Concerning Fibroscan Fibrosis Grades

Mean \pm SD	F0-F1 (n=8)	F2 (n=26)	F3-F4 (n=16)	P-value
Age (years)	52.25 \pm 17.24	55.77 \pm 17.24	57.19 \pm 8.12	0.874
BMI (kg/m ²)	22.80 \pm 1.72	23.53 \pm 1.05	23.07 \pm 1.45	0.635
Fibroscan (kPa)	4.68 \pm 1.20	8.66 \pm 0.21	12.19 \pm 1.12	< 0.00001
Viral load	1,511,700 \pm 1,196,509	1,400,648.34 \pm 1,154,236.60	1,400,648.31 \pm 1,154,236.60	0.951
Albumin (g/dl)	4.2 \pm 0.5	3.89 \pm 0.39	3.66 \pm 0.43	0.036
PT-INR	1.05 \pm 0.07	1.04 \pm 0.07	1.17 \pm 0.2	0.004
AST (U/L)	30.5 \pm 12.38	47.19 \pm 20.38	101.12 \pm 84.31	0.003
ALT (U/L)	42.87 \pm 27.05	47.19 \pm 30.5	87.75 \pm 66.79	0.124
ALP (U/L)	77.12 \pm 26.42	116.96 \pm 38.87	116.06 \pm 49.7	0.060
GGT (U/L)	27.62 \pm 13.04	30.27 \pm 11.40	37.87 \pm 11.70	0.096
LDH (U/L)	174 \pm 37.44	164.08 \pm 35.34	171.29 \pm 30.96	0.549
AFP (ng/mL)	7.88 \pm 1.77	7.98 \pm 2.46	9.71 \pm 3.63	0.132
RBP4 (mg/l)	41.24 \pm 3.08	39.32 \pm 1.43	35.31 \pm 0.50	< 0.00001
APRI	0.25 \pm 0.6	0.61 \pm 0.38	1.80 \pm 1.26	< 0.00001
Fib-4 score	0.79 \pm 0.34	2.01 \pm 0.65	4.60 \pm 1.92	< 0.00001
CDS	2.88 \pm 1.64	4.77 \pm 0.86	6.75 \pm 1.44	< 0.00001
Lok index	0.19 \pm 0.13	0.39 \pm 0.12	0.65 \pm 0.20	< 0.00001
GUCI	0.26 \pm 0.07	0.63 \pm 0.38	2.06 \pm 1.41	< 0.00001
Platelets (10 ⁹ /L)	297 \pm 86	205 \pm 57	136 \pm 52	< 0.00001
WBCs (10 ⁹ /L)	6240 \pm 1550	6040 \pm 1770	5440 \pm 2130	0.511
NLR	1.35 \pm 0.58	1.32 \pm 0.66	2.01 \pm 1.45	0.079
PLR	132.99 \pm 61.38	88.94 \pm 29.50	81.97 \pm 40.95	0.012

Notes: Bold: $P < 0.05$.

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate amino-transferase; AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GGT, gamma-glutamyl transpeptidase; GUCI, Göteborg University Cirrhosis Index; INR, international normalized ratio; LDH, kPa, kilopascal; lactate dehydrogenase; PT-INR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; RBP4, Retinol-binding protein 4; WBCs, white blood cells.

Table 3 Spearman Correlation Analysis Between Viral Load, AFP, RBP4, and PLR, and Noninvasive Fibrosis Indices; Fib-4, APRI, CDS, Lok Index, and GUCI

	Viral Load		AFP		RBP4		PLR	
	r	P-value	r	P-value	r	P-value	r	P-value
Age (years)	-0.041	0.6942	-0.035	0.733	-0.016	0.871	-0.1045	0.301
FibroScan (kPa)	0.312	0.0015	0.337	0.001	-0.5249	0.0001	-0.323	0.001
Viral load	-	-	0.639	0.0001	-0.781	0.0001	-0.0037	0.713
AFP (ng/mL)	0.639	0.0001	-	-	-0.632	0.0001	0.0395	0.696
RBP4 (mg/l)	-0.586	0.00001	-0.632	0.00001	-	-	0.154	0.126
APRI	0.192	0.0553	0.360	0.0002	-0.546	0.0001	-0.307	0.002
Fib-4 score	0.187	0.0630	0.277	0.005	-0.538	0.0001	-0.450	0.001
CDS	0.124	0.218	0.130	0.197	-0.405	0.0001	-0.520	0.0001
Lok index	0.133	0.1861	0.223	0.025	-0.481	0.0001	-0.518	0.0001
GUCI	0.186	0.0642	0.364	0.0002	-0.543	0.0001	-0.269	0.003

Notes: r: Spearman correlation coefficient; Bold: $P < 0.05$.

