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Study of Retinol Binding Protein 4 Level in Chronic Hepatitis C Patients and Correlate its Level with the Antiviral Therapy

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ABSTRACT

An association between hepatitis C virus (HCV) infection and lipid metabolism demonstrated that LDL-receptor is one of the HCV receptors and experiments in vitro showed competitive inhibition of binding between HCV and LDL-receptor by LDL-cholesterol. Lipoproteins play an important role in the process of complexing of the virus to VLDL-C or LDL-C could promote endocytosis of HCV via the LDL receptor.

Retinol-binding protein 4 (RBP4) is a protein serving as a carrier of retinol in the blood. Liver is the primary source of RBP4 synthesis and it has been reported as an adipocytkine contributing to insulin resistance.

Patients with chronic liver disease showed a significant different (RBP4) level. The relation between (RBP4) and HCV has been needed to be investigated and the impact of disease progression and severity on (RBP4) in HCV deserves to be clarified.

Results revealed that HCV RNA differences among the three groups showed that it is higher in responders and breakthrough than relapsers (574±351, 1542.9±651, 287.1±194.6 KIU respectively (P=0.001)) RBP4 before treatment was lower in the high viral load group vs the low viral load group (48.5±17.5 ng/ml vs 46.5±19 ng/ml respectively) and after treatment it was lower in high viral load vs the low viral load and that was statistically significant (t=-8.88,P<0.01). Variation of RBP4 level within each group showed that, in the responders group there is a progressive and significant decrease (62.7±7, 53±18, 41.5±11.7, 39.5±7.8 ng/ml respectively & F=5.9, P=0.002) before treatment, at 24th w, at 48th w, 72th w. This decrease may be due to recovery of the metabolic disturbance caused by the virus and that was coincident with viral clearance denated by PCR at 24, 48, 72 weeks. In the relapsers group, there was a significant reduction of the RBP4

level at 24th w but it peak at 48th w and that was statistically significant 32.9±6.8 vs 52.3±29 ng/ml respectively. In the breakthrough group, there is high level of RBP4 level at 24th w 39±12 ng/ml and more increase at 48th w and was statistically significant 55.6±18.9 ng/ml. Age, ALT, AST, PT, PLT, FBS, TGs, FRT, UA, RNA and albumin were considered as variables closely related to RBP4 level, from which we can predict its value as shown in table 6.

It can be concluded that, the metabolic state due to HCV can influence the level of RBP4 and indirectly affect and correlate with the response to therapy.

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of end-stage liver disease and hepatocellular carcinoma and one of the most common indications for liver transplantation[(1)]. HCV prevalence varies widely with geographical location and within population, therefore HCV is a major burden on healthcare resources[(2)].

An important aspect of HCV infection is its relation with the metabolism of glucose and lipids which negatively affects liver diseases progression and the response to treatment. The high prevalence of steatosis in HCV-infected patients is important issue as it enhances the progression of HCV infection to liver fibrosis and cirrhosis and decreases the response to antiviral therapy and also raising the possibility of multifaceted and clinically relevant interactions with the metabolic syndrome[(3)].

An association between HCV infection and lipid metabolism demonstrated that LDL-receptor is one of the HCV receptors and experiments in vitro showed competitive inhibition of binding between HCV and LDL-receptor by LDL-cholesterol[(4)]. Lipoproteins play an important role in the process of complexing of the virus to VLDL-C or LDL-C could promote endocytosis of HCV via the LDL receptor[(5)].

Retinol-binding protein 4 (RBP4) is a protein serving as a carrier of retinol in the blood, and belongs to the lipocalin family which are proteins that transport small hydrophobic molecules such as steroids, retinoids and lipids[(6)].

Liver is the primary source of (RBP4) synthesis and has been reported as an adipocytokine contributing to insulin resistance[(7)].

Patients with chronic liver disease showed a significant different (RBP4) levels. These findings may reflect the impact necro-inflammatory activity on (RBP4)[(8)]. The relation between (RBP4) and HCV has been needed to be investigated and the impact of disease progression and severity on (RBP4) in HCV deserves to be clarified.

PATIENTS AND METHODS

This study was carried out in Tropical Medicine, Biochemistry Departments, Faculty of Medicine, Zagazig University.

From 600 patients who where candidate for antiHCV combined therapy and who had been followed up of one year during their course of treatment and for 6 months post treatment, use selected 300 patients after exclusion of diabetes,

hypertension, and obesity. They were enrolled in our study after approval of the ethical committee of Zagazig University hospital. Written consent was obtained from patients for interview, anthropometric measurements and blood sampling.

