

## Association of A Novel Adipokine With Insulin Resistance And Disease Severity in HCV Infected Patients

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### Abstract:

Retinol-binding protein 4 (RBP4) has been identified as a protein contributing to insulin resistance (IR). As insulin resistance is present in nearly all patients with liver cirrhosis, we evaluated RBP4 in patients with chronic hepatitis C virus infected patients (CHC). This study aimed to evaluate the role of serum retinol binding protein 4 (RBP-4) as a predictor for early detection of insulin resistance (IR) in patients with CHC infection.

**Research Design and Methods:** Serum RBP4 was measured in 60 non diabetic CHC Patients classified according to Child- Pugh classification (Child A, Child B, Child C), 20 diabetic CHC patients and 20 age- and sex-matched healthy blood donors served as control subjects. RBP-4 correlation with the homeostasis model assessment of insulin resistance index (HOMA-IR) and metabolic factors was investigated.

**Results:** Serum RBP-4 levels were significantly lower in HCV infected patients and HCV diabetic patients compared with controls (HCV  $16545.17 \pm 10633.16$ , Diabetic HCV  $10310 \pm 4371.31$ , Control  $27820.0 \pm 9316.46$ ,  $P < 0.001$ ). In addition, serum levels of RBP-4 significantly decreased between all stages of cirrhosis, with the lowest level in Child C. HOMA-IR not significantly higher in HCV infected patients compared to control (HCV  $5.54 \pm 3.67$ , and Control  $4.11 \pm 2.42$ ). RBP-4 levels correlate positively with GGT ( $P < 0.01$ ), CHOL ( $P < 0.05$ ) and TG ( $P < 0.05$ ) in HCV Child C, additionally, it shows significant positive correlation with TG ( $P < 0.05$ ) in HCV Child B.

**Conclusions:** Disease severity may limit the role of RBP4 as a predictor of IR in CHC. These data demonstrate that RBP4 in CHC patients decreased due to reduced hepatic production, and it is not associated with insulin resistance.

Keywords: HCV, RBP4, Insulin Resistance

**Abbreviations:** **RBP4:** Retinol-binding protein 4, **IR:** insulin resistance, **HOMA-IR:** homeostasis model assessment of insulin resistance index, **CHC:** Chronic Hepatitis C Virus.

### Introduction

Chronic Hepatitis C Virus (HCV) infection is associated with a wide spectrum of liver histological lesions, ranging from mild chronic hepatitis to cirrhosis and hepatocellular carcinoma. The highest HCV prevalence in the world occurs in Egypt at an estimated 12% among the general population<sup>(13)</sup>. HCV infection is recognized as a systemic disease involving oxidative stress, insulin resistance, steatosis, fibrosis, apoptosis, altered gene expression and hepatocellular carcinoma<sup>(3)</sup>. Patients with chronic hepatitis have impaired glucose metabolism with hyperinsulinemia and insulin resistance. One explanation to the higher prevalence of DM is the presence

of HCV RNA in pancreatic  $\beta$  cells which has a direct cytopathic effect at the islet-cell level, accompanied by morphological changes and functional defects in insulin secretion<sup>(12)</sup>. Therefore, HCV infection is able to trigger autoimmune mechanism(s) against the insulin producing pancreatic beta cells leading to diabetes<sup>(10)</sup>.

Retinol-binding protein 4 (RBP4) has been identified as a novel adipokine that mainly secreted by hepatocytes (80%), but also by adipose tissue (20%). Several mechanisms link RBP4 to insulin resistance and type 2 diabetes. It was reported that serum RBP4 correlated positively with the presence of insulin resistance in individuals with

obesity, impaired glucose tolerance, or type 2 diabetes and was even increased in healthy individuals with a strong family history of diabetes <sup>(8)</sup>. On the other hand, serum RBP4 is reduced in liver cirrhosis and directly related to disease severity and liver's biosynthetic capacity <sup>(19)</sup>.

Hyperinsulinemia and glucose intolerance are present in nearly all patients with liver cirrhosis <sup>(1)</sup>, and insulin resistance is an established risk factor for disease progression and survival in patients with chronic liver disease (CLD) <sup>(15)</sup>. The aim of this study is to evaluate the role of serum retinol binding protein 4 (RBP-4) as a predictor for early detection of glucose intolerance or insulin resistance (IR) in patients with HCV infection.