Abbreviations: AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GUCI, Göteborg University Cirrhosis Index; PLR, platelet to lymphocyte ratio; RBP4, Retinol-binding protein 4.

hepatic injuries as fibrosis, cirrhosis, and hepatocellular carcinoma. The degree of liver fibrosis is the best indicator of disease progression.¹³

All CHC patients were genotype 4; this was in line with the inconsistent finding of others,^{14,15} and all treated patients achieved (SVR12).

Table 4 The Performance of Fibrosis Serum Markers in Detecting Fibrosis Stage

No or Mild Liver Fibrosis (F0-F1) Versus Significant and Advanced Liver Fibrosis (F2-F3-F4)											
	AUC	Cutoff	Sensitivity	95% CI	Specificity	+LR	-LR	PPV	NPV	Accuracy	P-value
FibroScan (kPa)	1	> 6.4	100	0.929–1	100	–	0	100	100	100	<0.001
FIB-4 score	0.985	> 1.17	95.24	0.902–1	100		0.048	100	80	97.62	<0.001
GUCI	0.948	> 0.37	90.48	0.846–0.991	100		0.095	100	66.7	95.24	<0.001
APRI	0.946	> 0.35	90.48	0.843–0.990	100		0.095	100	66.7	95.24	<0.001
LOK INDEX	0.921	> 0.18	100	0.809–0.978	75	4	0	95.5	100	87.5	<0.001
CDS	0.893	> 3	97.62	0.773–0.963	75	3.9	0.032	95.3	85.7	86.31	<0.001
RBP4 (mg/l)	0.811	≤ 40.6	90.48	0.675–0.908	62.5	2.41	0.15	92.7	55.6	76.49	0.004
PLR	0.738	≤ 115.8	85.71	0.594–0.852	62.5	2.29	0.23	92.3	45.5	74.11	0.042
AFP (ng/mL)	0.577	> 6	83.33	0.409–0.697	37.5	1.33	0.44	87.5	30	60.415	0.604
Mild & moderate (F0-F1-F2) versus advanced liver fibrosis and cirrhosis (F3-F4)											
	AUC	Cutoff	Sensitivity	95% CI	Specificity	+LR	-LR	PPV	NPV	Accuracy	P-value
FibroScan (kPa)	1.000	> 8.9	100	0.929–1	100	–	0	100	100	100	<0.001
RBP4 (mg/l)	1.000	≤ 35.9	100	0.929–1	100	–	0	100	100	100	<0.001
FIB-4 score	0.940	> 2.31	93.75	0.835–0.988	88.24	7.97	0.071	78.9	96.8	90.995	<0.001
GUCI	0.935	> 1.27	75	0.827–0.985	97.06	25.5	0.26	92.3	89.2	86.03	<0.001
APRI	0.923	> 0.59	100	0.811–0.979	76.47	4.25	0	66.7	100	88.235	<0.001
LOK INDEX	0.893	> 0.55	81.25	0.774–0.963	85.29	5.52	0.22	72.2	90.6	83.27	<0.001
CDS	0.882	> 5	75	0.760–0.956	94.12	12.75	0.27	85.7	88.9	84.56	<0.001
PLR	0.650	≤ 77.36	62.50	0.502–0.779	70.59	2.13	0.53	50.0	80.0	66.55	<0.001
AFP (ng/mL)	0.638	> 7	100	0.490–0.769	35.29	1.55	0	42.1	100	67.645	0.067

Note: Bold: $P < 0.05$.

Abbreviations: AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; AUC, area under the curve; CDS, cirrhosis discriminant score; CI, confidence intervals; Fib-4, fibrosis index based on the four factors; GUCI, Göteborg University Cirrhosis Index; NPV, NPV, negative predictive value; LR, likelihood ratio; PLR, platelet-to-lymphocyte ratio; PPV, positive predictive value; RBP4, Retinol-binding protein 4.

CHC patients had liver dysfunction with a significant increase in hepatic enzyme levels reflecting the extent of liver cell cytolysis, and the hepatic synthetic function worsens with progression of fibrosis and hepatocyte loss as revealed by a decreased in serum albumin, RBP4, and platelet count and increase of PT-INR, this was in agreement with other reports.^{16,17} Serum ALT values were slightly greater in the marked fibrosis-cirrhosis patient group.