The patients were classified into three groups according to their virological response:

I: Group 1 (responders):

It included 100 patients who achieved sustained virological response (SVR) which is defined as serum HCV-RNA negatively 6 months after the cessation of treatment. They were 80 males and 20 females, their mean age 35.3±12 years.

II. Group 2 (breakthrough):

It included 100 patients who attained HCV RNA negativity during treatment with recurrence of viremia while treatment is ongoing denoted by HCV RNA positivity at 24/or 48 weeks of treatments. They were 100 males and their mean age 36±10 years.

III. Group 3 (Relapses):

It included 100 patients who attained HCV RNA negativity at the end of therapy end of treatment response (ETR) with recurrence of viremia 6 months after discontinuation of treatment. They were 80 males and 20 females, their mean age 36.6±9 years.

All participants were selected according to HCV treatment protocol implanted by Egyptian Committee for control of viral hepatitis and they are received peg interferon α2a at fixed dose of 180 μg every week in combination with ribavirin at a dose according to body weight for 48 weeks. The criteria of hepatic encephalopathy, variceal bleeding, serum creatinine ≥2 mg/dl, serum AST, ALT more than 3 times of normal, HCC, autoimm-une liver disease, extrahepatic biliary diseases portal vein thrombosis on ultrasonogra-phy, history of alcohol use or hepatotoxic drugs within the last 6 months before enrollment were excluded.

All participants were undergo to thorough clinical examination, routine investigation as liver function tests, kidney function tests, complete blood count, fasting blood sugar, HCV Ab, HBsAg, TSH, ANA, AFP, Real time Quantitative PCR, it is done by (COBAS Ampliprep/Tagman HCV monitor, with detection limit is IU/ml Roche diagnostic systems), qualitative PCR it is done at 24th, 48th weeks of treatment and 6months after (CBBAS Amplicator Hepatitis C virus test, version 2.0, with dynamic range ≥ 50 IU/ml., abdominal ultrasonography using real time gray-scale device is done and liver biopsy for Histological grading and staging of liver histology were done.

Specific investigations including:

1- Serum RBP-4: Serum RBP levels were examined by Sandwich ELISA kit (Quantikine, R & D systems, USA) with 10 healthy controls being used for validation. It was measured at baseline, at week 24th, at week 48th, and 6 months after the end of treatment, and correlated with virological response.

2- Biomarkers claimed to be correlated with metabolic syndrome RBP4: They include serum uric acid, triglycerides, ferrtin levels and serum GGT level.

RESULTS

Table (1): Demographic, anthropometric and laboratory data of the three groups.

Group	Responders	Relapsers	Breakthrough	ANOVA
Gender (M/F)	80/20	80/20	100/0	
Age(years)	35.3±12	36.6±9	36±10	F=0.7, P=0.51
BMI(k/m^2)	26.3±3.1	26.2±2.5	24.6±1.8	F=1.11, P=4.35
ALT(IU/ml)	45.2±24.2	79±29	72±42	F=3.14, P=0.07
AST (IU/ml)	38.6±13.8	76±40.6	60.6±39	F=3.06, P=0.06
Albumin (g/dl)	4.3±0.4	4.2±0.42	4.1±0.5	F= 0. 102, P= 0.9
T. Bilirubin (mg/dl)	1.19±0.21	1.2±0.13	1.12±0.23	F=0.5,P=0.6

Table (1) shows demographic and anthropometric data among the three groups: the responders group included 80 males and 20 females, the relapsers included also 80 males and 20 females and the breakthrough group included 100 males. Regarding the age, it was higher in the relapsers than responders and breakthrough patients (36.6±9, 35.3±12, 36±10 years respectively) and that was nonsignificant (F=0.7, P=0.51).

As regards BMI, it was statistically non significant among the three groups (26.3±3.1, 24.6±1.8, 26.2±2.5 k/m^2 respectively& F=1.11, P=0.35)

The liver function tests data among the three groups. ALT was higher in relapsers than breakthrough and responders and was statistically non significant (79±29, 72±42, 45.2±24.2 IU respectively - F=3.14, P=0.07).

AST was higher in relapsers than breakthrough and responders and was statistically non significant (76±40.6, 60.6±39, 38.6± 13.8 IU respectively - F=3.06, P=0.06).

Serum Albumin was higher in responders than relapsers and breakthrough and was statistically non significant (4.3± 0.4, 4.2±0.42, 4.1±0.5 g/dl respectively - F=0.102, P=0.9).

