SERUM RETINOL BINDING PROTEIN 4 LEVEL IN PATIENTS WITH CHRONIC HEPATITIS C.

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ABSTRACT

Hepatitis C virus infection is one of the principle causes of cirrhosis and hepatocellular carcinoma.The aim of antiviral therapy is the cure of hepatitis C by sustained elimination of the virus. A large proportion of patients do not respond to Pegylated interferon plus which have unpleasant side effects and high costs.

Study aim

Investigation of the relationship between the changes in the serum RBP4 levels throughout the course of Pegylated interferon, ribavirin and the virological response pattern.Also the association between serum RBP4 and biochemical & histological characteristics of chronic hepatitis C patients.

Methods:

Our study included 30 patients with chronic HCV; they were followed up for 18 months. They were divided into three groups; the responders(n=10), the relapsers(n=10) and breakthrough group(n=10). All the patients were subjected to thorough history taking and clinical evaluation. They were exposed to All the investigations necessary before initiation of combined therapy. Serum RBP-4 was examined at baseline, week 24th, week 48th, and 6 months after the end of treatment, and correlated with liver histology and virological response

Statistical Analysis: Data were entered, checked and analyzed using SPSS for windows version 17, presented as mean±SD. Paired t test for comparison of data before and after therapy, ANOVA for multiple comparison, stepwise regressive analysis for determinimg independent variables affecting RBP4.

Results: Sustained virological response was associated with significant reduction in RBP4 level at the end of the treatment course, however failure to attain this reduction in RBP4 level, and the most important is persistent elevation in RBP4 at the end of treatment was coincident with breakthrough and relapse.

Conclusion: RBP4 a marker of insulin resistance can be used as predictor of SVR in patients with CHC under combined therapy

INTRODUCTION

Hepatitis C virus (HCV) is an outstanding cause of end-stage liver disease and hepatocellular carcinoma (HCC) and is now one of the most common causes of liver transplantation**.** [1] It was first identified in 1989 by immuno-screening of sera from patients with post-transfusion non-A, non-B hepatitis. [2] According to the World Health Organization there are 180 million people infected with the hepatitis C virus, corresponding to 3% of the world's total population.[3] Accumulating evidence demonstrates that HCV affects glucose and lipid metabolism. [4] Higher prevalence of HCV was seen in diabetic patients compared with matched controls, and a higher prevalence of diabetes in HCV-infected patients.[5] HCV infection shows significantly higher levels of fasting serum insulin, C peptide, and HOMA-IR compared to matched controls. [6] HCV may contribute to insulin resistance by causing an insulin signaling defect in hepatic insulin receptor substrate-1, Phosphatidyl Inositol 3-kinase. [7], hepatitis C core-induced suppression of cytokine signal 3(SOC3). Alternatively, HCV may elevate tumor necrosis factor-alpha (TNF-α) which induces serine phosphorylation of IRS-1, down regulation of GLUT4 or expression of protein phosphatase 2A (PP2Ac) to dephosphorylate PKB/Akt. [4]

Hepatic steatosis in case of HCV occurs in approximately 55.5% of liver biopsy .[8] Two discrete forms of steatosis may be found in patients infected with HCV; Metabolic steatosis in patients with obesity, hyperlipidemia, and insulin

resistance and HCV induced steatosis a result of the direct cytopathic effect of HCV genotype 3.[9] Steatosis may be due to the effects of NS5A protein and HCV core protein via reduction in PPAR α activity[10], inhibition of microsomal triglyceride transfer protein activity (MTP)[11], increased sterol regulatory element binding proteins (SREBP) which binds to SREBP response element (SRE) with increase in free fatty acid synthesis.[12] It was shown that chronic hepatitis C have higher HDL, lower total cholesterol, triglyceride, and LDL levels than matched healthy controls and sustained disappearance of HCV is associated with reduction of steatosis in genotype 3, and a correction of baseline low serum cholesterol and LDL , however this hypolipidemic effect was persisting in non-responders.[13]

