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Serum retinol-binding protein 4 as a predictor of fibrosis regression and response to direct-acting antiviral drugs in chronic hepatitis C virus patients

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Abstract

Background Hepatitis C virus is the underlying cause of chronic hepatitis which frequently progresses to cirrhosis and hepatocellular carcinoma. In addition, HCV is thought to cause steatosis, dyslipidemia, insulin resistance, diabetes, obesity, and cardiovascular events. The aim of this study is to evaluate the role of serum RBP-4 in the prediction of fibrosis regression and the response of treatment among chronic HCV patients receiving direct-acting antiviral agents.

Methods This study included 40 chronic HCV Egyptian patients, divided into two groups: Naive cases, 20 chronic HCV patients before starting first line of treatment; Relapser cases, 20 chronic HCV patients who were non-responders before starting second line treatment; and 10 healthy subjects as control. Laboratory investigations including complete blood count, full hepatic profile, fibroscan assessment, and retinol-binding protein-4 level at baseline and re-assessed 12 weeks after the end of treatment [sustained virological response SVR12]. Student *T* test, analysis of variance, chi-square, Tukey's test, and Pearson correlation coefficient tests were used for statistical analysis.

Results Baseline retinol-binding protein-4 level was significantly higher in the naïve case group than in the relapser and control groups with a *P* value of *P* value of < 0.001. All the naïve patients had 100% SVR12, only 90% of the relapser group achieved SVR12. A significant reduction in retinol-binding protein-4 and fibrosis staging and measurements by fibroscan among all studied patients were noted after receiving direct acting antivirals (*P* value < 0.001). Retinol-binding protein-4 levels before and after treatment were significantly lower among F4 patients in comparison to those of F1–F3 patients (*P* value 0.002, 0.009, respectively). The best cutoff value of retinol-binding protein-4 in the prediction of liver cirrhosis (F4) was ≤ 46 pg/ml with sensitivity of 100% and specificity of 66.67%.

Conclusion Serum retinol-binding protein-4 was found to be higher in chronic HCV infection with a significant reduction after successful eradication. Its level is much lower in cirrhotic patients [F4]. As a result, retinol-binding protein-4 may have a promising role in assessing direct acting antivirals response, as well as a prognostic value in predicting liver cirrhosis.

Keywords Retinol-binding protein-4, Chronic HCV, Direct acting antivirals

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Introduction

Hepatitis C virus (HCV) infection is a serious global health concern; it causes chronic infection in over 80 million people worldwide, with 3–4 million new infections and 350,000 deaths annually [1]. The recent emergence of potent direct acting antivirals (DAAs) has revolutionized HCV treatment, providing a high potential for achieving HCV eradication and consequently preventing disease progression [2].

Retinol-binding protein 4 (RBP4) is a lipocalin protein, [3] that is secreted primarily by hepatocytes (80%) and adipose tissue (20%) [4]. RBP4 was discovered to induce hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and to impair insulin signaling in mouse muscle [5]. It was therefore suggested to connect obesity-associated comorbidities, especially insulin resistance (IR), and certain components of the metabolic syndrome, such as nonalcoholic fatty liver disease (NAFLD), in either a retinol-dependent or retinol-independent way with RBP4 [5]; plasma RBP4 levels tended to decrease concomitantly with increased necro-inflammatory activity, NAFLD activity score, and fibrosis score in NAFLD patients [6, 7]. However, clinical data regarding the links among RBP4, IR, and NAFLD are conflicting.

Although the exact mechanisms that allow HCV infection to cause IR are not fully understood, HCV infection may provide a clearer perspective on RBP4's hidden role in the development of IR. Although, the relationship between HCV infection and RBP4 has been studied. Meanwhile, the role of disease progression and severity on RBP4 expression in HCV infection remains unknown [8].

The aim of this study is to evaluate the role of serum RBP-4 in the prediction of fibrosis regression and the response to treatment among chronic HCV patients receiving direct-acting antiviral agents.

Methods

During the period of January to October 2019, 40 Egyptian chronic HCV-infected patients (22 males and 18 females) > 18 years old eligible for antiviral treatment with DAAs and agreeing to regular follow up were recruited from the National Committee for Control of Viral Hepatitis (NCCVH) Regional Center in Ain Shams University Hospital after approval by the Ain Shams University ethics committee (FWA 000,017,585) and obtaining informed consent from all patients.

