Low Serum Adiponectin Correlates with Liver Fibrosis in Patients with Chronic Hepatitis C Infection

Nashwa Noreldin[1] and Mohamed M. Shareef [2]

[1]Department of Internal Medicine, College of Medicine, University of Tanta, Tanta, Egypt
[2] Department of pathology, College of Medicine, University of Tanta, Tanta, Egypt
nashwanor@hotmail.com

Abstract: Background/Aims: Chronic hepatitis C (CHC) virus infection was shown to be frequently associated with insulin resistance (IR). Adiponectin (AD), the adipocyte derived hormone, possesses insulin-sensitizing properties. Metabolic syndromes such as obesity and DM represent a risk factor for having IR and may co-exist in those patients. CHC patients with IR have higher rates of progression to liver fibrosis. Little is known about the role of adiponectin in CHC virus infection. The aim of our study was to find a relationship between serum adiponectin level and insulin resistance as well as different grades of steatosis and fibrosis in non obese non diabetic patients.
Methods: Thirty CHC patients (group H) were compared versus 15 controls (group C) regarding alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, prothrombin activity, serum cholesterol (Ch), and high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) triglycerides (TG), fasting blood glucose (FBG), fasting serum insulin level, IR calculated as Homeostasis model assessment (HOMA-IR) and serum AD. Liver biopsy was taken in CHC patients for grading of steatosis and fibrosis**. Results:** ALT, AST and bilirubin were significantly higher while albumin was significantly lower in group H compared to group C. There were significant increase in serum insulin level and HOMA-IR and significant decrease in serum AD in group H compared to group C. In group C, as well as group H, there was significantly increased AD level in females compared to males (P=0.00). Inverse correlation was shown between serum AD and each of HOMA-IR., steatosis and fibrosis while IR was directly correlated with each of steatosis and fibrosis while direct correlation was found between steatosis and fibrosis. **Conclusion:** Serum AD level was decreased while IR was increased with negative correlation in CHC, non-obese, non-diabetic patients. Steatosis and fibrosis stages were shown to be directly correlated with each other and with IR while inversely correlated with AD.
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1. Introduction

CHC virus infection may induce IR, regardless of the presence of liver cirrhosis. CHC patients with IR have higher rates of progression to liver fibrosis[1] and are less likely to respond to antiviral therapy [2].Liver steatosis due to disturbances in lipid metabolism, was found to be characteristic of CHC patients and tends to hasten liver fibrosis[3]. AD seems to play a pivotal role in the pathogenesis of CHC virus infection as it is a hepatic insulin sensitizer and has an anti-steatotic effect on the liver [4]. Metabolic syndromes such as obesity and DM represent a risk factor for having IR and may co-exist in those patients. The aim of the present work was to study the relationship between AD and IR and between them and different grades of liver steatosis and fibrosis in CHC virus infection in non-obese non diabetic patients.

2. Methods

In this study, and after approval of the local ethical committee and obtaining a written informed consent, we investigated 30 patients presented to the outpatient clinic of Tanta University Hospital with a confirmed diagnosis of CHC virus infection defined by positive polymerase chain reaction (PCR) and the presence of anti-CHC virus antibody. Thirty patients with CHC (group H) were compared with 15 healthy controls (group C). Exclusion criteria included obesity, defined as body mass index (BMI) >35, DM, other liver diseases that included, but not limited to, hepatitis B virus infection and hepatocellular carcinoma, history of cardio vascular disorders, chronic renal diseases, human immunodeficiency virus, history of alcoholism, drug abuse or intake of lipid-lowering medications.

Full history taking, clinical examination, abdominal ultrasonography, ECG and chest X ray were done for all candidates before enrollment into the study. Laboratory investigations included ALT, AST, total bilirubin, albumin, prothrombin activity, Ch, and HDL-C, LDL-C, TG, FBG, fasting serum insulin level, HOMA-IR and serum AD. Serum AD level was determined by a commercial ELISA assay (Human Adiponectin ELISA Kit, B-Bridge International, Inc., Mountain View, CA, USA). Serum insulin level was

measured by a specific immunoassay (Axsym; Abbott Laboratories, Rungis, France). IR was assessed by using the HOMA Method as follows: fasting insulin (mU/l)— fasting plasma glucose (mmol/l)/22.5.