RBP4 is a protein that belongs to the lipocalin family. [14] It is secreted mainly by hepatocytes (80%) and to lesser extent by adipose tissue (20%). It takes part in the control of metabolic and proliferative cell functions including steatogenesis by interacting with nuclear retinol X receptor (RXR).[15] A pathogenic link was proposed between insulin resistance, diabetes and high serum and adipose levels of RBP4.[16] Its secretion from adipocytes is regulated by glucose transporter 4 (GLUT4) that mediates glucose uptake into muscle and fat cells. In insulin-resistant states the expression of GLUT4 is reduced in adipocytes resulting in decreased influx of glucose and increased secretion of RBP4.[17]

It was demonstrated that patients with chronic liver disease and more advanced fibrosis carried a significantly decreased RBP4 than controls, reflecting the impact of hepatic necro-inflammatory activity on RBP4 [18]. **Iwasa et al., 2009** [19] studied changes in RBP4 levels following interferon therapy. RBP4 levels were lower in CHC patients than controls concomitant with the grade of fibrosis, activity, and steatosis.A study made by **Petta et al., 2008**[20] had shown a remarkable association between the degree of hepatic steatosis and RBP4 levels, restricted to genotype1 hepatitis C patients and unrelated to abnormal metabolic features

A sustained elimination of HCV is achieved if the HCV RNA is negative 6 months after the end of treatment [21]. Predicting the probable outcome of treatment in patients with HCV infection has been a challenge. Combined therapy eradicates the virus in approximately 60% of patients; HCV genotype 1 (42—51% response rates) and genotypes 2 and 3 (76—84% response rates) [22]. However, a significant number of patients do not respond to therapy or even relapse following discontinuation of treatment. Accurately predicting the patients who will respond to therapy is becoming increasingly important helping the decision to continue treatment in patients who will respond or stop in who are unlikely to respond.

the aim of our work is to investigate the changes in the level of RBP4 throughout the period of treatment and finding the association between retinol-binding protein-4 and pattern of response to combined therapy elucidating its role as a possible predictor of sustained virological response, and its relation to histological and biochemical characteristics of patients with chronic hepatitis C.

METHODS

A- Patient selection

From May 2009 to November 2010, 100 patients who were candidates for anti HCV combined therapy at the hepatology clinic-Internal medicine department-Zagazig univeristy were followed up for one year during their course of treatment and for 6 months post treatment. 40 patients achieved SVR, 30 patients were relapsers and 20 patients showed viral breakthrough during the treatment course, however 10 patients were non-responders. Of these patients, 30 patients were selected randomly after exclusion of diabetes, hypertension and obesity. They were enrolled in our study after approval of the ethical committee of Zagazig university

hospital. Written informed consent was obtained from patients for interview, anthropometric measurements and blood sampling. A questionnaire regarding the medical history, drug history, and family history was obtained. The patients were classified into three groups according to their virological responses:

I: Group1 (responders): It included 10 patients who achieved sustained virological response. They were 8 males and 2 females, their main age 35.3±12 years.

II: Group2 (breakthrough): It included 10 patients who attained HCV RNA negativity during treatment with recurrence of viremia while treatment going on denoted by HCV RNA positivity at 24 /or 48 weeks of treatment. They were 10 males and their main age 36±10 years.

III: Group3 (Relapsers): It included 10 patients who attained HCV RNA negativity at the end of therapy with recurrence of viremia 6 months after discontinuation of treatment. They were 8 males and 2 females, their main age 36.6±9 years**.**

Inclusion criteria: All Patients were previously untreated aged 18–60 years, seropositive for HCV antibodies. They had undergone liver biopsy within 6 months before entry, the patients were non diabetic, non obese (BMI ≤ 30) ,HBsAg –ve.

Exclusion criteria: A history of hepatic encephalopathy or variceal bleeding, serum Cr ≥ 2 mg/dl, serum AST or ALT more than 3 times normal, hepatocellular carcinoma, evidence of active autoimmune liver disease, history of alcohol use, or use of hepatotoxic drugs within the last 6 months before enrollment.

Community based control group:

This group included 10 healthy subjects after exclusion of HCV, HBV, D.M and hypertension.

B- Methods: All the patients were subjected to thorough medical history taking and clinical examination including general examination. Clinical signs of portal hypertension and liver cell failure were evaluated.

