Changes in Adipocytokines and Insulin Sensitivity during and after Antiviral Therapy for Hepatitis C Genotype 4

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Abstract

Background: Hepatitis C virus (HCV) infection, especially genotypes 1 and 4, is associated with wide metabolic disarrangements. **Aim**: To assess whether host metabolic factors influence sustained virological response (SVR) in patients with chronic hepatitis C genotype 4 (HCV-4) treated with peginterferon / ribavirin and to evaluate the impact of antiviral therapy on insulin resistance (IR) and serum levels of adipocytokines. **Methods**: Changes in levels of adiponectin, leptin, TNF-α and the homeostasis model assessment for insulin resistance (HOMA-IR) on antiviral combination in patients with HCV-4 were analyzed and effect on response was studied. **Results**: 107 patients were included (M/F 86/21; mean age 41.4±5.6 years). Neither serum adipocytokines nor HOMA-IR was correlated with viral load. SVR was achieved by 57% of patients and was associated with fibrosis score (odds ratio: 6.5; P = 0.001) and adiponectin level (odds ratio: 1.3; P = 0.01). At the end of follow-up, HOMA-IR, adiponectin, leptin and TNF-α were reduced, all these changes unrelated to predicting the outcome of treatment. At follow-up, HOMA-IR and adiponectin continued to decrease in patients with SVR, but remained unchanged significantly in patients who did not response or relapse. **Conclusions**: Serum adiponectin at baseline appears to be an independent predictor for the achievement of SVR and can be utilized as an additional predictive marker. Changes of IR and adipocytokines occur under treatment which is more evident with the resolution of HCV infection, suggesting that HCV could have a direct role in these metabolic changes.

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Key words

Hepatitis C – insulin resistance – HOMA – adiponectin – interferon.

Introduction

Chronic hepatitis C (CHC) virus infects ~ 170 million individuals worldwide, and hepatitis C genotype 4 (HCV-4) is the cause of ~ 20% of this number [1, 2]. In addition to causing liver disease, CHC infection is associated with wide metabolic disarrangements. HCV interacts with lipid metabolism leading to steatosis, causing wide adipocytokines changes and impairs glucose metabolism leading to increased prevalence of insulin resistance (IR) and type 2 diabetes [3]. This association is important, because several studies have shown that the presence of IR is associated with increased rates of fibrosis [4] and lower rates of rapid and sustained response to antiviral therapy [5]. However, the mechanisms of metabolic syndrome-induced IFN resistance are not completely understood.

To date, the action of adipocytokines in CHC is still controversial. Our recent findings show that among BMI, age, and gender matched HCV negative controls, chronic HCV-4 infected patients have higher serum leptin, adiponectin and high molecular weight (HMW) adiponectin, and these changes are independently correlated with IR [6]. We found adiponectin to be correlated with the different stages of liver injury (steatosis, inflammation and fibrosis) [7]. However, the relationship between interferon therapy and serum adipocytokines has not been investigated in detail especially in patients infected with HCV-4. The impact of these adipocytokines changes in the reported negative effect of IR and/or hyperinsulinemia by reducing the response of antiviral therapy with pegylated interferon/ribavirin (Peg-IFN/RBV), and the potential role of differential genotype effect is not completely understood. Accumulating evidence suggests that different HCV genotypes are associated with specific metabolic marker derangements. Unfortunately, HCV-4 has not been the subject of widespread research. Therefore, the aims of this study were to examine baseline

clinical, biochemical and histological factors associated with adipocytokines changes, IR and the effects of antiviral therapy during and after treatment, comparing patients with successful outcomes to those who did not respond or relapsed in genotype HCV 4 patients and usefulness as a predictor of response to treatment

Patients and methods

Eligible patients

The study assessed 107 treatment-naïve patients with compensated CHC who attended the liver unit of Minia University Hospital, Minia, Egypt and were treated with combination Peg-IFN-alfa-2a/b and weight based ribavirin during the period between February 2008 to January 2011 and completed their treatment. Eligible patients were ≥18 years of age who had CHC based on the presence of anti-HCV and detectable serum HCV-RNA for 6 months or more, in combination with a liver biopsy obtained within 12 months of enrollment with findings compatible with CHC using the Metavir score [8]. All patients were infected with HCV-4; HCV genotype was detected using either a line probe assay or reverse hybridization (InnoLipa; Innogenetics, Genetics, Gent, Belgium). Exclusion criteria included patients with diabetes mellitus diagnosed according to the American Diabetes Association classification criteria [9] and patients with other causes of liver disease including: concomitant hepatitis B virus, human immunodeficiency virus, Schistosoma co-infection, autoimmune hepatitis and patients with alcohol intake >40 g per day in the last 6 months prior to the start of antiviral treatment.