They were divided into two groups: Naïve cases included 20 patients: 13 males and 7 females with a mean age of 43.55 ± 11.03 treated with Sofosbuvir 400 mg + Daclatasvir 60 mg ± Ribavirin1000–1200 mg; the relapse case group included 20 patients: 9 males and 11 females with a mean age of 40.2 ± 4.23 treated with Sofosbuvir 400 mg + Qurevo + Ribavirin 1000–1200 mg as second regimen according to the Egyptian NCCVH protocol [9]; and 10 healthy, age and sex-matched volunteers served as a control group (7 males and 3 females).

Exclusion criteria were co-infection with HBV or HIV, Child C, thrombocytopenia below 50,000/mm^3, hepatocellular carcinoma, or other malignancies with exception of 2 years illness free, obesity (BMI > 30), pregnancy, uncontrolled diabetes (HbA1c > 9%), previous or current heavy alcohol intake, patients on statins or fibrates, organ transplant recipients, previous bariatric surgeries, drugs causing steatosis like amiodarone, corticosteroids, tamoxifen, and valproic acid.

All participants underwent a relevant history and clinical examination, as well as biochemical investigations, including complete blood count, liver function tests, kidney function tests, fasting, and 2 h postprandial blood glucose level, HBA1c, lipid profile, HBsAg, HIV Ab using ELISA technique, and α-fetoprotein. HCV quantitative RNA was measured using real-time polymerase chain reaction (PCR) at baseline and 12 weeks after the end of treatment to document SVR (SVR 12).

Serum RBP4 was measured by quantitative sandwich ELISA kits (Quantikine, R&D Systems, Minneapolis) according to the manufacturer's instructions. RBP4 was assessed for patient groups at baseline and at SVR 12.

Abdominal ultrasonography was performed after overnight fasting (8 h) with emphasis on liver size, splenic bipolar diameter, and portal vein diameter.

Transient elastography was performed using the FibroScan device (FibroScan; Echosens, Paris, France) at baseline and at SVR12, and it was expressed in kPa.

Liver stiffness measurement (LSM) was used to estimate the METAVIR fibrosis stage as follows: F0-F1: 2.5–6.9 kPa; F2: 7.0–9.4 kPa; F3: 9.5–12.4 kPa; and F4: greater than or equal to 12.5 kPa [10].

Statistical analysis

The collected data were analyzed using IBM SPSS statistics software version 20. Quantitative data were expressed as mean ± SD.

In quantitative data, independent *t* test, paired *t* test, ANOVA, and post hoc test and Tukey's test were used. While in qualitative data, chi-square test was used. Correlations were done using Pearson correlation coefficient test. A *P* value less than 0.05 was sufficient to show statistical significance.

Results

Regarding biochemical parameters, the mean values of PCR, platelet count, and prothrombin time before treatment were statistically significantly lower in the relapser group compared to naïve group (Table 1).

Sonographic parameters and fibrosis degree measurements by fibroscan before treatment showed no statistically significant difference between naïve and relapser patient groups (Table 2).

The mean values of baseline RBP4 were significantly higher in naïve case group in comparison to relapse and control groups (Table 3).

All naïve patients had cure (SVR12 was 100%). While in the relapser group, SVR12 was 90%, as two patients failed to achieve SVR12.

For the naïve group, prothrombin time, AST, and ALT showed a significant reduction, while platelet

Table 1 Comparison between naïve and relapse groups regarding socio-biochemical parameters before treatment