C- Laboratory analysis:

All patients underwent a 12-h overnight fast before blood tests which included:

a- Routine investigations preliminary to combined therapy:

As liver function tests, prothrombin time, prothrombin concentration (%), Kidney function tests, complete Blood Count and fasting blood sugar**.** According to the American Diabetes Association criteria 2010, Prediabetes is considered if FBS was between 100 and 126 mg/dl.

-HCV antibody, HBsAg, T.S.H, A.N.A, Serum AFP

-Real time Quantitative PCR is done at 12th week (COBAS Ampliprep/Taqman HCV monitor, with detection limit 15 IU/ml; Roche Diagnostic Systems. Qualitative PCR done at 24th, 48th weeks of treatment and 6 months after termination of treatment using a standardized automated qualitative reverse transcription polymerase chain reaction assay (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0, with dynamic range ≥ 50 IU/ml).

-Abdominal ultrasonography: The patients were examined after 6 hours fast. Criteria of cirrhosis were excluded. Criteria of portal hypertension as Portal vein diameter more than 13mm, splenic bipolar diameter more than 130mm , splenic vein diameter> 10mm **,** together with a platelet count less than 100000 with platelet count/splenic diameter ratio ≤909[23] predicts oesophageal varices and necessitates performing upper GIT endoscopy to exclude varices , the presence of which is a contraindication to combined therapy.

-Liver biopsy**:** liver biopsy specimen of at least 2 cm in length was taken and fixed in 10% formalin buffer. Biopsy samples were stained with hematoxylin-eosin to elucidate histological grading based on histological activity index (HAI) of **Knodell et al., 1981**[24]. Staging of liver histology into F0–4 according to the Metavir scoring systems: F0 = none, F1= portal expansion, F2= bridging fibrosis, F3 = bridging fibrosis with lobular distortion, and F4 = cirrhosis**.** [25]

b - Specific investigations

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 liver histology and virological response.

2-Biomarkers claimed to be correlated hepatic steatosis and RBP4: They included Serum Uric acid, triglycerides, ferritin levels, and Serum GGT level

D: STATISTICAL ANALYSIS: Data were analyzed using SPSS version 17 software. Continuous variables are presented as mean±SD. Correlations were analyzed using Spearman's rank correlation test. Stepwise regression analysis was performed to identify independent predictable of plasma RBP4 levels. The analysis of variance was performed using Scheffe's F-test for multiple comparisons among the three groups. Paired t-test was used to compare mean values before and after treatment with combined therapy. P value of <0.05 was considered statistically significant.

RESULTS

The control subjects were 5 males and 5 females with mean age of 30.4 ± 6.1 years, their mean BMI was 27.3 ± 3.3 kg/m^2. All had normal ALT (24.1± 4.6 IU/L), RBP-4 was 35 ±6 ng/mL and they were non diabetic, not hypertensive; FBS: 87.9 ± 8.3 mg/dl, TGs 100 ± 18.34 mg/dl.

The characteristics of the study patients are summarized in **Table (1)**. The cohort included 26 men and 4 women. The mean BMI was 25.7 ± 2.6 kg/m2. Their age was 37.6±10.2 years. ALT was elevated 65.3± 34.7 IU/L, AST 58.4 ± 36 IU/L, GGT as a marker of steatosis[27] was 46.8 ± 25.7IU/L , albumin 4.2 ± 0.42 g/dl , total bilirubin 1.16± 0.2 mg/dl, prothrombin time 11.8 ± 1.2, platelet count was 153±47.4x10^3 /µl, AFP 11.5 ± 16.7ug/dl, HCV RNA 801.4 ± 448 KIU/l

Table (1): Anthropometric and laboratory data of the study patients. (n=30)

	Age	BMI	AST	ALT	γ-GT	ALB	T.BIL	P.T.	PLT	AFP	HCV RNA
Mean	37.6	25.7	58.4	65.3	46.8	4.2	1.16	11.8	153	11.5	801.4
SD	10.2	2.6	36	34.7	25.7	0.42	0.2	1.2	47.4	16.7	258.4