Informed consent

The study protocol was approved by the Institutional Ethics Committee of participating centers, and all patients gave informed consent to participate in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Treatment

Patients were treated either with Peg-IFN-α-2a 180 mcg/week or Peg-IFN-α-2b 1.5 mcg/kg/week plus ribavirin (1000 mg or 1200 mg/day for body weight ≤ or > 75 kg, respectively) during the study period for 48 weeks independent of the rapid virological response (RVR). Patients who showed a < 2 $\log_{10}$ drop in viral load at week 12 as compared with baseline and those in whom HCV-RNA level was positive at week 24 discontinued treatment on the basis of international guidelines. Conversely, patients who had a > 2 $\log_{10}$ drop in viral load at week 12 as compared with baseline and those whose HCV-RNA level was negative at week 24 continued the treatment schedule for a total of 48 weeks.

Early virological response (EVR) was defined as >2 $\log_{10}$ decline in serum HCV-RNA level from baseline or undetectable HCV-RNA in serum at treatment week 12. End of treatment (EOT) response was defined as undetectable serum HCV-RNA at the end of treatment. SVR was defined as undetectable serum HCV-RNA 24 weeks after the end of treatment.

Liver histology

Liver biopsy specimens not less than 15 mm in length or the presence of at least 10 complete portal tracts were required. A detailed descriptive scoring sheet was employed; necroinflammation and fibrosis were assessed using the METAVIR score [8]. Necroinflammation activity (A) was graded as A0 (absent), A1 (mild), A2 (moderate), and A3 (severe). Fibrosis stage (F) was scored as F0 (absent), F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis) and F4 (cirrhosis). Advanced hepatic fibrosis was defined as the presence of F3 or F4. Steatosis was recorded and classified as absent, minimal (0-5%), mild (5–33%), moderate (33–66%) and severe (>66%), predominant zonal distribution of fat (pericentral, periportal, azonal, diffuse), clarification/ballooning of liver cells (0, absent; 1, mild; 2, marked), perisinusoidal fibrosis (0, absent; 1, mild; 2, marked), Mallory hyaline (0, absent; 1, present) and presence of glycogenated nuclei (0, absent; 1, present) as described by Kleiner et al [10]. Lastly, patients were classified as CHC alone, CHC with steatosis but without NASH (CHC+steatosis), or CHC with NASH (CHC+NASH). The criterion for steatosis was grade 1 or higher. NASH was defined according to a score made by the addition of clarification/ballooning and perisinusoidal fibrosis semiquantitation. A score >3 was considered diagnostic for NASH. Neither steatosis nor lobular inflammation was required for the diagnosis of NASH, because both could be related to CHC alone.

Clinical and laboratory assessment

The following data were collected at the time of liver biopsy: age, gender, daily alcohol intake in the past 6 months (g/day), route of HCV transmission, body mass index (BMI), waist circumference. BMI was calculated as weight divided by the square of the height (kg/m²). Waist circumference was measured to the nearest 0.5 cm at the shortest point below the lower rib margin and the iliac crest.