CHC patients showed a significant decrease in the mean platelet count related to disease severity and the hepatic fibrosis grade, which improved after therapy. This was in line with other authors.^{14,18–21} Moreover, platelet count correlated positively with RBP4 and negatively with the fibrosis grade and the five non-invasive fibrosis scores. This certified that platelets add to the inflammatory reaction after the liver injury that contributes to both liver fibrogenesis and regeneration.²²

TE fibroscan is a reliable, objective technique to measure hepatic tissue elasticity,²³ which is correlated with the stages of liver fibrosis in CHC.²⁴ Yet, several limitations

could affect its clinical usefulness. The cost can bar its widespread availability, and it has a decreased accuracy in specific patient groups (obese or patients with ascites or hepatitis flare) that may limit its use in assessing the degree of liver fibrosis in CHC patients.²⁵

Fibroscan was useful for monitoring fibrosis progression in untreated CHC cases and showed significant fibrosis regression in patients achieving SVR after therapy; this was in-line with others.^{1,26,27}

Several non-invasive blood test indices developed to predict hepatic fibrosis to overcome liver biopsy limitations. In this study, we evaluated the performance of five indices in predicting liver fibrosis grade, and we found that with the increasing hepatic fibrosis severity, as estimated by fibroscan; the values of non-invasive indices increased significantly together with the rise of AFP and the decrease of albumin, RBP4, PT-INR, platelet count, and PLR. This implies that all can objectively evaluate the various stages of liver fibrosis in CHC patients. This was in agreement with many authors.^{28–32}