Comparing laboratory Data in the CHC patients and controls showed that, Retinol-binding protein 4 level was higher in the study patients than in controls (47.8±16.9 ng/ml vs. 35 ±6 ng/mL; **t = 5.62, P <0.001**), higher ALT in the study patients (65.3±34.7 vs. 24.1± 4.6 IU/L, **t=2.47, P= 0.04**), higher AST (58.4±36 IU/L vs. 24.5± 9.7 IU/L, **t = 2.75, P= 0.02**).The study patients showed higher FBS level (105±12.3 vs. 87.9 ± 8.3 mg/dl, **t= 3.35, P= 0.01**), higher TGs level (118 ±44 vs.100 ± 18.34 mg/dl, **t= 44.01, P<0.001**)

The metabolic profile of the patients was evaluated as follows: serum RBP4 was 47.8±16.9 ng/ml, serum TGs 117.7± 44 mg/dl, serum ferritin as a marker of iron overload, inflammatory activity and insulin resistance was 461.3mg ± 309.2 mg/dl, fasting blood sugar as an indicator of insulin resistance and pre-diabetic state was 105±12.3 mg/dl and uric acid as a marker of metabolic disturbance, steatosis [28] was 5.23±1.8 mg/dl.

Table (2): Relation between RBP4 and BMI & FBS

BMI (kg/m^2)	N	Mean	SD	RBP4	T	P
<25	14	23.5	0.9	51.6 ±17	**9.7**	**<0.001**
25–30	16	27.2	1.4	44.4 ± 18		
FBS≤100mg/dl	7	88.6	10.8	39.1±12.7	**13.1**	**<0.001**
FBS>100mg/dl	23	110.4	7	50.4±18.5		

Comparing the characteristics of the three groups as shown in **tables (3)**. Regarding the age (36.6±9, 35.3±12, 36±10 years respectively & F=0.7, P=0.51), BMI (26.3±3.1, 24.6±1.8, 26.2±2.5 km/m^2 respectively & F=1.11, P=0.35). ALT was higher in relapsers than breakthrough and responders (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 (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 (4.3±0.4, 4.2±0.42, 4.1±0.5gm/dl respectively & F=0.102, P=0.9). Total Bilirubin level (1.19±0.21, 1.12±0.23, 1.2±0.13 mg/dl respectively & F=0.5, P=0.6) and these variables showed no significant difference among the three groups.

14 patients had BMI<25 with RBP4 51.6 ±17 ng/ml, 16 patients had BMI 25 – 30 with RBP4 44.4 ±18 ng/ml ; it was shown that RBP4 was higher in lean patients and that was statistically highly significant.**(t = 9.7, P<0.001).** As regards to FBS, on the basis of impaired FBS as a marker of pre-diabetes and insulin resistance, we classified our patients into two groups; group1 with FBS≤100mg; they were 7 patients with FBS 88.6 ± 10.8 ; this group showed a RBP4 level 39.1±12.7ng/ml, and group 2 with FBS >100mg/dl, they were 23 patients with FBS 110.4±7, with a RBP4 level 50.4±18.5 ng/ml which is higher than the former group and that was statistically highly significant declaring that RBP4 is influenced by FBS and insulin resistance (**t = 13.1 , p <0.001) (table 2)**

Table (3) shows the liver function variations among the three groups

Group	Responders	Relapsers	Breakthrough	ANOVA
ALT(Iu/ml)	45.2 ± 24.2	79 ± 29	72 ± 42	F=3.14, P=0.07
AST	38.6 ± 13.8	76 ± 40.6	60.6 ± 39	F=3.06, P=0.06
γ-GT	31 ± 13	65 ± 20	45 ± 31	F=6.27, **p=0.01**
Albumin	4.3 ± 0.4	4.2 ± 0.42	4.1 ± 0.5	F= 0.102 P= 0.9
T. Bilirubin	1.19 ± 0.21	1.2 ± 0.13	1.12 ± 0.23	F=0.5 P=0.6
P.T	11.1 ± 0.8	13 ± 1.4	11.5 ± 0.8	F=7.28 **P=0.003**
α- FP	3.1 ± 1.9	16 ± 19.8	16 ± 20	**F=6.6** **P=0.005**
Platelet count	168 ± 33	122.7 ± 55.8	168 ± 39	F=3.57 **P=0.04**