On the morning of the liver biopsy, venous blood was drawn after a 12 hour overnight fast to determine the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, bilirubin, platelet count, international normalized ratio, total cholesterol, HDL cholesterol, triglycerides, glucose, insulin, C-peptide, TNF-α, leptin, and adiponectin. Serum glucose, ALT, AST, total and HDL cholesterol and triglycerides were determined by automated procedures Serum insulin and C-peptide was determined by electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, Indiana, USA). Insulin resistance was then investigated in all patients by the homeostasis model for the assessment of IR (HOMA-IR) using the standard formula: HOMA-IR = fasting insulin (uU/mL) x fasting glucose (mmol/L)/22.5. Serum TNF-α was measured using an enzyme-linked immunosorbent assay (Quantikine ELISA; R&D System, Minneapolis, MN). The leptin and adiponectin levels were measured in duplicate by

Luminex xMAP technology using a multiplex assay (Linco Research, St. Charles, MO). The mean values of duplicate measurements were used in the analyses.

Assessment of HCV-RNA load, viral kinetics

Serum HCV-RNA levels were assessed in all patients at baseline and then at weeks 4, 12, 24, 48, and 72 by use of quantitative reverse-transcription polymerase chain reaction (Abbott M2000, USA; lower limit of detection at 12 IU/ml).

Statistical analyses

Symmetrically distributed continuous variables were summarized as a mean ± standard deviation (SD). The median and interquartile ranges were used for skewed continuous variables. Categorical variables were presented as frequency and percentage. Comparisons between groups were made by using the Mann–Whitney U test or the Student *t* test for continuous variables and the χ^2 or Fisher exact probability test for categorical data. The two-tailed, paired Student's *t*-test was used to test for significance of differences between baseline and posttreatment adiponectin, leptin, TNF-α and HOMA-IR values. The changes from baseline, at 12 and at follow-up were compared in all patients and between virological responders (those with SVR and relapse) and patients with non-response using paired two-tailed, unpaired Student's *t*-test. Spearman rank correlation was used to quantify the association between continuous or ordered categorical variables. Prior to regression analyses, the skewed variables were log transformed to approximate normality. Logistic regression analysis was used to model the association between baseline adiponectin and other covariates and to determine the factors associated with sustained response. HOMA-IR was not included in the model due to its correlation with adiponectin. A probability value of $P < 0.05$ was considered statistically significant. SPSS software for Windows version 12 (SPSS Inc., Chicago, IL) was used to perform all analyses.

Results

Demographic, biochemical and metabolic characteristics of HCV-infected patients

The demographic, biochemical, metabolic and histological characteristics of 107 patients with chronic HCV are detailed in Table I. The mean age was 41.4±5.6 years (range 30-50 years), 86 patients were male and the mean BMI was 25.2±2.5 kg/m² (range 22-34 kg/m²). Fifty-two cases (48.6%) had CHC alone, 46 (42.9%) had CHC with steatosis but without NASH (CHC+steatosis), and 9 (8.4%) had CHC with NASH (CHC+NASH).

Association of serum adipocytokines with demographic, metabolic, and viral factors in the study population

Serum adiponectin at baseline showed significant negative correlations with male gender, BMI, waist/hip ratio, serum glucose, serum insulin, serum C-peptide, LDL-C and HOMA-IR), while this was positively correlated with HDL (Table II). There was no association between viral load and serum adiponectin (fig. 1A, B). By multiple linear regression analysis, factors independently associated with adiponectin levels were serum leptin, HOMA-IR and HDL-C (Table III).

Serum leptin at baseline showed a significant positive correlation with age (rs= 0.20, P=0.03), male gender (rs= - 0.49, P = 0.001), BMI (rs= 0.40, P = 0.001), waist/hip ratio (rs= 0.29, P = 0.002), serum insulin (rs= 0.59, P = 0.001), LDL-C (rs= 0.57, P = 0.001), and HOMA-IR (rs= 0.93, P= 0.0001). There was a significant negative correlation between serum adiponectin and leptin (rs= -0.39, P = 0.001).

Serum TNF-α at baseline showed a significant positive correlation with age (rs= -0.20, P = 0.02). There was no significant correlations among baseline TNF-α and leptin levels, adiponectin levels, BMI, HCV RNA levels and liver function tests.