	Groups				Chi-square or *T* test	
	Naïve		Relapser			
	N	%	*N*	%	X^2 or *T*	*P* value
Gender						
Male	13	65.00	9	45.00	1.616	0.204
Female	7	35.00	11	55.00		
Age						
Range	21–63		34–48		1.267	0.213
Mean ± SD	43.550 ± 11.038		40.200 ± 4.238			
PCR (IU/ml)						
Range	335,040–3,132,444		10,100–1,003,700		5.425	< 0.001*
Mean ± SD	1,532,926.05 ± 963,954.961		310,582.200 ± 293,212.793			
Total leucocytic count (X 10^9 cells/L)						
Range	4.3–10		4.5–10		0.069	0.945
Mean ± SD	7.050 ± 1.558		7.015 ± 1.629			
Hemoglobin (g/dl)						
Range	11–17.9		12.1–16		1.131	0.265
Mean ± SD	14.500 ± 1.540		14.020 ± 1.108			
Platelet (X 10^9cells/L)						
Range	109–321		87–230		2.137	0.039*
Mean ± SD	166.350 ± 53.524		135.250 ± 37.022			
Prothrombin Time						
Range	11.5–14		11–13		3.116	0.003*
Mean ± SD	12.590 ± 0.713		11.943 ± 0.595			
AST (U/L)						
Range	16–89		23–88		− 0.117	0.907
Mean ± SD	46.550 ± 17.707		47.200 ± 17.374			
ALT (U/L)						
Range	22–62		25–77		− 1.655	0.106
Mean ± SD	38.300 ± 12.520		45.600 ± 15.250			
Total bilirubin (mg/dl)						
Range	0.5–1.4		0.4–1.7		− 0.287	0.776
Mean ± SD	0.930 ± 0.236		0.956 ± 0.329			
Albumin (g/dl)						
Range	3.7–4.5		3.6–4.4		0.286	0.777
Mean ± SD	4.030 ± 0.232		4.010 ± 0.210			
Creatinine mg/dl						
Range	0.34–1.36		0.57–1.2		1.07	0.124
Mean ± SD	0.98 ± 0.25		0.92 ± 0.19			

*significant

Table 2 Comparison between naïve and relapse groups regarding ultrasound parameters and fibroscan before treatment

	Groups				Chi-square or *T* test	
	Naïve		Relapser			
	N	%	*N*	%	X^2 or *T*	*P* value
Echogenicity						
Bright	17	85	13	65	2.133	0.144
Coarse	3	15	7	35		
Spleen size (cm)						
Range	12–18		12–18		−0.158	0.875
Mean ± SD	14.55 ± 2.038		14.65 ± 1.954			
Portal vein diameter (mm)						
Range	10–16		13–18		−1.423	0.163
Mean ± SD	13.8 ± 3.238		14.95 ± 1.605			
Liver size (cm)						
Range	12–16		13–31		−0.889	0.380
Mean ± SD	14.05 ± 1.063		14.85 ± 3.884			
Fibroscan						
F1	8	40	4	20	3.210	0.360
F2	6	30	7	35		
F3	3	15	2	10		
F4	3	15	7	35		
Fibroscan kPa (kilopascal)						
Range	4.8–20.6		4–20.6		−1.412	0.166
Mean ± SD	8.93 ± 3.809		10.755 ± 4.349			

Table 3 Comparison between naïve and relapse groups with controls as regards the RBP4 level before treatment

	Groups			ANOVA		Tukey's test		
	Naïve (*N*)	Relapser (*R*)	Control (*C*)	*F*	*P* value	N&R	N&C	R&C
Range	45–73	29–49	25–39	66.115	< 0.001*	< 0.001*	< 0.001*	0.037*
Mean ± SD	59.025 ± 8.012	39.45 ± 6.134	32.8 ± 4.662					

*significant

count showed a significant elevation at SVR12 in comparison to their baseline levels (Table 4).

However, in the relapser group, a significant reduction in ALT and AST levels was noted together with a significant elevation in platelet count and serum albumin on achieving SVR12 (Table 4).

Moreover, in comparison to their baseline level, naïve and relapser patients experienced a significant reduction in RBP4 levels at SVR12 (Table 4).

An overall reduction in the fibroscan measurements was noted among the naïve and relapser groups after receiving DAAs in comparison to their baseline levels. Yet, this reduction was insignificant in the relapser group (Table 5). Also, a significant reduction in fibrosis measurements as well as staging among all patients collectively was noted after receiving DAAs (Table 6).

RBP4 level before and after treatment was significantly lower among F4 patients in comparison to its level in F1–F3 patients (Table 7).

In the Naïve group, a significant negative correlation was found between baseline RBP4 and the fibrosis degree measurements by fibroscan, with an insignificant correlation with all other parameters (Table 8).

In the relapser group, a significant positive correlation was found between baseline RBP4 and age, total cholesterol, triglyceride, LDL, and ALT, with an insignificant correlation with all other parameters (Table 9).