Characterization of RBP4 level throughout the study

RBP-4 among the three groups:

A: As regard to the difference among the three study groups:

- **Pre treatment (Baseline) RBP4:** was higher in responders than relapsers and breakthrough and that was statistically highly significant (64.9±13, 38±9, 40.4 ±16.2 ng/ml respectively & F=13.38, P<0.001 – **ANOVA test**)

At 24th Week: RBP4 was still higher in responders than relapsers and breakthrough but the level was lower compared to the baseline value, and that was statistically highly significant (54±12, 36 ± 6, 41 ±16 ng/ml respectively & F=9.3, P<0.01 – **ANOVA test)**

At the end of treatment (48th W): RBP4 level was higher in breakthrough and relapsers than responders (53.7±17.4, 50.5±28, 45.5±14.7ng/ml & F=0.376, P=0.7 – **ANOVA**). This may be explained by the correction of metabolic derangement in the responders which is still persistent in the breakthrough and relapsers groups and the cause of the emergence of viral resistance.

At 72nd Week: RBP4 was significantly lower in responders than relapsers (43.7±8.9 vs. 52.1± 32 ng/ml respectively & t= 5.12, p= 0.001 - t test)

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

Study groups / RBP4	Responders	Relapsers	Breakthrough	Significance
RBP4-Pretreatment	64.9±13	38± 9.8	40.4±16.2	F=12.1,P<0.001 (ANOVA)
RBP4 at 24thW	54 ±12	30.7 ± 7.9	41±10	F=9.3,P<0.01 (ANOVA)
RBP4 at end of treatment	45.5 ±14.7	50.5± 28	53.5 ±17.4	F=0.376, P=0.7 (ANOVA)
RBP4 at 72W	43.7±8.9	52.1 ± 32		T= 5.12,p=0.001 (T test)
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	W24-72: t =-2.45 p= 0.038	W24-48: t= -2.52, p= 0.032	

B: Variations of RBP4 level within each group thoughout the study:

1- The responders: RBP4 level was higher in responders than controls [64±9 vs. 35 ±6 ng/mL, t= 15.2, p<0001). However within

the group, tracing the level of RBP4 through the period of study; it showed a progressive and significant decrease (W0: 64.9±13, W24: 54±12, W48: 45.5±14.7, W72: 43.7±8.9 ng/ml respectively, this decrease may be due to recovery of the metabolic disturbance caused by the virus**.**

At 24thW: A statistically highly significant decrease was noted (64.9 ± 13 →54± 12 ng/ml& t= -5.7, p = 0.001)

At 48thW: more statistically significant reduction (64.9±13 → 45.5 ± 14.7& t= 3.5, p= 0.01)

At 72nd W (64.9±13 → 43.7±8.9 & t= 3.63, p= 0.005 respectively – paired t test) and that was highly significant. From these observations, there's a tendency towards decrease in RBP4 level due to recovery of the metabolic abnormalities induced by HCV and that was coincident with viral clearance denoted by PCR at 24, 48, 72 weeks**.**

2- In the relapser group: RBP4 level was slightly higher than controls [38 ± 9.8 vs. 35 ±6 ng/mL, t= 0.88, p<0.4). Following RBP-4 changes through the course of the study revealed that:

At 24th W: there was a significant reduction of the RBP4 level when compared to baseline level. (38 ± 9.8 → 30 ±6 ng/mL, t=8.3, p= 0.001)

At 48th W: it showed a peak when compared to the base line and 24th W level (39±9.8, 30±6 → 50.5± 28ng/ml respectively& t = -2.54, p= 0.03)

At 72th W: it showed a further increase (50.5± 28 → 52.1± 32ng/ml respectively & t= -0.58, p=0.57) but was statistically non significant from 48th week value. It was declared that failure to achieve significant reduction of RBP4 level at 48th week may predict the relapse of viremia at 72nd week which was proven when compared to HCV PCR at 48th and 72nd weeks of these patients.