Table I. Demographic and baseline characteristics of patients

Variable	Patient (n=107)
Mean age (years)	41.4±5.6 (30-50)
Male/ female	86 (80.4%) / 21(19.6%)
Mean BMI (kg/m²)	25.2±2.5 (22-34)
Systolic blood pressure (mmHg)	123.3±10.8 (100-140)
Diastolic blood pressure (mmHg)	79.7±5.9 (70-90)
Waist /hip ratio	0.93 ± 0.03 / (0.90-0.96)
Platelets (x 10^9/L)	213.3±42.2 (135-322)
Serum adiponectin (µg/L)	14.8±1.6 (11.5-17.5)
Albumin (g/L)	4.4±0.4
ALT (IU/L)	44.5±35.4 (10-300)
Fasting glucose (mmol/L)	4.5±0.6 (3.3-5.9)
Insulin (µU/ml)	13.7±5.1 (7-26)
C-peptide (Pmol/L)	1.5±0.9 (0.7-4.7)
HOMA-IR	2.9±1.2 (1.2-5.4)
Triglycerides (mg/dl)	62.7±21.4 (31-116)
HDL-C	42.9±3.7 (37-53)
LDL-C	112.6±8.7 (100-131)
Cholesterol (mg/dl)	151.4±33.1 (110-310)
Viral load (log_{10})	374,924.2±855,782.7 (1,249-5,600,000)
Steatosis	
CHC alone	52 (48.6%)
CHC + steatosis	46 (42.9%)
CHC + NASH	9 (8.4%)
Histopathological activity (Metavir score) F1	50 (46.7%)
F2	41 (38.3%)
F3	11 (10.3%)
F4	5 (4.7%)
METAVIR activity grade	
Mild (A1)	62 (57.9%)
Moderate (A2)	35 (32.7%)
Severe (A3)	10 (9.3%)
Leptin (ng/mL)	10.1±1.5 8.1-19.4
AST (IU/L)	48.3±33.2 (18-220)
TNF-α (pg/ml)	3.7±1.01 (2-6.4)

Table II. Association of serum adiponectin with demographic, metabolic, and viral factors in the study population

Variable	Adiponectin	
	Spearman's r	P-value
Age	-0.16	0.09
Male sex	-0.63	0.001
BMI (kg/m^2)	-0.46	0.001
Waist/Hip ratio	-0.19	0.04
Glucose (mmol/L)	-0.54	0.001
Insulin (µU/ml)	-0.74	0.001
C-peptide (Pmol/L)	-0.69	0.001
HCV RNA (log 10 IU/ml)	0.001	0.9
Cholesterol (mg/dl)	-0.15	0.08
Triglycerides (mg/dl)	-0.11	0.2
HDL (mg/dl)	0.20	0.03
LDL-C (mg/dl)	-0.67	0.001
WBCs	-0.002	0.9
Hemoglobin (g/dL)	-0.01	0.9
ALT (IU/L)	-0.12	0.3
AST (IU/L)	-0.13	0.2
Bilirubin (mg/dL)	-0.17	0.2
Albumin (g/L)	-0.13	0.1
HOMA-IR	-0.76	0.001
Leptin (ng/mL)	-0.39	0.001
TNF-α (pg/mL)	- 0.15	0.07

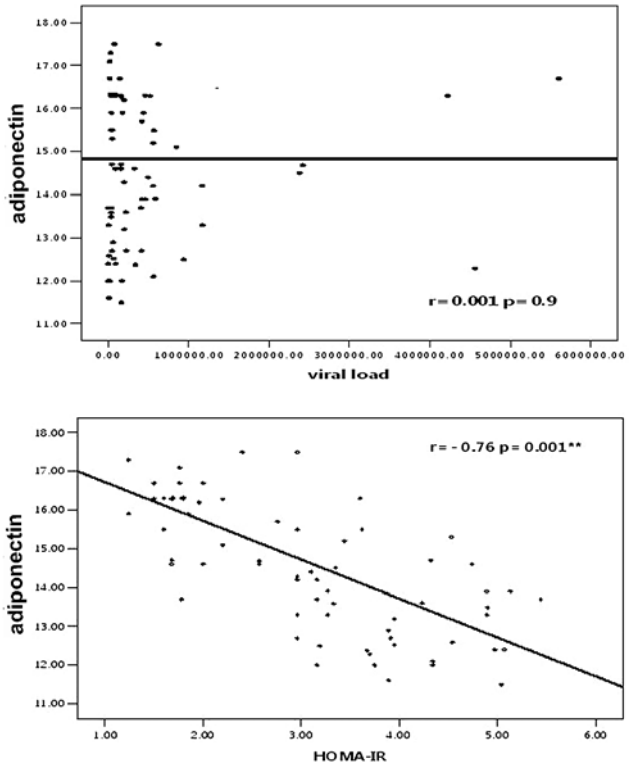

Fig 1. A) the correlation between adiponectin and viral load; B) the correlation between adiponectin and HOMA.

