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BRIEF ARTICLE

Significance of serum leptin and adiponectin levels in Egyptian patients with chronic hepatitis C virus associated hepatic steatosis and fibrosis

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Abstract

AIM: To study serum levels of leptin and adiponectin in patients with chronic hepatitis C virus infection genotype-4 (HCV-4) related steatosis and fibrosis.

METHODS: We prospectively studied 45 untreated men with chronic HCV-4, with proven steatosis (group Ⅰ, 30 patients), and fibrosis (group Ⅱ, 15 patients), on liver biopsy. In addition, 15 healthy men (group Ⅲ), matched for age, and body mass index were included. However, we excluded another five patients with steatohepatitis, and six patients with cirrhosis. We measured total serum leptin and adiponectin levels, as potential predictors for liver steatosis and fibrosis. Also, a correlation between these adipokines and various clinical and laboratory data were evaluated. All subjects were selected from Tropical and Internal medicine departments, Menoufiya University Hospital, Menoufiya, Egypt, during the period from February 2010 to August 2011.

RESULTS: In group Ⅰ, severity of hepatic steatosis was mild, moderate, and severe, in 19 patients (63.5%), 8 patients (26.5%), and 3 patients (10%), respectively. In contrast, in group Ⅱ, hepatic fibrosis was found to be in stage 1, 2, and 3, in 6 patients (40%), in 6 patients (40%), and in 3 patients (20%), respectively. On comparing group Ⅰ with group Ⅱ, there was a significant decrease in serum adiponectin levels (131.4 ± 7.91 pg/mL *vs* 436 ± 9.75 pg/mL, $P < 0.001$), while there was no significant difference between both groups regarding serum leptin levels (34.69 ± 7.69 ng/mL *vs* 35.17 ± 1.06 ng/mL, $P > 0.05$). However, in the same group, when compared with group Ⅲ, there was a significant increase in serum leptin levels (34.69 ± 7.69 ng/mL *vs* 10.69 ± 0.84 ng/mL, $P < 0.001$), while there was a significant decrease in serum adiponectin levels (131.4 ± 7.91 pg/mL *vs* 342.4 ± 44.48 pg/mL, $P < 0.001$). In contrast, in group Ⅱ, when compared with group Ⅲ, there was a significant increase in serum leptin and adiponectin levels (35.17 ± 1.06 ng/mL *vs* 10.69 ± 0.84 ng/mL, $P < 0.001$, and 436 ± 9.75 pg /mL *vs* 342.4 ± 44.48 pg/mL, $P < 0.05$, respectively), while there was no significant difference between both groups regarding serum creatinine (0.83 ± 0.34 *vs* 0.89 ± 0.24, $P > 0.05$). On the other hand, serum leptin was not correlated with serum adiponectin in group Ⅰ and in group Ⅱ ($r = 0.09$, $P > 0.05$, and $r = -0.1$, $P > 0.05$, respectively). However, serum adiponectin was significantly negatively correlated with serum aspartate transaminase in group Ⅰ, but no correlation detected in group Ⅱ ($r = -0.39$, $P > 0.05$, and $r = -0.03$, $P > 0.05$).

CONCLUSION: In male patients with chronic HCV-4, serum adiponectin levels are elevated in hepatic fibro-

sis, but decreased in steatosis. Therefore, in contrast to leptin, adiponectin may be used as a non-invasive marker.

Key words: Leptin; Adiponectin; Hepatitis C virus; Hepatic steatosis; Hepatic fibrosis

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INTRODUCTION

Leptin and adiponectin are the main metabolic products of adipose tissue. The former is expressed also in the stomach, placenta and mammary gland, while the latter is also secreted by hepatocytes[1,2].

Currently, there is an increasing interest in the role of these adipokines in the development of hepatic steatosis, and fibrosis, particularly in patients with nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis C virus (HCV) infection[3].

Chronic HCV genotype-4 (HCV-4) is known to be endemic in Egypt, Central Africa and in the Middle East[4]. However, several recent studies carried out in Europe have indicated changes in genotype distribution and have underlined the increasing prevalence of HCV-4[5,6]. There are controversial data about the relationship between serum leptin levels and HCV-related steatosis[7,8].