3-In the breakthrough group: RBP4 level was higher than controls [40.4 ± 16.2 vs. 35 ±6 ng/mL, t= -0.907, p =0.39). Following RBP-4 changes through the course of the study revealed that:

At 24th W: there was a slight non significant increase of the RBP4 level when compared to baseline level. (40.4 ± 16.2 → 41 ± 10 ng/mL & t=-1.35, p= 0.2)

At 48th W: it showed an unexpected significant increase when compared to the base line and 24th W level (40.4±16.2→ 41±10→ 53.5±17.4ng/ml respectively& t = -2.45, p= 0.04). Non significant decline of RBP4 at 24th week with unexpected rise at 48th week was associated with reappearance of viremia at 48th week.

As regards to metabolic profile data among the three groups Uric acid was high normal in the three groups (5.4± 1.5, 5.1± 1.6, 5.2± 2.3 mg/dl respectively & F=0.09, P=0.914). TGs were lower in the responders exhibiting a hypotriglyceridimic state (95±26, 129.5± 55, 130± 41 mg/dl respectively - F=2.36, P=0.12). Serum Ferritin was higher in the relapsers and breakthrough than responders (568±355, 462±334, 364±204mg/dl respectively & F=0.88, P=0.43). FBS was higher in the responders and breakthrough than relapsers (108±7.8, 106±17, 102±10.7mg/dl respectively & F=0.59, P=0.6)**,** thus these variables showed statistically non significant difference among the three groups**.**

Variables correlated with RBP4:

Spearman rank correlation used to detect variables closely correlated to RBP-4 as shown:

A: Responders Group

Pretreatment RBP4 (0) **ALT** has a significant negative correlation i.e. higher RBP4 is associated with lower ALT level (r= -0.709, P=0.01).

-RBP 24 thweek **ALT** has a significant negative correlation i.e. higher RBP4 is associated with lower ALT level (r= -0.598, P=0.03)

-RBP4 48th week: **AST** has a significant negative correlation i.e. higher RBP4 is associated with lower AST level (r= -0.596, P=0.04). **Platelets (PLT)** has significant positive correlation (r= 0.598, 0.03).

- RBP4 72nd week: **Age** has a significant positive correlation (r= 0.564, P=0.045),

and **TGs** has significant positive correlation (r= 0.669, 0.02)

B: Relapsers Group

-Pretreatment RBP4 (0): **Prothrombin time (PT)** has a significant positive correlation i.e. (r= 0.571, P=0.04), and **albumin** has a significant negative correlation(r= -0.593, P=0.035)

- RBP4 at 24th week: **PT** has a significant positive correlation with RBP4 level (r= 0.566, P=0.04)

- RBP4 at 48th week: **Albumin** has a significant negative correlation i.e. lower RBP4 is associated with higher ALB level (r= -0.644, P=0.02)

- RBP4 at 72nd week: **PT** has a significant positive correlation i.e. higher RBP4 is associated with higher PT level (r= 0.574, P=0.04)

C: Breakthrough Group

- Pretreatment RBP4 (0): **Fasting blood sugar (FBS)** has a significant positive correlation with RBP4 (r= 0.608, P=0.03)

- RBP4 at 24th W: **Fasting blood sugar (FBS)** has a significant positive correlation with RBP4 (r= 0.565, P=0.04)

- RBP4 48th week: **Ferritin (FRT)** has a significant negative correlation with RBP4 (r= -0.588, P=0.04)**,** and **RNA** has significant negative correlation. (r= -0.55, P=0.049)

As regards the all groups:

Baseline or pretreatment RBP4 was highly correlated with **ALT**, it showed a significant negative correlation in all groups i.e. higher RBP4 is associated with lower ALT level (r= -0.423, P=0.01) and **FBS** where it showed a significant positive correlation i.e. higher fasting blood sugar is associated with higher RBP4. (r= 0.340, P=0.03).

RBP4 at 24th week was highly correlated with **ALT**, it showed a significant negative correlation in all groups (r=-0.336, P=0.04) and **FBS** where it showed a significant positive correlation i.e. (r= 0.370, P=0.02).

RBP4 at the end of treatment (48th week): has a significant negative correlation with serum albumin i.e. lower RBP4 is associated with higher albumin level (r= -0.380, P=0.02).