Finally, our study demonstrated the best cutoff value of baseline RBP4 in the detection of liver cirrhosis (F4) among all studied patients as ≤ 46 pg/ml with 100% sensitivity, 66.67% specificity, 50% PPV, 100% NPV, and an overall accuracy of 80.5% (Fig. 1).

Table 4 Comparison between biochemical profile of naïve and relapse groups before and after treatment

	Naïve group		Paired test		Relapser group		Paired test	
	Before treatment	After treatment 12 Weeks	*T*	*P* value	Before treatment	After treatment 12 weeks	*T*	*P* value
Total leucocytic count (X 10^9c /L)								
Range	4.3–10	5–8.9	0.867	0.397	4.5–10	4.9–8.9	1.000	0.330
Mean ± SD	7.050 ± 1.558	6.755 ± 0.966			7.015 ± 1.629	6.600 ± 0.956		
Hemoglobin (g/dl)								
Range	11–17.9	13–16.5	-0.441	0.664	12.1–16	12.7–16	−2.039	0.056
Mean ± SD	14.500 ± 1.540	14.600 ± 0.901			14.02 ± 1.108	14.675 ± 0.846		
Platelet (X 10^9c /L)								
Range	109–321	100–317	-5.266	< 0.001*	87–230	95–287	−6.654	<0.001*
Mean ± SD	166.350 ± 53.524	216.050 ± 53.331			135.25 ± 37.022	206.65 ± 49.290		
Prothrombin time								
Range	11.5–14	11–12.9	3.575	0.002*	11–13	10–13.6	−1.491	0.152
Mean ± SD	12.590 ± 0.713	11.925 ± 0.495			11.943 ± 0.595	12.28 ± 0.861		
AST (U/L)								
Range	16–89	11–24	6.725	< 0.001*	23–88	11–29	7.185	<0.001*
Mean ± SD	46.550 ± 17.707	19.550 ± 3.692			47.2 ± 17.374	19.5 ± 3.791		
ALT (U/L)								
Range	22–62	17–33	5.506	< 0.001*	25–77	17–42	6.462	<0.001*
Mean ± SD	38.300 ± 12.520	24.400 ± 4.593			45.600 ± 15.250	25.85 ± 5.976		
Total bilirubin (mg/dl)								
Range	0.5–1.4	0.5–1.1	0.914	0.372	0.4–1.7	0.5–1.9	−1.287	0.214
Mean ± SD	0.930 ± 0.236	0.874 ± 0.172			0.956 ± 0.329	1.035 ± 0.283		
Albumin (g/dl)								
Range	3.7–4.5	4–4.7	-2.008	0.059	3.6–4.4	3.7–4.7	−2.101	0.049*
Mean ± SD	4.030 ± 0.232	4.170 ± 0.200			4.010 ± 0.210	4.150 ± 0.240		
RBP4 (ng/ml)								
Range	45 –73	37–65	17.528	< 0.001*	29–49	21–47	10.957	<0.001*
Mean ± SD	59.025 ± 8.012	49.875 ± 7.323			39.450 ± 6.134	34.075± 6.697		
Creatinine mg/dl								
Range	0.34–1.36	0.6–1.1	0.370	0.713	0.57–1.2	0.7–1.1	0.542	0.590
Mean ± SD	0.98 ± 0.25	0.853 ± 0.130			0.92 ± 0.19	0.891 ± 0.100		

*significant

Table 5 Comparison between fibroscan measurements of naïve and relapse groups before and after treatment

	Naïve group		Paired test		Relapser group		Paired test	
	Before treatment	After treatment 12 Weeks	*T*	*P* value	Before treatment	After treatment 12 Weeks	*T*	*P* value
Fibroscan kPa								
Range	4.8–20.6	4–14.1	5.850	< 0.001*	4–20.6	3–20.3	2.074	0.052
Mean ± SD	8.930 ± 3.809	7.095 ± 2.983			10.755 ± 4.349	9.655 ± 5.149		

*significant

Discussion

Chronic HCV is an important health concern globally. It accounts for about 400,000 annual fatalities worldwide, primarily due to end-stage complications and HCC [11].