The role of leptin in hepatic fibrosis is also less clear[9]. Moreover, the levels of adiponectin in patients with different stages of liver diseases, particularly in those with NAFLD and chronic HCV (especially genotype 4) infection, have been partly unraveled[10,11].

Therefore, the aim of this study was to measure serum leptin and adiponectin levels, as potential predictors of liver steatosis and fibrosis, for use in clinical practice, in patients with chronic HCV-4 infection, associated steatosis and fibrosis. Moreover, a correlation between these adipokines and different clinical and laboratory data were evaluated.

MATERIALS AND METHODS

Patients and study design

A total of 45 (30 with hepatic steatosis, and 15 with hepatic fibrosis) untreated Egyptian male patients with chronic HCV-4, who had undergone liver biopsy, were prospectively included in this study. We excluded another five patients with steatohepatitis, and six patients with cirrhosis, on liver biopsy. Patients were selected from Tropical and Internal medicine departments, Menoufiya University Hospital, Egypt, during the period from February 2010 to August 2011.

In addition, a control group comprised 15 healthy males matched for age and body mass index (BMI), from the same hospital. They were considered healthy on the basis of history, physical examination and laboratory tests. None received any medication and had normal liver enzymes and no clinical, laboratory or imaging evidence of liver disease.

We excluded all female patients, those with chronic HCV non-genotype 4, steatohepatitis or cirrhosis on liver biopsy, other causes of chronic liver disease (hepatitis B virus infection, alcoholism, Wilson's disease, haemochromatosis, and autoimmune hepatitis), seropositivity for anti-human immunodeficiency virus, evidence of cirrhosis or hepatocellular carcinoma, decompensated liver disease (evidence of ascites, variceal bleeding, or hepatic encephalopathy), history of heart failure, diabetes mellitus, thyroid diseases, abnormal renal function, obesity (*i.e.*, BMI $\geqslant$ 30), previous treatment with metformin, a thiazolidinedione, or interferon-based antiviral therapy, and use of drugs known to induce liver steatosis (corticosteroids, amiodarone, tamoxifen, valproic acid) within the last 6 mo.

Chronic HCV was defined as a positive serological test for HCV by at least a fourth-generation enzyme-linked immunosorbent assay (ELISA), positive HCV RNA results by polymerase chain reaction assay and compatible liver biopsy[9].

Clinical and laboratory data

A complete medical history was taken and physical examination carried out in all patients and controls. BMI was calculated according to the following equation: BMI = weight (in kilograms)/height^2 (in meters). Overweight was defined as a BMI of 25-29.9 kg/m^2, and obesity was defined as a BMI $\geqslant$ 30 kg/m^2[12].

Laboratory investigations included: liver function tests [serum aspartate transaminase (AST), alanine transaminase (ALT), prothrombin activity, serum proteins and albumin, total serum bilirubin and alkaline phosphatase (ALP)] kidney function tests (serum urea and creatinine), total serum leptin and adiponectin levels.

Sample collection and assay

After gaining the consent of all subjects studied, 10 mL of venous blood was withdrawn from all subjects after fasting for at least 10 h. 1.8 mL whole blood was added to 0.2 mL sodium citrate, then centrifuged at 4000 *g* for 5 min, then plasma was used for measuring prothrombin concentration using Fibrintimer Ⅱ instrument of Behring, Germany Using Sysmex K-21, Japan.

7 mL of venous blood was transferred slowly into a plain tube, allowed to clot, and then centrifuged for ten minutes. The clear supernatant was separated in several aliquots, kept frozen at -20 ℃, until analysis of the fol-

lowing: kinetic determination of ALT and AST[13], serum total bilirubin by a timed endpoint Diazo method[14]; serum albumin, using a method of enhanced specificity of bromcresol purple for albumin[15]; serum ALP activity by a kinetic method using a 2-amino-2-methyl-1- propanol buffer[16]; serum total protein by using the modified method of Biuret reaction[17]; colorimetric kinetic determination of serum creatinine, and colorimetric determination of serum urea[18].