Total Bilirubin level differences among the three groups was statistically non significant (1.19± 0.21, 1.12± 0.23, 1.2± 0.13 mg/dl respectively - F=0.5, P=0.6).

Table (2): Variables were significantly different among the study groups.

Variable	Responders Mean±SD	Relapsers Mean±SD	Breakthrough Mean±SD	Significance
γ-GT	31±13	65±20	45±31	F=6.27,P=0.01
PT	11.1±0.8	13±1.4	11.5±0.8	F=7.3,P=0.003
PLT	168±33	122.7±55.8	168±39	F=3.57,P=0.04
α-FP	3.1±1.9	16±19.8	16±20	F=6.6,P<0.01

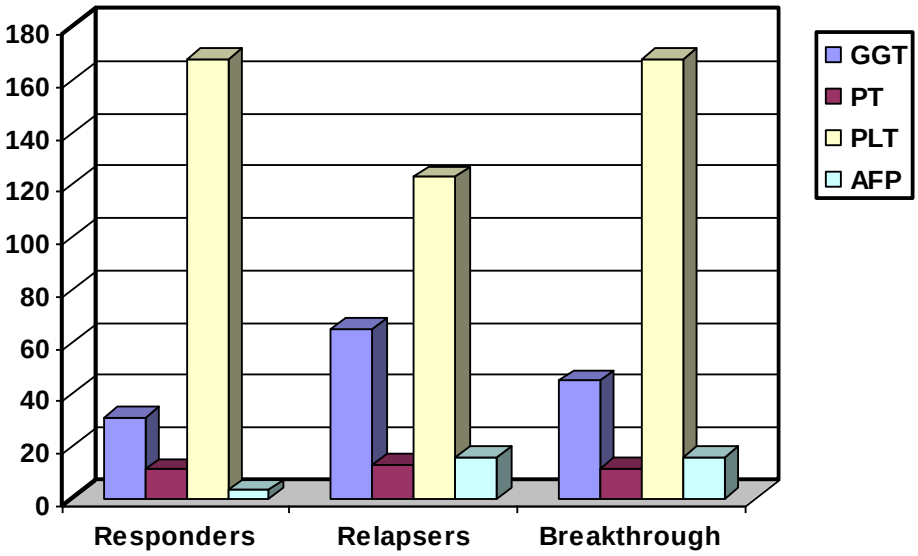

Fig. (1): Variables significantly associated with response to therapy among the three groups.

Table (3): Metabolic profile data among the three groups.

Variable	Responders Mean±SD	Relapsers Mean±SD	Breakthrough Mean±SD	Significance
Uric acid (mg/dl)	5.4±1.5	5.1±1.6	5.2±2.3	F=0.07,P=0.93
TGs (mg/dl)	95±6	129.5±55	130±41	F=2.36,P=0.12
Ferritin (mg/dl)	364±64	568±112	462±106	F=11.03,P<0.001
FBS (mg/dl)	108±7.8	102±10.7	106±17	F=0.59,P=0.06
RBP4	49.1±11.12	43.8±21	44.7±14	t=5.12,P=0.001

As regards to metabolic profile data mong the three groups as shown in table 3. Serum ferrtin was the only marker has statistically significant difference among the three groups (P<0.001).

Table (4): Changes in RBP4 before initiation and after termination of treatment in CHC patients with high and low viral load.

	High viral load (4000,000) (n=110)	Low viral load (<4000,000) (n=190)	T	P
RBP4 (0) (ng/ml)	46.5±19	48.5±17.5	8.1	0.01
RBP4 (48) (ng/ml)	46±17	52±22	8.88	0.01

a- Pre treatment RBP4: was lower in the high viral load group (46.5±19 vs. 48.5±17.5 ng/ml respectively) in which it showed a significant difference (t=8.1, p=0.01).

b- RBP4 at end of treatment (ET): RBP4 was lower in high viral load vs. low viral load group (46±17 vs. 52±22 respectively and that was statistically significant (t=8.88, P<0.01)