Timely introduction of DAAs targeted at eliminating HCV by 2030. DAAs have demonstrated safety and efficiency. Consequently, initial treatment expansion initiatives have been adopted by various countries, including Egypt [12].

Table 6 Comparison between fibroscan measurements of all 40 studied patients before and after treatment

Fibro scan	Before treatment		12 weeks after treatment		Chi-square	
	N	%	*N*	%	X^2	*P* value
F1	12	30.00	24	60.00	13.759	0.003*
F2	13	32.50	2	5.00		
F3	5	12.50	8	20.00		
F4	10	25.00	6	15.00		
Total	40	100.00	40	100.00		

		Total						Paired test	
		Before treatment			12 weeks after treatment			*t*	*P* value
Fibroscan (kPa)	**Range**	4	-	20.6	3	-	20.3	4.738	< 0.001*
	Mean ± SD	9.843	±	4.14	8.375	±	4.351		

*significant

Table 7 Relation between RBP4 levels before and after treatment and fibroscan stages

	Fibro scan stage all patients						*T* test	
	F1–F3 (*n* = 30)			F4 (*n* = 10)			*T*	*P* value
RBP4 before treatment								
Range	29	-	73	31	-	46	3.267	0.002*
Mean ± SD	52.483	±	12.043	39.5	±	5.74		
RBP4 after treatment 12 weeks								
Range	21	-	65	27	-	41	2.773	0.009*
Mean ± SD	44.45	±	10.89	34.55	±	4.634		

*significant

Glucose intolerance is more common among chronic liver disease patients, especially those with HCV, than in the general population. IR seems to be a key characteristic of HCV-induced glucose intolerance. HCV eradication with DAAs has been found to reduce insulin resistance [13].

Gouthamchandra et al. [14] studied whether RBP4 is involved in HCV replication and stated that RBP4 may have a suppressive effect. RBP4 is known to modulate the insulin receptor signaling pathway and hence gluconeogenesis and lipogenesis. RBP4 reduction promotes the mTORC1 (mammalian target of rapamycin complex 1) pathway, leading to elevated SREBP-1 (sterol regulatory element-binding protein 1) levels and thus lipogenesis. Because lipid raft formation is essential for HCV replication and assembly, RBP4 suppression could be beneficial to the virus.

Because the liver is still the main source of RBP4 synthesis, any changes in liver function have a significant impact on RBP4 levels [15].

The aim of this study was to evaluate the role of serum RBP-4 in the prediction of fibrosis regression and the response to treatment among chronic HCV patients receiving direct-acting antiviral agents.

The current study revealed that all the naïve patients had been cured (SVR12 was 100%). While in the relapser group, SVR12 was 90%, as two patients failed to achieve SVR12.

The present study showed that platelet count was significantly lower among the relapser group than naïve group; this was in keeping with results published by Osada et al. [16], who showed that platelets are significantly decreased in chronic hepatitis due to reduced production, endothelial dysfunction, splenic sequestration, and autoimmune destruction.

Subsequently, there was a significant improvement in platelet count at SVR12 in the naïve and relapser groups ($P < 0.001$) which was similar to Dahal et al. [17], who studied the effect of DAAs on platelets in patients with pretreatment thrombocytopenia. as well as van der Meer et al. [18], who discovered an improvement in platelet count in patients achieving SVR.

The current study showed significant improvement of ALT and AST in all patients at SVR12 with a *P* value of 0.001. This was in agreement with van der Meer et al. [18], who found that ALT, AST, and alkaline phosphatase were significantly reduced 12 weeks after successful DAA treatment.

Table 8 Correlation between baseline RBP4 level with different data among naive groups

Correlations		
Naïve patients	**RBP4 before treatment**	
	R	***P* value**
Age	0.108	0.651
Spleen size (cm)	−0.246	0.296
PV diameter (mm)	−0.038	0.872
Liver size (cm)	0.272	0.246
Total cholesterol	0.034	0.885
Triglycerides	−0.292	0.211
HBA1C	0.220	0.352
HDL	0.377	0.101
LDL	−0.240	0.309
PCR (IU/ml)	0.266	0.257
Total leucocytic count (X 10^9 cells/L)	0.049	0.839
Hemoglobin (g/dl)	−0.239	0.310
Platelet (X 10^9 cells/L)	0.025	0.915
Prothrombin time	−0.140	0.557
AST (U/L)	−0.197	0.404
ALT (U/L)	0.164	0.491
Total bilirubin (mg/dl)	−0.121	0.611
Albumin (g/dl)	0.026	0.912
Serum creatinine (mg/dl)	0.126	0.236
Fibroscan (kPa)	−0.562	0.010*