#### **Patients and methods:**

The study was carried out on 60 chronic hepatitis C virus infected patients without diabetes classified according to Child-Pugh classification into 3 subgroups [Child A, Child B, Child C] (40 males and 20 females) their ages ranged between 29 and 65 years, 20 chronic HCV infected patients with diabetes mellitus (15 males and 5 females) their ages ranged between 30 and 65 years, and 20 apparently healthy control subjects (11 males and 9 females) aged 29 to 60 years. Subjects were excluded if they had hepatocellular carcinoma, associated other viral hepatitis infections, history of alcohol intake, history of antiviral therapy as Interferon, active parasitic infections as Shistosomiasis. Patients were selected from the inpatient and the outpatient clinics of the hepatology department in the National Liver Institute, Menofiya University.

All patients and control subjects underwent a 12-h overnight fast before blood tests, which included Viral (HCV-Ab) test, Liver function tests, Lipid profile test, fasting plasma glucose (FPG), fasting insulin and RBP4 levels. IR was calculated on the basis of FPG and insulin levels, according to the homeostasis model assessment (HOMA) method. The formula for the HOMA model of IR is as follows:  $(\text{HOMA-IR}) = \text{FPG (mg/dL)} \times \text{fasting insulin level (IU/mL)} / 405$ .

#### **Laboratory Investigations:**

Anti-HCV was detected using the Innostest HCV Ab which is a 4th generation enzyme immunoassay (EIA) for the detection of antibodies to hepatitis C virus in human serum or plasma (Italy), Liver function (ASAT and ALAT, GGT, total and direct bilirubin, Alb, ALP, TP), Lipid profile (Chol, TG, HDL-C) and serum FBG, were all measured using the Beckman Coulter (Synchron Cx 9 ALX) Clinical Autoanalyzer, USA.

Serum insulin and RBP-4 were measured by enzyme linked immunosorbent assay method (ELISA). Insulin ELISA kit, Monobind Inc, Lake Forest, USA was used for insulin. Serum RBP4 was measured by Human RBP4 ELISA kit, R&D systems, Inc. Minneapolis, (USA). The procedures were done according to the manufacturer instructions.

#### **Statistical Analysis:**

The data collected were tabulated and analyzed by SPSS (statistical package for the social science software) 11.0 statistical package. All results were expressed as mean and standard deviation (X+SD). ANOVA test for analysis of variance (f-test) was used for comparison of more than two groups of normally distributed variables; and krauskal Wallis test was used for comparison of more than two groups of none normally distributed variables. Correlations between parameters were assessed by using Pearson's correlation analysis.

#### **Results:**

##### **1. Patient clinical data compared with controls (Figure 1):**

##### **Serum RBP4 levels in cirrhosis are decreased due to reduced hepatic production**

Serum RBP-4 levels were significantly lower in HCV infected patients and HCV diabetic patients compared with controls (Figure 2) and decreased significantly in HCV patients compared with HCV diabetic patients ( $p < 0.05$ ). In addition, serum levels of RBP-4 significantly decreased between all stages of cirrhosis, with the lowest level in Child C (Figure 3)

## 2. Circulating RBP4 levels in cirrhosis are not correlated with insulin resistance

HCV diabetic patients were clearly insulin resistant (HOMA-IR) compared with controls and HCV patients (Figure 4). HOMA-IR not significantly higher in HCV infected patients compared to control.

HOMA-IR has been calculated to assess insulin resistance in patients with liver cirrhosis. In patients with Child A-C cirrhosis, plasma RBP4 did not correlate with plasma glucose, plasma insulin. In addition, no association between circulating RBP4 levels HOMA-IR ( $P > 0.05$ ).

## 3. Correlations of RBP4 with metabolic factors

RBP-4 levels correlate positively with GGT ( $P < 0.01$ ), CHOL ( $P < 0.05$ ) and TG ( $P < 0.05$ ) in HCV Child C (Table 1), additionally, it shows significant positive correlation with TG ( $P < 0.05$ ) in HCV Child B. On the other hand, RBP-4 showed no correlation with insulin and HOMA-IR.

### Discussion:

In this study, serum RBP-4 was highly significant decreased in HCV and HCV diabetic groups compared to controls due to decrease hepatic RBP-4 production. In addition, our study showed that serum RBP-4 was highly significant decreased in HCV child B and C compared to HCV child A subgroups. The decrease in RBP4 was directly related to the stage of liver cirrhosis, as defined by the Child-Pugh score. Our finding showed that RBP4 significantly decreased between all stages of cirrhosis, with the lowest level in Child C cirrhosis. *Yagmur et al* <sup>(19)</sup>., demonstrated that serum RBP4 is linked to hepatic function and hepatic RBP4 mRNA expression, 3.5-fold lower in experimental cirrhosis compared with normal liver tissue.