Liver biopsy was taken from group H patients and specimens were fixed in 10% buffered formalin, paraffin-embedded and stained with haematoxylin–eosin– safran and sirius red. Steatosis was graded as follows: mild (grade 1), 10–30% of hepatocytes affected; moderate (grade 2), 30–60%; severe (grade 3), more than 60% of hepatocytes affected. Liver biopsy was scored according to presence of fibrosis was graded as follows: 0, absent; 1, perisinusoidal/ pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis.

Sample size was calculated according to a pilot study done on 5 controls and 5 CHC patients, who were not included later in the study. A sample size to detect a change in serum AD and IR in CHC patients compared to controls was calculated to be 13, 7 cases respectively with a 90% chance to detect a significant difference between the two groups at the 5% level of significance. To estimate the Pearson's correlation coefficient between IR and AD in group H, a sample size of 26 patients was required to achieve 90% power using a 2-sided test at a significance level of 0.05. To compensate for dropout cases, 30 cases were studied in group H and 15 in group C.

Data were analyzed using SPSS, version 16 (IBM, Somers, NY, USA). Data are expressed as mean ± SD. Unpaired t test was used to compare normally distributed and continuous data. Correlation analysis between nonparametric variables was calculated with Pearson's correlation test. To assess the significance between the obtained point biserial correlation coefficient (rpb) rank correlations was used. A P value <0.05 was considered significant.

3. Results

Thirty eligible CHC patients and 15 control subjects were included in our study. No patient was excluded for any reason. Age, gender and BMI were comparable in both groups (Table 1). Serum insulin level, HOMA-IR, ALT, AST and total bilirubin were significantly increased while albumin and AD were significantly decreased in group H compared to group C. No significant differences were detected between groups regarding prothrombin activity, Ch, HDL-C, LDL-C, triglycerides and FBG (Table 2). Comparison of AD level between female and male of the studied groups showed that it was significantly increased in the females of both groups compared to males (Table 3).

There was significant negative correlation between serum AD and HOMA-IR., steatosis and fibrosis. IR was significantly positively correlated with each of steatosis and fibrosis. Also, steatosis was significantly in direct relation to fibrosis (Table 4, Fig. 1).

Table 1. Demographic characteristics

Variable	Group C (n=15)	Group H (n=30)	*P*-value
Age (years) (Range, mean ± SD)	29.00-52.00 41.20±6.70	33.00-54.00 41.60±6.41	0.85
Sex M/F Number,/%	10(66.67%)/5(33.33%)	20(66.67%)/10(33.33%)	0.73
BMI (kg/m2) (Range, mean ± SD)	18.00-24.50 21.21±2.32	17.90-25.00 21.12±2.44	0.91

Table 2. Laboratory data in both groups

Parameter	Group C (range, Mean±SD)	Group H (range, Mean±SD)	*P*-value
ALT U/L	25 - 44, 35.27 ± 6.09	42-87, 64.75±14.33	0.00*
AST U/L	29 – 55, 35.93 ± 6.73	43 – 82, 64.63±12.23	0.00*
Bilirubin mg|dl	0.50 – 1.1, 0.73±0.19	0.80 – 1.50, 1.13±1.5	0.00*
Albumin g|dl	3.9-5.1, 4.47±0.40	3.52 – 4.40, 3.86±0.19	0.00*
prothrombin activity	80.00 – 99.00, 92.73±5.90	73.00 – 98.00, 89.80±6.28	0.14
serum cholesterol mg\dl	110.00 –160.00, 136.27±12.11	114.00 – 162.00, 138.77±9.62	0.46
HDL-C mg\dl	40.00 – 82.00, 57.80±11.96	40.00 – 84.00, 63.63±13.37	0.16
LDL-C mg\dl	40.00 -85.00, 60.27±16.06	40 -82, 58.10±12.94	0.63
serum triglycerides mg\dl	66.00- 140.00, 96.87±22.58	68.00- 143.00, 96.43±22.52	0.95
glucose mg/dl	69.0 –102.00, 89.33±10.61	69.00 – 104.00, 86.30±11.54	0.40
Insulin MIU/ml	5.20 – 9.70, 7.37±1.57	11.50 – 22.50, 15.29±3.02	0.00*
HOMA IR	1.05 – 2.30, 1.59±0.43	2.20 – 5.60, 3.29±1.00	0.00*
AD (µg/ml)	16.50 – 29.00, 22.46±4.29	7.50 – 18.40, 12.38±3.01	0.00*