*significant

Table 9 Correlation between baseline RBP4 level with different data among relapse group

Correlations		
Relapse patients	**RBP4 before treatment**	
	R	***P* value**
Age	−0.536	0.015*
Spleen size (cm)	−0.149	0.532
PV diameter (mm)	−0.110	0.645
Liver size (cm)	0.091	0.702
Total cholesterol	0.663	0.001*
Triglycerides	0.596	0.006*
HBA1C	−0.023	0.923
HDL	−0.178	0.454
LDL	0.628	0.003*
PCR (IU/ml) before treatment	0.082	0.732
Total leucocytic count (X 10^9 cells/L)	0.386	0.092
Hemoglobin (g/dl)	0.284	0.224
Platelet (X 10^9 cells/L)	0.257	0.274
Prothrombin time	0.320	0.170
AST (U/L)	0.315	0.176
ALT (U/L)	0.592	0.006*
Total bilirubin (mg/dl)	−0.235	0.319
Albumin(g/dl)	−0.188	0.429
Serum creatinine (mg/dl)	0.322	0.678
Fibroscan (kPa)	−0.120	0.613

*significant

Using transient elastography (fibroscan) to assess fibrosis revealed a significant regression of fibrosis degree after treatment in the naïve group (from 8.930 to 7.09 kPa) with a *P* value of < 0.001, yet an insignificant reduction in the relapser group (from 10.75 to 9.75 kPa) with a *P* value of 0.052.

This was consistent with the findings of a study conducted on 549 HCV patients by Bachofner et al. [19], who found a rapid decline in fibrosis degree by fibroscan during and shortly after DAAs completion; their median measurement prior to DAA was 12.65 kPa and decreased to 8.55 kPa post-treatment.

Elsharkawy et al. [20] agreed stating that treatment of chronic HCV patients improved inflammation and fibrogenesis leading to improvements in liver stiffness and laboratory parameters [20].

With a *P* value of < 0. 001, the baseline RBP4 level was statistically significantly higher in naïve group (59.025 ± 8.012 ng/ml) than in the relapser group (39.45 ± 6.134 ng/ml) than in the healthy control group (32.8 ± 4.66 ng/ml). This was in conformity with ElRazik et al. [21], who studied RBP4 in 30 HCV patients, including 10 responders, 10 relapsers, and 10 breakthrough patients and found that serum RBP4 levels were 64.9 ± 13 ng/ml for the responder group versus 38 ± 9.8 ng/ml in the relapser group.

This was supported by a study by Seo et al. [22], who hypothesized that RBP4 levels are elevated in liver diseases other than cirrhosis, such as non-alcoholic steatohepatitis (NASH) and chronic HCV.

Although one study of nondiabetic, nonobese patients with genotype 1 chronic HCV showed that serum RBP4 level was positively linked to viral steatosis and chronic HCV patients had higher RBP4 levels than the controls, other chronic HCV studies either failed to link RBP4 positively with steatosis or demonstrated a lower RBP4 level than the controls. Given the inconclusive relationship between HCV infection and RBP4 levels, how RBP4 affects metabolism, including IR, in chronic HCV patients remains even more unclear [5].

These results could be explained by several lines of evidence that demonstrate the involvement of HCV in the retinoid pathway: HCV core protein stimulates cell growth by antagonizing all-trans retinoic acid and enhancing retinoid X receptor-α-dependent transcriptional activity via epigenetic downregulation of retinoid