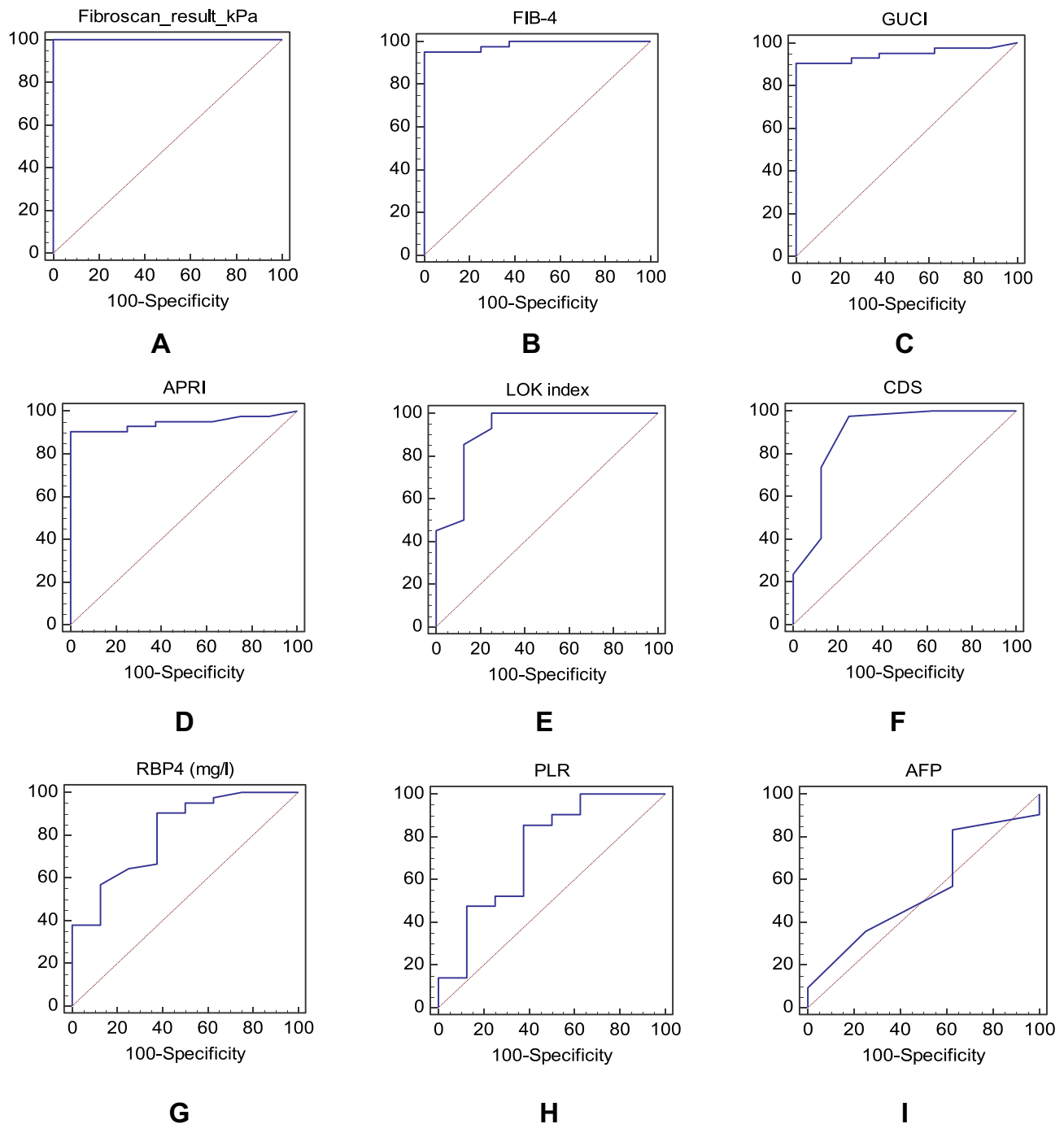


Figure 1 The area under the receiver operating characteristic (ROC) curves of RBP4, FIB-4, GUCI, APRI, LOK index, CDS, PLR, and AFP for the diagnosis of advanced liver fibrosis using Fibroscan as the reference.

The viral load correlated positively with the hepatic fibrosis severity and AFP, whereas RBP4 correlated positively with the platelet count, and negatively with fibrosis stage, viral load, AFP, and the five non-invasive fibrosis indices (APRI, CDS, Fib-4, GUCI, Lok index). PLR correlated negatively with fibrosis stage and the five non-invasive fibrosis indices. This was in line with the finding of other authors.^{29–35}

RBP4 is the most superior, likewise fibroscan, with an outstanding performance in predicting significant and advanced fibrosis, followed by Fib-4 score, GUCI, APRI, LOK index, CDC, and PLR. This is in line with other authors.^{8–12}

In this study, 68% of CHC patients had higher AFP levels, in contrast to former studies that observed higher