Data are mean ± SD. * = significant

Table 3. Male and female comparison of AD level between of the studied groups

AD (µg/ml)	Female			Male			*P*-value
Group C	26.680	±	2.061	20.350	±	3.458	0.001*
Group H	15.260	±	1.658	10.935	±	2.437	0.000*

Data are mean ± SD. * = significant

Table 4. Correlation between different parameters

Factors	*r*	*P P*-value
AD (ug/ml)/ HOMA IR	-0.842	<0.001*
AD (ug/ml)/ Steatosis	0.840	0.000*
AD (ug/ml)/ Fibrosis	0.818	0.000*
HOMA IR/ Steatosis	-0.826	0.000*
HOMA IR/ Fibrosis	-0.914	0.000*
Steatosis/ Fibrosis	0.735	0.000*

* = *significant*

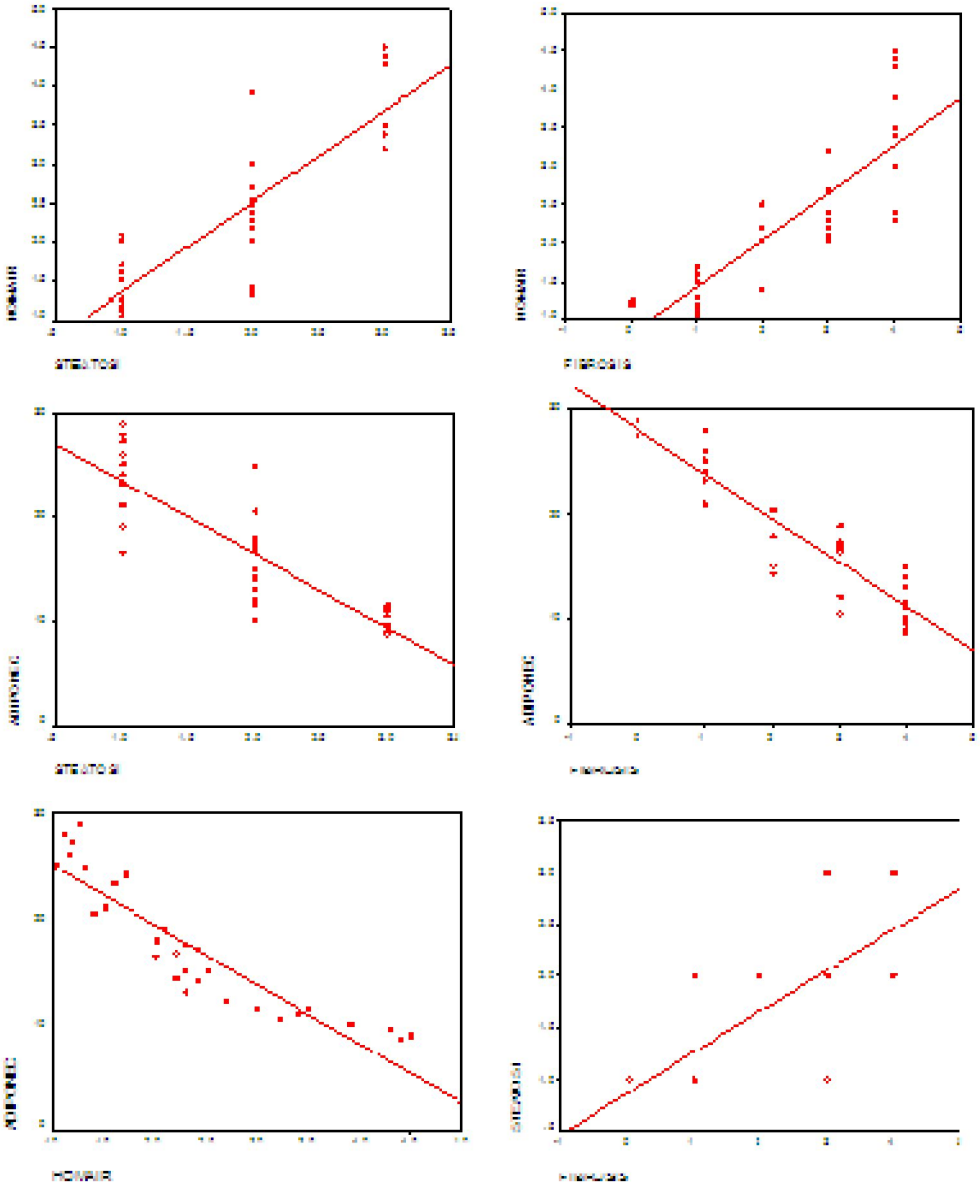

Figure 1. Correlation between AD, HOMA IR, steatosis and fibrosis

4. Discussion

In this study, CHC virus infection was associated with significantly increased IR and decreased serum AD. Serum AD inversely correlated with IR and grades of steatosis and fibrosis while IR directly correlated with each them. Furthermore, steatosis we found to be directly correlated with fibrosis.

Our results agreed with those who stated that IR has been associated with CHC virus infection independently of metabolic factors and severity of liver disease[5]. Mechanisms underlying the development of IR in patients with CHC virus infection have not been fully understood, however, the following factors have been raised: change in insulin signaling pathways due to viral proteins; [6-8] overproduction of tumor necrosis factor and consequent phosphorylation of ISR-1 serine residues;[9,10] lower expression of genes related to glucose metabolism, and degradation of insulin receptor substrates. [11]

AD is an insulin-sensitizing adipocytokine mainly secreted by adipose tissue and acts on hepatocytes improving the insulin-induced inhibition of hepatic glucose production[12] and has a central role in glucose and lipid metabolism with antidiabetic and antiatherogenic activities. Previous studies with regard to serum AD level in chronic CHC virus infection, showed an apparent discrepancy as some[13-16] found that chronic CHC patients have reduced circulating AD levels than healthy controls while others[17,18] pointed to increased serum AD in patients with CHC infection. This discrepancy might be due to the effect of gender, metabolic syndromes such as obesity and DM and different virus genotypes. In addition, circulating AD concentrations may also be affected by renal clearance, as AD levels are elevated in states characterized by impaired renal function, such as macroalbuminuria. [19] Moreover, **Korah et al** [20]denoted that serum AD levels were elevated in those patients with hepatic fibrosis, and that