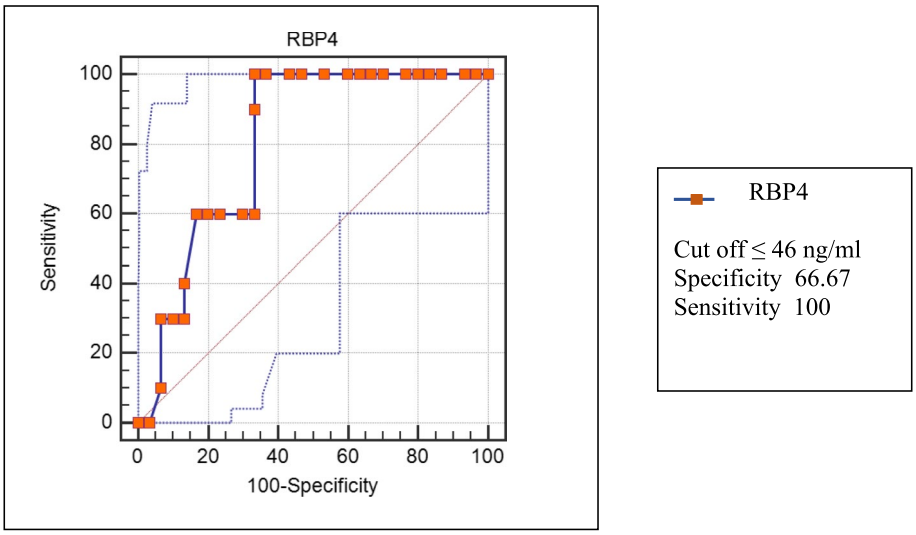

Fig. 1 The ROC curve of RBP4 in detecting F4 cirrhosis

acid receptor-β2; HCV NS3/4A protein disrupts retinoic acid-inducible gene I signaling pathways to halt HCV defense. However, the relationship between HCV infection and RBP4 remained elusive [5].

RBP4 levels were even higher among healthy subjects with a strong family history of diabetes. All of these findings imply that RBP4 could be used as an IR marker. HCV infection itself may provide a better perspective on the involvement of RBP4 in the development of IR [23].

The current study showed a significant reduction in serum RBP4 level 12 weeks after the end of treatment with DAAs (P value < 0.001) in both naïve (from 59.025 to 49.875 ng/ml) and relapsers (from 39.45 to 34.07 ng/ml), and this reduction may be attributed to successful eradication of HCV in both naïve and relapser groups.

This was in partial agreement with the study conducted by Emam et al. [8], where RBP4 declined in the responder group from 64.9 ± 13 to 45.5 ± 14.7 ng/ml, while it increased in the relapser group from 38 ± 9.8 to 50.5 ± 28 ng/ml, and this may possibly be due to failure to achieve SVR.

Also, this was in accordance with El Razik et al. [21], who displayed that RBP4 at the 72nd week was significantly lower in responders than relapsers and described this normalization in the responders as reflecting the recovered metabolic abnormalities; however, the increased level of RBP4 in relapsers reflects again the disturbed metabolic background of this group.

Gastaldi et al. [11] explained these results by stating that complete suppression of HCV replication in patients without significant fibrosis improves insulin sensitivity and that HCV alters the circulating levels of factors involved in the development of reduced insulin sensitivity.

A significant reduction in RBP4 at the end of treatment may predict SVR; however, failure to achieve this reduction and, more importantly, persistent elevation in RBP4 at the end of treatment were linked to breakthrough and relapse [21].

On the contrary, Yagmur et al. [23] and Iwasa et al. [24] showed that RBP4 levels were lower in chronic hepatitis C (CHC) patients than in controls (34.6 ± 12.3 μg/mL vs. 46.2 ± 10.5 μg/mL; $P \leq 0.001$). They also declared that only patients with SVR had significantly elevated RBP4 levels after treatment, reaching levels seen in healthy subjects, and that these levels continued to rise after 6 months to become significantly higher than the baseline, whereas RBP4 levels in relapsers and non-responders remained unchanged during the follow-up period. In addition, they discovered no link between plasma RBP4 levels and hepatic steatosis in CHC patients.

Iwasa et al. [24] explained their results by stating that there is a discrepancy between the studies as regard the degree of steatosis that was measured in only 5 (12%) out of 41 HCV patients in their study, and in most of their cases, steatosis was associated with more severe fibrosis.

This controversy could be attributed to the differences in patient characterization, especially the histopathologic features of the liver and the viral genotype [21].