Table III. Multiple linear regression analysis for factors associated with serum adiponectin in patients with chronic HCV infection (n = 107)

Predictors of adiponectin levels	Regression Coefficient	SE of Regression	P-Value Coefficient
Leptin	-0.35	0.07	0.001
HOMA-IR	-0.41	0.23	0.02
HDL-C	0.19	0.024	0.004

Changes in serum adipocytokines and insulin resistance during therapy and follow up

The kinetics of adiponectin, leptin, TNF-α and HOMA-IR during IFN-α treatment and follow-up are displayed in Table IV. The mean adiponectin levels before treatment (14.8±1.6) significantly decreased during the first 12 weeks of therapy (14.4±0.4; P=0.01). Once Peg-IFN /RBV were stopped, serum adiponectin tended to increase, but did not reach baseline by 24 weeks after stopping treatment (14.5±0.2; although the mean of the difference is small it yielded quite a statistical significance P=0.05). When analyzed by the response group, the adiponectin decreased during the first 12 weeks of treatment in patients who had achieved clearance of HCV RNA at 12 weeks (from 16.3±0.8 to 15.4±0.8; P =0.001), but it remained unchanged in patients with HCV RNA positive at 12 weeks (13.2±0.8 to 13.04±0.7; P = 0.1). In nonresponders, treatment was stopped, and adiponectin remained unchanged on follow-up (13.1±0.2). In sustained responders, adiponectin continued to decrease, and the paired *t* test indicated statistical significance when comparing baseline adiponectin with the values at the end of follow-up (16.3±0.8 vs. 15.09±0.9; P =0 .0001). Conversely, in relapsers, the adiponectin did not change significantly from 12 weeks to the end of follow-up (15.3±0.1)

Table IV. Changes in serum adipocytokines (adiponectin, leptin and TNF-α) and insulin resistance during therapy and follow up

Variable	Baseline	12 weeks		72 weeks	
	Mean±SD	Mean±SD	P-value[1]	Mean±SD	P-value[1]
Adiponectin (µg/L)	14.8±1.6	14.4±04	0.01	14.5±0.2	0.05
Leptin (ng/mL)	10.1±1.5	9.5±1.6	0.005	8.1±1.5	0.0001
TNF-α (pg/ml)	3.7±1.01	3.5±0.1	0.04	2.9±1.09	0.001
HOMA-IR	2.9±1.2	2.6±0.3	0.01	1.3±0.6	0.001

[1] Changes from baseline. HOMA, homeostasis model assessment

Serum leptin and TNF-α levels before treatment were 10.1±1.5, 3.7±1.01, respectively. These values significantly decreased during the first 12 weeks of therapy (9.5±1.6; 3.5±0.1: P=0.005, 0.04 respectively), and continued to decrease by the follow up 24 weeks after stopping treatment (8.1±1,5; 2.9±1.09: P=0.001 for both). When analyzed by the response group, the leptin and TNF-α levels decreased during the first 12 weeks of treatment in patients who had achieved clearance of HCV RNA at 12 weeks (from 9.5±1.1 to 8.6±1.03; P =0.001 and from 3.5±0.6 to 2.7±0.5; P= 0.005;

respectively). Leptin and TNF- α levels showed no significant decrease in patients with HCV RNA positive at 12 weeks (11.05±1.6 to 10.93 ±1.5; P = 0.5; 3.8±0.7 to 3.7±0.6; P = 0.2, respectively). In sustained responders, leptin and TNF-α continued to decrease (9.5±1.1 vs. 8.06±0.9; P = 0.0001, 3.5±0.6 vs. 2.3±0.5, P=0.003, respectively). Conversely, in relapsers, the leptin increased from 12 weeks to the end of follow-up (8.9±1.5; P =0.08), while it TNF- α did not change significantly (2.8±0.4, P=0.5) while in nonresponders, leptin and TNF-α remained unchanged on follow-up (10.95±1.4, 3.7±0.1, p= non significant).