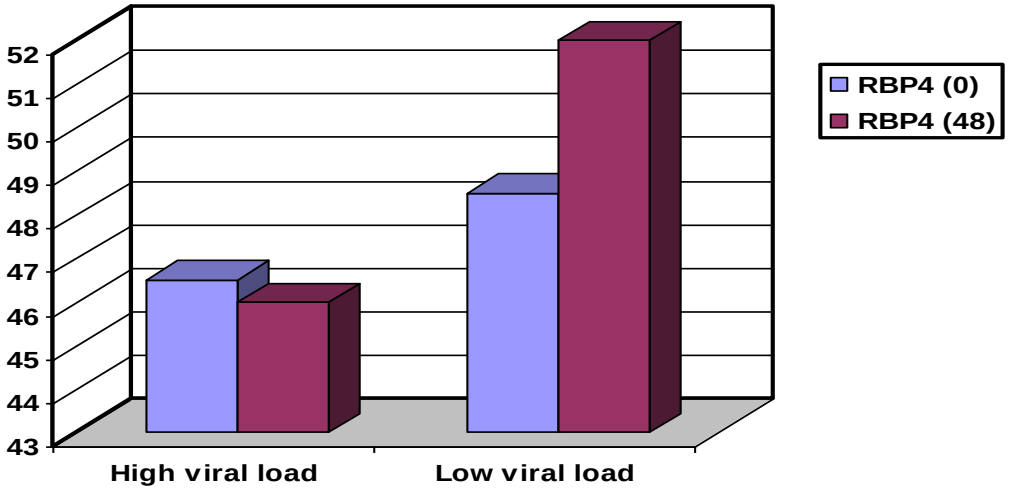

Fig. (2): RBP4 level is patients with high and low viral load.

Table (5): Serum RBP-4 levels among the three groups throughout the study.

Variable	Responders Mean±SD	Relapsers Mean±SD	Breakthrough Mean±SD	Significance
RBO4-Pretreatment	62.8±7	35±7	39.6±11.2	F=12.1,P<0.001
RBP4 at 24th W	53±18	32.9±6.8	39±12	F=9.3,P<0.01
RBP4 at end of treatment	41.2±11.7	52.3±29	55.6±18.9	F=0.376,P=0.7
RBP4 at 72W	39.5±7.8	55.2±42		t=5.12,P=0.001
ANOVA	F=5.9,P=0.002	F=2.32,P=0.09	F=2.84,P=0.08	
Paired t test	W0-24: t=5.7, P<0.001	W0-24: t=12.35, P<0.001	W0-24: t=1.35, P=0.91	
	W0-48: t=3.5, P=0.01	W24-48: t=2.5, P=0.03	W0-48: t=-2.45, P=0.036	
	W0-72: t=3.6, P=0.005	W42-72: t=3.45, P=0.038	W0-72: t=-2.52, P=0.032	

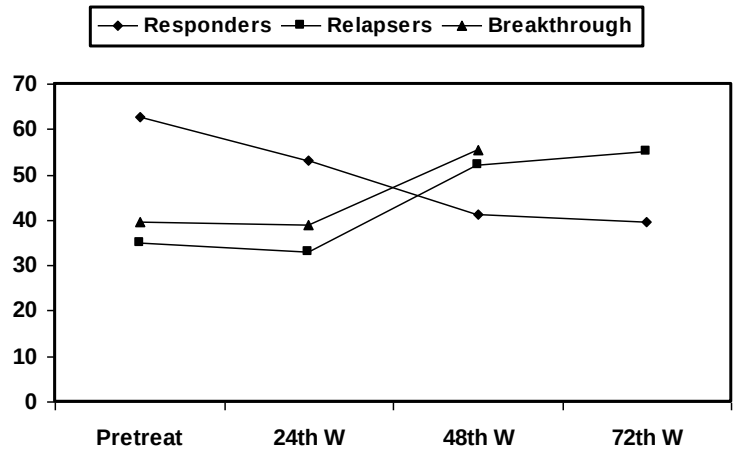

Fig. (3): Tracing the changes of serum RBP-4 levels in the three groups throughout the study.

Table (6): Stepwise multiple regression analysis of the variables associated with RBP4.

Stepwise regression	r	P
Step I – age	0.008	0.269
Step II – ALT	-0.07	0.03
Step III – AST	0.0021	0.61
Step IV – Prothrombin time	-0.087	0.149
Step V – Platelets	0.0014	0.534
Step VI – Fasting sugar	-0.007	0.279
Step VII – TG_3	-0.004	0.03
Step VIII- Ferritin	0.0002	0.476
Step IX- Uric acid	0.024	0.593
Step X- HCV RNA	0.004	0.484
Step XI – Albumin	-0.037	0.863

Age, ALT, AST, PT, PLT, FBS, TGs, FRT, UA, RNA, albumin were considered as variables closely related to RBP4 serum levels. Stepwise multiple regression analysis was performed to identify variables independently associated with RBP4 from which we can predict its value as shown in table 6.