hypoadiponectinemia is a good predictor of hepatic steatosis in HCV patients. Also, **Derbala et al**[18] found that, the elevation in plasma AD levels in patients with liver cirrhosis is proportionate to the severity of disease. Our results also was also in accordance with Fartoux et al who stated that steatosis in CHC virus infection has been repetitively associated with increased fibrosis[21]. A protective role of AD against hepatic fibrosis has been demonstrated in AD knock-out mice where they showed an increased development of carbon tetrachloride-induced liver fibrosis that was reverted by AD overexpression.[22,23]

Our study demonstrated significantly higher ALT, AST, Bilirubin , serum insulin levels and significantly lower albumin level in group H compared to group C while comparable blood lipid profile and serum glucose Likewise, previous repots showed that IR as well as obesity and low HDL levels occurring in CHC patients were associated with hypoadiponectinemia.[14,15,24] Also, on the other hand, it was suggested that in the absence of metabolic disorders such as obesity, DM and hepatosteatosis disorders, CHC virus infection alone does not affect insulin sensitivity and AD concentration and that IR, AD are potentially modifiable risk factors. [25,26] Future studies are needed to assess whether normalization of those risk factors could decelerate liver fibrogenesis and cirrhosis.

Our results showed, as previous studies, [24,27] that serum AD levels were characterized by gender difference both in chronic HCV-infected patients and in healthy controls with males showing significantly lower AD levels than females. However, the ratio of females to males in each group was equal (33.33%) and there was no significant differences between both females and males in both groups and, hence, the effect of gender differences is not likely to affect our data.

This work is not without limitation. Statistical correlation can be misleading. We should remember to think beyond the numerical association between two variables, and not to infer causality too easily. A spurious correlation can arise from the presence of lurking variable rather than direct causation. Yet, in our study, we tried to control other possible confounding factors by excluding patients with DM and obesity

Conclusion:

CHC patients devoid of metabolic disorders had decreased AD and increased IR with inverse correlation observed between them. Degrees of steatosis and fibrosis were found to be directly correlated with each other and with IR while inversely correlated with AD.

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