*Huang et al* <sup>(4)</sup> ., reported that, in contrast with the parallel increment of IR dependent on histological grading and staging, RBP4 level was inversely correlated with both hepatic necroinflammatory activity and fibrosis stage.

This study showed that no correlation between RBP-4 and HOMA-IR and insulin in HCV infected patients with or without diabetes. It was reported that RBP4 levels in CHC patients are not associated with insulin resistance because of the negative correlation between RBP4 and disease severity <sup>(4)</sup>.

*Kwon et al* <sup>(7)</sup>., reported that serum RBP4 level was a distinguishing factor at the early stage of chronic liver disease CLD between chronic hepatitis and Child A cirrhosis and was correlated with histological fibrosis score and several biochemical factors. Also the author showed RBP4 as serologic marker for disease severity in patients with CLD. Due to that RBP-4 can be used as a useful early marker of CLD and of the relative success of antiviral therapy.

In this study, the mean serum blood glucose, insulin and HOMA IR was higher in chronic HCV patients compared to controls. Our study indicated that HOMA-IR in CHC patients with DM was significantly higher than in those without diabetes. *Maeno et al* <sup>(11)</sup>., showed that insulin resistance was elevated among HCV-infected patients compared with control group, which may be due to decreased liver carbohydrate metabolism and hypersecretion of insulin-resistant cytokines.

The present study showed that, the mean of HOMA-IR was increased (not significantly) in HCV child (B and C) subgroups than HCV child (A) subgroup this may match *Huang et al* <sup>(4)</sup>., who observed a significant linear increase in HOMA-IR depending on liver fibrosis state (histological grade) from F0 to F4. Insulin resistance increased with the development of glucose intolerance and with the development of liver fibrosis <sup>(9)</sup>.

*Moucari et al* <sup>(14)</sup>., reported that IR is a specific feature of HCV genotypes 1 and 4 even in non-diabetic patients and is associated with significant fibrosis that is independent from steatosis. Hyperinsulinemia, in HCV patients, due to diminished hepatic insulin degradation rate can lead to a false increase of the HOMA-IR<sup>(17)</sup> Furthermore, HCV infection by itself can lead to IR as HCV core protein induces hepatic steatosis and

interferes with the insulin-signaling pathways<sup>(2)</sup>.

**Petit *et al*** <sup>(16)</sup>, demonstrated that insulin resistance in nondiabetic HCV-infected patients correlates with the staging of liver fibrosis but may occur at an early stage in the course of HCV infection, even in nondiabetic patients. A possible mechanism is that HCV core-induced suppressor of cytokine signalling 3 (SOCS3) which promotes proteosomal degradation of insulin receptor substrates (IRS1 and IRS2), leading to insulin resistance <sup>(10)</sup>.

Interestingly, the study was also found that there was an association between serum RBP4 and some components of metabolic syndrome as GGT, Triglycerides and total Cholesterol. Serum RBP4 level was positively correlated with GGT, Triglycerides and total Cholesterol. **Seo *et al*** <sup>(18)</sup>, reported positive correlation between RBP4 and GGT, LDL-C, Triglycerides and total Cholesterol in patients having nonalcoholic fatty liver disease.

Our findings indicate that RBP4 may not be a clinically useful marker for indicating insulin resistance in the presence of liver dysfunction. This result matched with **Yagmur *et al*** <sup>(19)</sup> and **Huang *et al*** <sup>(4)</sup>.