RBP4 at 72nd W of treatment **of the two groups (responders and relapsers)** had a significant positive correlation with serum triglycerides i.e. higher RBP4 is associated with higher TGs level (r= 0.446, P=0.03), and higher serum uric acid (r= 0.617, P=0.002) and indeed that was linked to metabolic abnormalities which mostly caused the relapse or viral resistance.

Stepwise multiple regression analysis was performed to identify variables independently associated with RBP4 from which we can predict its value**.** It was revealed that both ALT (r = -0.007, p= 0.003) and TGs (-.004 , p = 0.03) respectively are independent predictables for level of RBP-4 i.e. higher RBP4 is associated with lower TGS and ALT and 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 and it is believed that it should be recovered into even hypertriglyceridemia after successful eradication of HCV. In consistent with our results; **Ramcharran et al., 2010** [29] postulated that higher rates of SVR were associated with lower triglyceride and higher low-density lipoprotein cholesterol.

Relation of necro-inflammation and fibrosis to virological response: In responders, 3 patients (30%) had A1F1 in liver biopsy and 7 patients (70%) had A2F2, however in breakthrough patients 2patients (20%) had A1F1, 5 patients (50%) had A2 F2, and 3 patients (30%) had A3F3, in relapsers 7 patients (70%) had A2F2, 3 patients (30%) had A3F3**.** The relation of liver histology to virological response in our study patients was analyzed between the three groups and was only significant when responders compared to relapsers. (**Chi square = 6.1, p = 0.049**)

Relation of RBP4 to liver histology: as shown in (**table 8**), in patients with mild necro-inflammation and fibrosis (A1F1) RBP4 was significantly higher than those with A2F2 and those with A3F3 (57±18 vs. 48±17 & 38.8±12.7ng/ml respectively & F= 3.41, P=0.04 - **ANOVA test**). It was declared that with progression of necro-inflammation and fibrosis there is significant reduction in the level of RBP4 and that is consistent with diminished hepatic production.

DISCUSSION

An important aspect of HCV infection is its idiosyncratic relationship with the metabolism of glucose, which negatively affects liver disease progression and the response to IFNα-based therapies. [30] HCV infection has been shown to accelerate the development of type 2 diabetes in predisposed individuals**.** [31] **Moucari et al**., **2010** observed improvement in HOMA-IR and the decrease in serum HCV RNA in the group received 2-week course of danoprevir targeting the non-structural3 serine protease, while serum HCV RNA and HOMA-IR remained unchanged in patients receiving placebo. [32]

RBP is a member of lipocalins encoded by the RBP 4 gene that maps to chromosome 10q23-q24 and linked to increased risk for type2 diabetes in different populations. Genetic deletion of RBP 4 enhances insulin sensitivity [33]. It inhibits insulin signaling in skeletal muscle and upregulate PEPCK in the liver. [34] **Stefan et al., 2007** [35] documented that High circulating retinol binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat, this is further supported by **Seo et al 2008** [36] and **Wu et al 2008** [37]. In patients with chronic liver disease, Circulating RBP4 level is probably dependent on liver protein synthesis capacity and effective hepatic blood flow [38]**.** As our patients selected for combined therapy are Child Pugh class A, so theoretically it is expected that RBP4 level should be normal due to exclusion of influence of hepatic biosynthetic capacity on RBP4 level, and its level will correlate with serum glucose and insulin secretion and affected by insulin resistance. Though the study patients showed a within normal RBP4 level; patients with advanced necro-inflammation and fibrosis stage (A3F3) exhibited lower RBP4 level than A1F1 and A2F2 patients (38.8±12.7, 57±18, 48±17ng/ml) and this was consistent with **Tacke et al., 2008** [39] who confirmed that the degree of liver fibrosis and cirrhosis was the major histological parameter associated with reduced serum RBP4. **Alkhouri et al., 2009** [40] identified a novel association between serum RBP4 levels and hepatocellular injury**. Nobili et al., 2009** found that RBP4 level inversely correlated with degree of liver damage. [41]