With regard to IR, the mean HOMA-IR levels before treatment significantly decreased during the first 12 weeks of therapy (P=0.01), and by the end of follow up 24 weeks after stopping treatment (P=0.001) (Table IV). When analyzed by the response group, the HOMA-IR index decreased during the first 12 weeks of treatment in patients who had achieved clearance of HCV RNA at 12 weeks (from 2.03±0.6 to 1.6±0.5; P =.001), but it remained unchanged in patients with HCV RNA positive at 12 weeks (4.05±0.8 to 3.95 ± 0.8; P =0.3). In nonresponders, treatment was stopped, and HOMA-IR remained unchanged on follow-up (3.77± 0.6). In sustained responders, HOMA-IR continued to decrease, and the paired t test indicated statistical significance when comparing baseline HOMA-IR with the values at the end of follow-up (2.03±0.6 vs. 1.2±0.3; P =0 .01). Conversely, in relapsers, the HOMA-IR increased from 12 weeks to the end of follow-up (2±0.4)

By 12 weeks of therapy, BMI decreased (from 25.2±2.5 to 24±2.1, p=0.0002). When analyzed by the response group, BMI decreased in both groups from 24.1±2.8 kg/m² to 23.96±2.7 kg/m² (P =0.7) in HCV RNA–negative patients and from 24.6 ± 3.1 kg/m² to 24.3± 2.6 Kg/m² (P = 0.5) in HCV RNA–positive at 12 weeks of treatment. In relapsed and SVR groups, BMI increased 24 weeks later after stopping treatment but did not reach baseline.

The odds of increase or decrease in serum adiponectin, TNF-α, leptin or HOMA-IR during or after treatment, during treatment and follow up were not significantly predicted by patients' demographic, biochemical, histological parameters, viral factors or the type of peginterferon administered. Alterations in serum adiponectin, TNF-α, leptin or HOMA were not associated with the response to antiviral therapy.

Pretreatment variables associated with SVR

Overall, 61 patients achieved an SVR (57%). We examined the baseline disease and demographic characteristics of the patients in the study according to SVR status (Table V); subjects who achieved an SVR were significantly younger, had lower HOMA-IR scores, less viral load, less fibrosis, lower leptin levels, higher HDL-C and higher adiponectin levels compared with non-SVRs. There was no association between gender, BMI, ALT value, cholesterol, portal / periportal activity, or steatosis and in relation to SVR. When the data were analyzed by multivariate logistic regression, our results suggested that fibrosis (odds ratio: 6.5; P = 0.001) and basal adiponectin level (odds ratio: 1.3; P = 0.01) were independent factors associated with SVR in genotype 4 CHC patients treated with Peg-IFN plus RBV (Table VI).

Table V. Factors associated with sustained virological response in 107 patients with hepatitis C virus genotype 4 (HCV-4): univariate analysis.