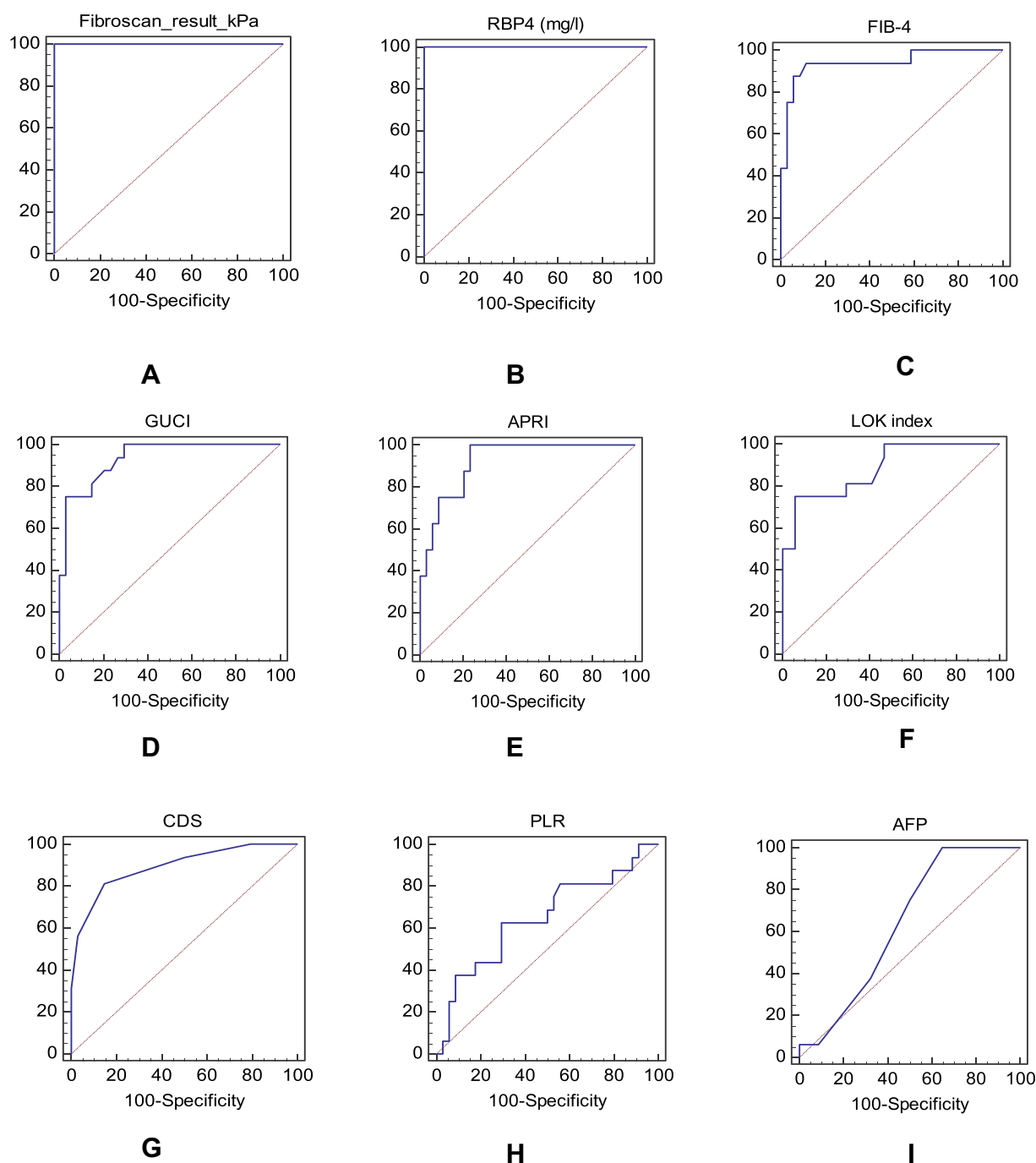


Figure 2 The area under the receiver operating characteristic (ROC) curves of RBP4, FIB-4, GUCI, APRI, LOK index, CDS, PLR, and AFP for the diagnosis of advanced liver fibrosis using Fibroscan as the reference.

Abbreviations: AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GUCI, Göteborg University Cirrhosis Index; PLR, platelet-to-lymphocyte ratio; RBP4, retinol-binding protein 4.

serum AFP levels with a prevalence ranging from 15% to 58% of patients with CHC and 11% to 47% of patients with cirrhosis, and they settled that AFP levels increase during regeneration of liver cell, especially after massive hepatic necrosis and this correlates with raised ALT.^{17,36,37}

CHC patients had a higher serum AFP, related to the loss of normal hepatic architecture arrangements and altered hepatocyte–hepatocyte interaction that correlated to fibrosis and cirrhosis extent, with a significant drop after DAAs and SVR achievement, reflecting the

improvement in disease activity and the amelioration of both inflammatory-necrosis and hepatocellular injury process. This was in agreement with other authors.^{16,38–41}

AFP showed a positive correlation with fibrosis stage, viral load, and four non-invasive fibrosis indices (APRI, Fib-4, GUCI, and Lok index) and had a negative correlation with WBC, neutrophil, lymphocyte count, and RBP4. In contrast, Tai and his coworkers found that AFP levels correlated positively with the age, ALT elevation, fibrosis grade, inflammation score, and negatively with platelet count. However, in our study, it has poor discrimination power to differentiate the stage of liver fibrosis.⁴²

In this study, the CHC patient had a significant reduction in the mean RBP4 that improved after therapy. This was in agreement with many authors.^{43–47} The inverse association between RBP4 and disease severity could be explained by the fact that retinoic acid is a suppressor of type I collagen expression by hepatic stellate cells and hence lower RBP4 levels were involved in activating stellate cells to overexpress and deposit type I collagen in the liver.⁴⁸

In CHC patients, RBP4 is a valuable serological marker to assess disease severity, inflammatory activity, and fibrosis grade evaluation, which could be valued as a sign of DAA success. For the diagnosis of significant fibrosis, the performances of fibroscan and serum biomarkers from routine blood tests were comparable. So in resource-limited countries, non-invasive tests are recommended for hepatic fibrosis assessment instead of invasive or expensive methods. To increase the diagnostic accuracy, fibroscan and the non-invasive fibrosis index combination (either stepwise or sequential) proposed through using an algorithm starting with a screening test using non-invasive fibrosis indices and restricting fibroscan or liver biopsy in patients classified as (F0–F1) by non-invasive serological tests.^{27,49–51}

This study had some limitations: 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

Serum RBP4 rises after successful HCV eradication considered a marker of inflammation regression and DAA treatment success. RBP4 showed excellent accuracy, sensitivity, specificity, PPV, and NPV. Likewise, fibroscan has a superior diagnostic performance in predicting advanced fibrosis grads in CHC patients and hence can replace expensive invasive procedures.

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Disclosure

The authors have declared no potential conflicts of interest in this work.

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