DISCUSSION

Hepatitis C virus (HCV) is one of the principle causes of cirrhosis and hepatocellular carcinoma and has a strong impact on public health worldwide[9].

HCV infection has metabolic derangements affecting mainly glucose and lipid metabolism which negatively affects liver disease progression and the response to therapies[10].

HCV infection has been shown to accelerate the development of type 2 diabetes in predisposed individuals and curing of HCV infection results in a reduced incidence of new onset type 2 diabetes independently of other risk factors[11].

Retinol binding $protein_4$ (RBP4) is a protein that belongs to the lipocalin family whichy are proteins that transport small hydrophobic molecules such as steroid retinoids, and lipids, it is mainly secreted by hepatocytes (80%) and to less extent by adipocytes (20%)[12].

RBP4 is encoded by RBP4 gene that maps to chromosome 10q23-q24. Transgenic over-expression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance. Conversely genetic deletion of RBP4 enhances insulin sensitivity[13].

Blood concentrations of RBP4 were associated with hepatic production of RBP4 and correlated with circulating levels of albumin, cholinesterase and coagulation factors so, its level is affected by efficiency of liver function being lower in patients with hepatic impairment[14].

As our patients selected for combined therapy of HCV are Child Pugh class A, so it is expected that RBP4 level should be normal due to exclusion of the influence of hepatic biosynthetic capacity on RBP4 level and any alteration of its level will by

correlated to the effect of HCV, serum glucose, insulin resistance.

In our study, RBP4 levels before therapy were higher in responders (62.8±7 ng/ml) which may be linked to the abnormal metabolic state caused by the virus before treatment and also higher in responders than breakthrough and relapsers (62.8±7, 39.6±11.2, 35±7 ng/ml, respectively).

At 48th week RBP4 was higher in the breakthrough than relapsers and responders (55.6±18.9, 52.3±29, 41.2±11.7 ng/ml, respective-ely) which may reflect the residual metabolic abnormality which remained throughout the course of therapy and caused viral persistence. This is supported by study made by(15) who postulated that RBP4 is elevated in liver diseases not associate with cirrhosis as chronic hepatitis C virus.

At 72nd week, RBP4 was significantly lower in the responders than than the relapsers (39.5±7.8, 55.2±42 ng/ml, respectively). The normalization in responders may reflect the recovered metabolic abnormalities.

We classified our study patients into a group with FBS 88.6±10.8 mg/ml, their RBP4 level was 39.1±12.7 ng/ml and group with FBS 110.4±7, their RBP4 level was 50.4±18.5 ng/ml, so declaring that RBP4 is infuenced by FBS and insulin resistance in our study (t = 13.1, P=0.001).

RBP4 concentrations were significantly higher in participants with isolated impaired fasting glucose than in those with normal glucose regulation(15).

Our study revealed that, ALT, AST, PT, PLT, FBS, TGs, serum ferritin, serum uric acid were considered as variables closely related to RBP4, so stepwise multiple regression analysis was performed to identify variables independently associated with RBP4 and revealed that triglycerides and ALT independently predict RBP_4 and is consistent with(16) who showed that triglycerides, ALT and cholesterol independently predicted RBP4 level, this is can be explained by the fact that lower ALT with good liver function is associated with higher RBP4 level, however as higher RBP4 should be associated with higher TGs level reflecting the disturbed metabolic state, here it is associated with lower TGs due to the hypotriglyceridimic state that are seen in HCV state.

Persistent elevation of the RBP4 associated with breakthrough and relapse may be to the associated insulin resistance which observed in HCV.

Our study showed that before treatment, RBP4 was higher in the responder group than the other groups and that was highly significant, however, at the end point of treatment, RBP4 was higher in breakthrough and relapsers than responders, 6 months post treatment the RBP4 level was lower in responders than relapsers and that was highly significant (P = 0.001).

GGT, AFP level, at the end of treatment were highly significant in predicting the response to treatment being higher in the relapsers and breathrough groups than the responder group and that were consistent with RBP4 level and response to therapy (P = 0.085), (P=0.03) respectively,

Our study revealed that the achievement of significant reduction in RBP4 level at the end of the treatment course could predict sustained virologic response, while failure to attain this

reduction in RBP4 level and persistent elevation in RBP4 at the end of treatment was coincident with breakthrough and relapse.

Finally, it can be shown from our study the metabolic state due to HCV can influence the level of RBP4 and indirectly affect and correlates with the response to antiviral therapy.

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