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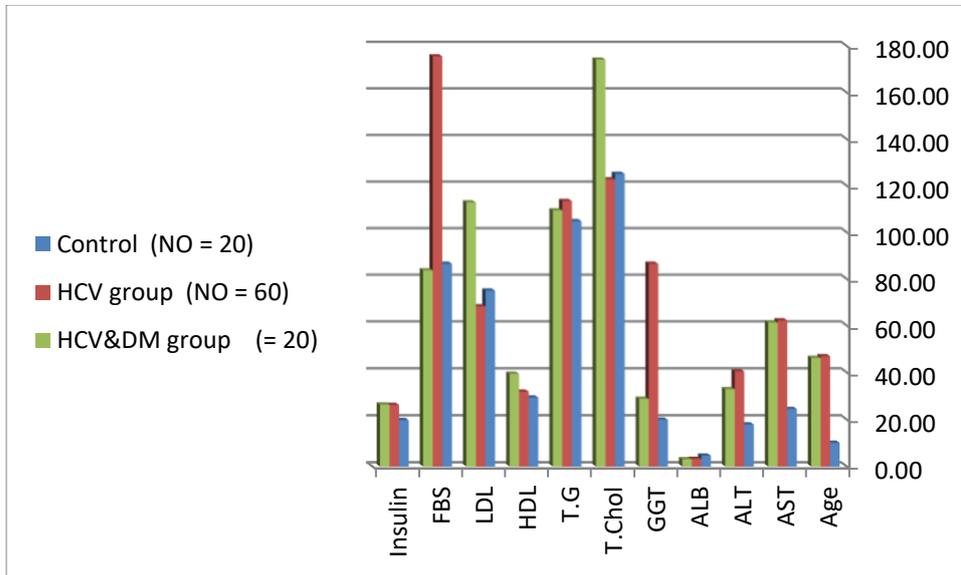


Figure (1) illustrates clinical data and laboratory parameter between studied groups

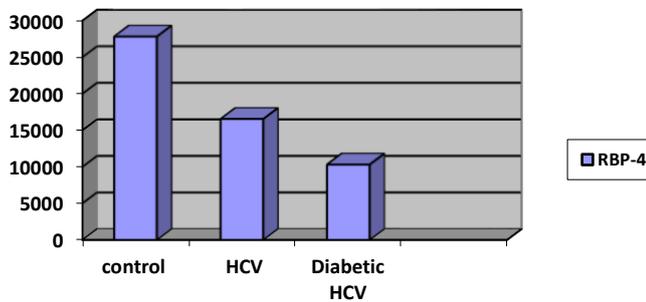


Figure (2): Shows RBP-4 levels in studied groups

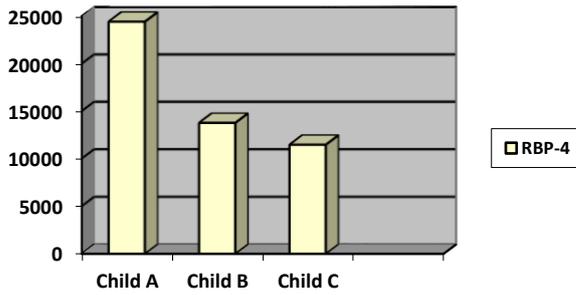


Figure (3): Illustrates RBP-4 levels between HCV studied subgroups.

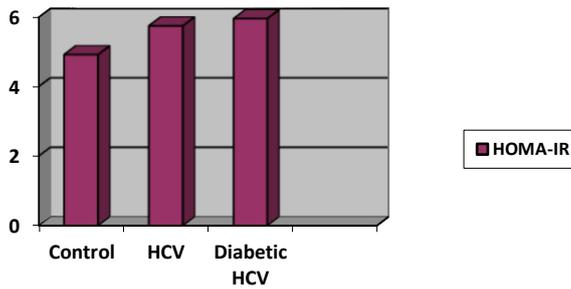


Figure (4): Illustrates HOMA-IR between studied groups

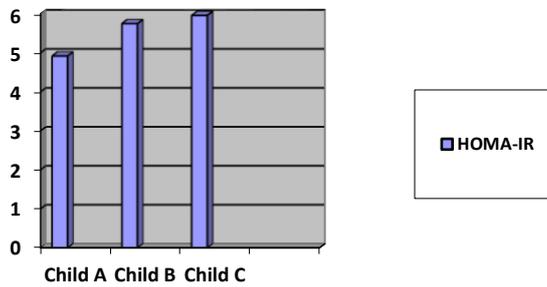


Figure (5): Shows HOMA-IR between HCV studied subgroups.

Table (1): Pearson correlation between RBP-4 & other parameters among HCV subgroups:

Parameter	(RBP-4)	
	Child B	Child C
<b>GGT</b>	> 0.05	< 0.01
<b>CHOL</b>	> 0.05	< 0.05
<b>TG</b>	< 0.05	< 0.05
<b>Insulin</b>	> 0.05	> 0.05
<b>HOMA-IR</b>	> 0.05	> 0.05