On applying fibrosis stage assessment by fibroscan among the studied patients, the baseline RBP4 level was significantly lower among F4 (39.5 ± 5.74 ng/ml) than in those with F1–F3 (52.48 ± 12.04 ng/ml) with a P value of 0.002. Also, there was a significant negative correlation

between RBP4 level and fibroscan stage and measurement (*P* value 0.010) among naive patients. This was consistent with Tacke et al. [25], who highlighted that the degree of liver fibrosis and cirrhosis was the main histological factor linked to lower RBP4 levels. Because the liver is the primary source of circulating RBP4, hepatic function must be considered when assessing RBP4 levels [8].

These results were supported by Alkhouri et al. [15], who studied RBP4 in patients with NAFLD and found a stepwise decline in RBP4 levels from patients without fibrosis to those with cirrhosis and a significant negative correlation between fibrosis stage and RBP4 levels. They also found that for every stage increase in fibrosis, mean RBP4 levels decreased by 3.06 mg/L, with the lowest RBP4 levels reported in patients with advanced fibrosis and cirrhosis, implying that serum RBP4 is directly linked to liver function.

The current study showed a significant positive correlation between baseline RBP4 and total cholesterol and triglyceride (TG) levels among relapser group of patients, this goes along with Graham et al. [26], who conducted a study on 311 chronic liver disease patients and discovered that RBP4 was significantly correlated with total cholesterol in cirrhotic patients and was linked to features of the metabolic syndrome as increased BMI, waist to hip ratio, serum triglyceride levels, systolic blood pressure, and low HDL levels.

RBP4 may have a role in hepatic TG production and the development of hepatic insulin resistance, as well as the metabolic implications of obesity. However, a recent study found that RBP4 has a causal role in hepatic lipogenesis: RBP4 enhanced intracellular triglyceride synthesis in cultured human HepG2 cells in a dose-dependent manner, and the effect was verified in vivo using animal models [27].

The present study showed that the best cutoff level of RBP4 in the prediction of hepatic fibrosis stage F4 was ≤ 46 ng/ml with sensitivity of 100%, specificity of 66.67%, PPV of 50%, NPV of 100%, and test accuracy of 80.5%.

This agreed with ElRazik et al. [21] who found that patients with advanced necro-inflammation and fibrosis stage (A3F3) exhibited lower RBP4 levels than those who had (A1F1) and (A2F2) in liver biopsy (38.8 ± 12.7, 57 ± 18, and 48 ± 17 ng/ml, respectively).

Conclusion

Serum RBP4 levels were found to be higher in chronic HCV infection, with a significant reduction after successful eradication. Its level is much lower in cirrhotic patients [F4]. As a result, RBP4 may have a promising role in assessing and predicting DAAs response, as well as prognostic value in liver cirrhosis prediction.

Abbreviations

ANOVA	Analysis of variance
BMI	Body mass indexs
CHC	Chronic hepatitis C
DAAs	Direct acting antivirals
GLUT4	Glucose transporter 4
HCV	Hepatitis c virus
HCC	Hepatocellular carcinoma
IR	Insulin resistance
LSM	Liver stiffness measurement
NCCVH	National Committee for Control of Viral Hepatitis
NPV	Negative predictive value
PPV	Positive predictive value
RBP4	Retinol-binding protein 4
SD	Standard deviation
SVR12	Sustained virologic response after 12 weeks
TG	Triglyceride

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Authors' contributions

Rasmy HS collaborated on the manuscript and methodology's conceptualization and editing. ElMalatawy MA contributed to the study by revising and drafting the work and participated in writing the manuscript. Abdelwareth EY assisted in the collection of data. Elkarmoty KZ made a significant contribution to the manuscript's writing and critical revision.. Isaac A made a substantial contribution to writing the manuscript and revising it critically for important intellectual content All authors have read and approved the submitted version of the manuscript. Each author agreed to be personally accountable for their own contributions and ensured that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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Availability of data and materials

The datasets that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Ethics approval and consent to participate

This study was performed according to the ethical standards for human experimentation and in accordance with the ethical principles of the 1975 Declaration of Helsinki. Patients included in this study signed an informed written consent to participate and all the procedures were in accordance with the standards of the Research Ethics Committee (REC) of the Faculty of Medicine, Ain Shams University (FWA 000017585).

Consent for publication

Consent was taken from each author for publication.

Competing interests

The authors declare that they have no competing interests.

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