Variable	SVR (N=61)	Non SVR (N=46)	P-value
Age (years)	40.4±5.8	42.8±5.1	0.02
Sex:			
Male	52 (85.2%)	34 (73.9%)	0.2
Female	9 (14.8%)	2 (26.1%)	
Systolic BP (mmHg)	128.7±9.4	130.7±7.7	0.1
BMI (kg/m²)	24.1 ± 2.8	24.6 ± 3.1	0.2
Waist/hip ratio	0.87 ± 0.03	0.92 ± 0.04	0.1
HOMA-IR	2.03±0.6	4.05±0.8	0.001
Cholesterol (mg/dL)	144.3±29.2	150.8±16.1	0.1
Triglycerides (mg/dL)	71.3±13.7	70.2±10.1	0.5
HDL-C (mg/dL)	43.6±3.4	41.9±3.8	0.01
LDL-C (mg/dL)	113.1±7.4	118.5±6.4	0.3
WBCs	5.2±1.1	5.4±1.1	0.4
Hemoglobin (g/dL)	13.6±0.9	13.9±1.07	0.1
Platelets (x 10^9 /L)	219.8±42.6	204.8±40.7	0.06
ALT (IU/L)	137 ± 9	139 ± 11	0.2
Baseline HCV-RNA:			
> 400,000 IU/ml /	25 (40.9%)	26 (56.5%)	
≤ 400,000 IU/ml (%)	36 (59%)	20 (43.4%)	0.02
Bilirubin	0.9±0.1	0.9±0.2	0.1
Leptin (ng/mL)	9.5±1.1	11.05±1.6	0.001
Adiponectin (µg/L)	16.3±0.8	13.2±0.8	0.001
TNF-α (pg/ml)	3.5±0.6	3.8±0.7	0.09
Fibrosis: 1	43 (50.7%)	7 (15.2%)	
2	15 (24.6%)	26 (56.5%)	0.003
3	3 (4.9%)	8 (17.4%)	
4	0	5 (10.9%)	
Inflammation 1	27 (44.2%)	19 (41.3%)	0.5
2	18 (29.5%)	14 (30.4%)	
3	16 26.2%)	13 (28.2%)	
Steatosis			
No steatosis	29 (47.5%)	19 (41.3%)	
Moderate	24 (39.3%)	19 (41.3%)	0.08
Severe	8 (13.1%)	8 (17.3%)	

Table VI. Factors associated with sustained virological response (SVR) in 107 patients with hepatitis C virus genotype 4 (HCV-4): multiple logistic regression analysis.

Variable	Odds Ratio	95% CI	P
Fibrosis -METAVIR score (F1 – F2, F3 – F4)	6.5	4.9-8.8	0.001
Adiponectin*	1.3	1.1-1.5	0.01

*Serum adiponectin concentration before Peg-IFN-α treatment

Factors associated with the severity of hepatic fibrosis and predicting NASH in chronic HCV-4 infection

Factors associated with the severity of hepatic fibrosis and associated with the presence of NASH in patients with CHC, in the whole cohort of 107 HCV-4 patients were

assessed using multivariate models (Table VII). By multiple ordinal regression analysis, independent factors associated with the stage of fibrosis were HOMA-IR, portal/periportal inflammation grade and age. BMI, triglyceride, HOMA-IR and adiponectin were predictors for NASH in HCV-4 patients (Table VII).

Table VII. Multiple Ordinal Regression analysis for factors associated with hepatic fibrosis and predicting NASH in Egyptian patients with genotype 4 chronic hepatitis C (n = 107).

Variable	Odds Ratio	95% CI	P
Fibrosis			
Age	3.78	1.50–15.34	0.008
HOMA-IR	1.87	1.09–8.29	0.04
Inflammatory grade	2.71	1.03–7.27	0.04
NASH			
BMI (kg/m²)	5.08	1.35–19.11	0.01
HOMA-IR	1.22	1.03–1.52	0.05
Adiponectin (µg/ml)	1.37	1.014-1.86	0.04
Triglycerides	2.43	1.13–5.53	0.01

Discussion

To our knowledge, this is the first study that has assessed the evolution of IR and adipocytokines levels kinetic during antiviral treatment and follow up in chronic HCV-4 patients and their impact on SVR in these patients. The results of this study indicate that on combination with antiviral therapy, among the host factors, basal serum adiponectin was significantly higher in patients who achieved an SVR with therapy, and identified as a new independent predictor of SVR in HCV-4 patients. Our findings show that antiviral therapy produces significant changes in IR and adiponectin levels, leptin levels and TNF-α. At the end of follow-up, HOMA-IR and adipocytokines continued to decrease in patients with SVR, but remained unchanged in patients who did not response or relapsed. All these changes were unrelated to predicting the outcome of treatment.