RBP4 levels before therapy were higher in responders than breakthrough and relapsers (64.9±13, 40.4 ±16.2, 38±9ng/ml). At 48th week RBP4 was higher in the breakthrough than relapsers and responders (53.5±17.4, 50.5±28, 45.5±14.7 respectively). This is supported by a study made by **Seo et al 2008** [36] who postulated that RBP4 is elevated in liver diseases not associated with cirrhosis as NASH, and chronic hepatitis C. The most recent study by **Petta et al., 2011** [42] who documented that RBP4 serum levels in NASH and CHC genotype 1 patients were higher than normal. At 72nd week, RBP4 level was significantly lower in the responders than the relapsers (43.7±8.9 vs. 52.1±32ng/ml). The increased level of RBP4 in relapsers reflects the disturbed metabolic background of this group mainly insulin resistance which can be predicted by elevated fasting blood sugar above 100mg/dl according to ADA. [43] To prove this association we classified our study patients into a group with FBS≤100mg, their FBS was 88.6 ± 10.8mg/dl, this group showed a RBP4 level 39.1±12.7ng/ml, and group 2 with FBS >100mg, their FBS was 110.4 ±7, with a RBP4 level 50.4±18.5 ng/ml which is significantly higher than the former group declaring that RBP4 is influenced by FBS

and insulin resistance (t = 13.1, p <0.001**).** This is consistent with that reported by **Lecube et al., 2007** who postulated that glucose abnormalities, adversely influence the rate of SVR in HCV infected patients treated with interferon and ribavirin. [44]

Ferritin was higher in relapsers and breakthrough patients than responders (568±112, 462±106 vs. 364±64 respectively) their corresponding RBP4 level was (50.5±28, 53.5±17.4, 45.5±14.7ng/ml) being higher in breakthrough, relapsers than responders respectively. This is consistent with **Fernández-Real et al., 2008** [45] who found that serum RBP4 concentration was higher parallel to increased ferritin levels. GGT at the end of treatment as indirect marker of steatosis and insulin resistance[[46], it was also higher in relapsers and breakthrough groups than responders confirming our data.(65±20, 45±31, 31±13 IU/L respectively). The role of uric acid involved in metabolic syndrome as a predictor of response to treatment was investigated by **Pellicano et al., 2008**. Serum uric acid level ≥5.8 mg/dl is predictive of poor response to HCV treatment [47]. In our study Uric acid was high normal in the three groups (5.4±1.5, 5.1±1.6, 5.2±2.3 mg/dl respectively - F=0.09, P=0.914) this is consistent with **Hwang et al., 2011**[48] who postulated that increased uric acid concentrations, even within the normal range, were independently associated with the presence of NAFLD

Serum RBP4 concentrations were significantly higher in participants with isolated impaired fasting glucose than in those with normal glucose regulation and was associated with the risk of microalbuminuria[49]**. Chavez et al 2009**[50] found that Plasma RBP4 was significantly elevated in impaired glucose tolerance/T2DM compared with NGT lean or obese subjects. A study made by **Sulkowski et al., 2010**[51] who postulated that impaired fasting glucose strongly associated with lower SVR and higher relapse rates.

According to our study, the achievement of significant reduction of RBP4 at the end of the treatment course could predict sustained virological response, however failure to attain this reduction in RBP4 level, and the most important is persistent elevation in RBP4 at the end of treatment was coincident with breakthrough and relapse. Stepwise multiple regression analysis revealed that Triglycerides and ALT independently predicted RBP4, this was consistent with **Iwasa et al., 2009** [19] who showed that triglycerides, ALT and cholesterol independently predicted RBP4 level. And that reported by **Mallat et al., 2009** [52] who found that RBP4 was higher in study group than controls and strongly associated with TGs. Finally it can be shown from our study that both the metabolic state and stage of fibrosis can influence the level of RBP4 depending on which of them is more predominant. In our patients the metabolic disturbance was the more predominant with higher levels of RBP4 than expected. Predicting and correction of the metabolic abnormality may improve the response to therapy for example adding metformin or pioglitazone which was shown to reduce the level of RBP4 and improve insulin sensitivity, a fact was shown by **Lin et al., 2008** [53] and **Aigner et al., 2009** [54] who concluded that the addition of pioglitazone could significantly lower serum RBP4 and HOMA-IR values.

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