Peg-IFN plus RBV is the standard treatment for chronic HCV and will likely continue to be used in combination with direct-acting antiviral agents. In this study, similarly to the large clinical trials of anti-viral therapy using Peg-IFN and ribavirin [11], SVR was achieved in 57% of patients. We confirmed our previous observation of the role of HOMA-IR and fibrosis score as predictive factors of therapy-induced viral eradication [5] and, for the first time, showed that lower serum adiponectin at baseline is a negative independent predictor of SVR. These data confirm the importance and the complexity of metabolic aspects in HCV patients, and suggest a new therapeutic target in this setting of patients. These findings are in accordance with the results of Zografos et al [12], who found that pretreatment serum adiponectin was an independent predictor of the response rate to IFN-α. But, it was in contrast to Lo Iacono et al [13] who failed to find an association between adiponectin and virological response and this may be due to the number of patients enrolled in this study, which is relatively small, and the different enrolled HCV genotype. As levels of adiponectin seemed to be associated with the treatment response, we looked for correlations with adiponectin; as expected, adiponectin correlated positively with HDL, and negatively with leptin and HOMA-IR. Although leptin levels were significantly lower in patients who attained an SVR, multivariate analysis could not identify serum leptin level as an independent predictive factor associated with SVR. These findings are in accordance with several previous reports [7, 8, 14].

The question why the mean levels of adiponectin were higher in patients who attained an SVR than in non responders, remains to be addressed. In an attempt to resolve some of these issues, we also studied the kinetics of IR and of adipocytokines during and after antiviral therapy. Importantly, in this study the degree of decrease in adipocytokines levels while patients are on therapy differ significantly by virological response at baseline, being higher in virological responders (who became HCV RNA negative by 12 weeks) than in patients with non-response (who did not). Similarly, the improvements in HOMA-IR follow the same pattern. By the end of follow up, patients who achieved an SVR with therapy maintained improvements in HOMA-IR values and decrease of the adipocytokines levels. While importantly, in relapsers' HOMA-IR values and adipocytokines tended to worsen again and returned to baseline in most patients by 24 weeks after stopping therapy. These findings are in accordance with data that showed insulin sensitivity improvement during IFN treatment restricted to patients with SVR [15, 16]. Similiarly, two papers demonstrated, respectively, leptin levels reduction during antiviral therapy [9] and adiponectin decrease during IFN treatment only in virological responders [14].

Although the underlying pathogenic mechanisms of these changes are not widely known, these findings suggest that HCV-4 replication is directly involved in inducing these wide metabolic changes and affecting insulin sensitivity, and that clearance of HCV-4 with antiviral therapy can improve insulin sensitivity and induce wide metabolic changes. Actually, previous data from our unit [6] and some other reports [17, 18] have suggested that CHC patients have higher serum adiponectin, leptin, TNF-α, HOMA-IR in comparison with BMI, age, and gender matched HCV negative controls. Importantly, these findings provide another support that these changes may be secondary to the response to viral infection and so viral eradication may affect adipocytokines levels in addition to the insulin response. An additional factor supporting this explanation is that we elucidated that HOMA-IR and adipocytokines kinetics during and after the treatment appeared not related to weight changes, indicating that sustained viral eradication has important effects on adipocytokines levels independently from other common influencing factors. Another possible explanation is that the improvement in liver disease with improvement in hepatic function and decrease in inflammation that occurs with an SVR is more directly responsible for the improvements in

insulin sensitivity and reduction of adipocytokines levels.

Although IFNs have been reported to impair glucose tolerance [19] and although some types of IFN-alfa could induce alterations in lipoprotein profile [20], in this study, the metabolic changes that occur during and after treatment were not related to the type of Peg-IFN (alfa-2a or alfa-2b) or any other viral, demographical or biochemical factors.

Conclusion

Among host factors, adiponectin is a new useful additional predictor of SVR rates to combination antiviral therapies for HCV-4 hepatitis patients. Resolution of HCV infection was associated with improvement in insulin sensitivity and wide changes of adipocytokines levels among patients with HCV-4, suggesting the magnitude of this metabolic pathway in HCV infection. It is underlined by therapy-induced modification of adipocytokines levels and by the influence on treatment outcome and provides strong support for recommending therapy in patients with CHC and insulin resistance or metabolic syndrome. The implication of these findings and their long term impact on liver disease outcome remain unknown and deserve further evaluation.

Conflicts of interest

None to declare.

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