Leptin and adiponectin assay

Serum leptin levels were determined by a solid phase ELISA based on the sandwich principle. The microlitre wells were coated with a monoclonal antibody directed towards a unique antigenic site on the leptin molecule. An aliquot of patient sample containing endogenous leptin was incubated in the coated well with a specific rabbit anti-leptin antibody. A sandwich complex was formed. After incubation the unbound material was washed off and anti-rabbit peroxidase conjugate was added for detection of bound leptin, the substrate solution was added and the intensity of color obtained was proportional to the concentration of leptin in the patient sample. (BioSource Europe S.A. 8 B-1400 Nivelles Belgium)[19]. For leptin, the intra-assay coefficient of variation (CV) was 6.91%, while inter-assay CV was 8.66%.

Regarding serum adiponectin levels, they were estimated by Human Adiponectin ELISA kits. This assay employed an antibody specific for human adiponectin coated on a 96-well plate. Standards, samples and biotinylated anti-human adiponectin were pipetted into the wells. Then, adiponectin was captured by the antibody immobilized to the wells and by the biotinylated adiponectin-specific detection antibody. After washing away unbound biotinylated antibody, horse radish peroxidase-conjugated streptavidin was pipetted into the wells. The wells were washed again. Following this second wash step, tetramethylbenzidine substrate solution was added to the wells, resulting in color development proportional to the amount of adiponectin bound. The Stop Solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm (FIN-00790 Helsinki, Finland)[20]. For adiponectin, the intra-assay CV was > 10%, while inter-assay CV was < 12%.

The study was approved by the Ethical Committee of our hospital. All patients and control subjects gave their verbal informed consent, and consented to the use of clinical data and serum for research purposes.

Liver histological assessment

The degree of hepatic fibrosis was assessed according to the modified Knodell scoring system[21]. Steatosis was identified and graded according to the histopathological criteria described by Burnt *et al*[22]. Based on the percentage of hepatocytes containing fat droplets, steatosis was graded as mild (< 33% of hepatocytes affected), moderate (33%-66% of hepatocytes affected) and severe (> 66% of hepatocytes affected)[22].

Statistical analysis

Results were collected, tabulated and statistically analyzed using an IBM personal computer and statistical package SPSS version 16 (SPSS Inc. Chicago, Illinois, United States). Student's *t* test was used for comparison between two groups having quantitative variables. analysis of variance (*F*) test was used for comparison among three groups having quantitative variables. Pearson correlation (*r*) was used to detect association between quantitative variables. χ^2 test to compare the qualitative data between different groups. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

In group Ⅰ, severity of hepatic steatosis was mild, moderate, and severe, in 19 patients (63.5%), 8 patients (26.5%), and 3 patients (10%), respectively. In contrast, in group Ⅱ, hepatic fibrosis was found to be in stage 1, 2, and 3, in 6 patients (40%), in 6 patients (40%), and in 3 patients (20%), respectively.

Table 1 shows the clinical and laboratory data of patients and controls studied. There was no significant difference between all groups studied regarding age and BMI ($P > 0.05$). In group Ⅰ, when compared with group Ⅱ, there was a significant increase in serum AST, prothrombin activity (PT), and albumin ($P < 0.001$), while, there was a significant decrease in serum total bilirubin, and serum adiponectin ($P < 0.001$, for each). There was no significant difference, between both groups regarding serum ALT, proteins, ALP, blood urea, serum creatinine, and leptin levels ($P > 0.05$). In contrast, for group Ⅰ, when compared with group Ⅲ, there was a significant increase in serum AST, ALT, total bilirubin, ALP, blood urea, and serum leptin levels ($P < 0.001$, 0.001, 0.001, 0.001, 0.05, 0.001, respectively), while, there was a significant decrease in serum PT, proteins, and serum adiponectin ($P < 0.001$, for each). There was no significant difference, between both groups regarding serum albumin, and serum creatinine levels ($P > 0.05$). Finally, in group Ⅱ, when compared with group Ⅲ, there was a significant increase in serum AST, ALT, total bilirubin, ALP, blood urea, serum leptin and adiponectin levels ($P < 0.001$, 0.001, 0.001, 0.001, 0.05, 0.001, 0.05, respectively), while, there was a significant decrease in serum PT, proteins, and albumin ($P < 0.001$, for each). There was no significant difference, between both groups regarding serum creatinine ($P > 0.05$).

Table 2 shows the correlation between serum leptin and other parameters in the patients studied. In group Ⅰ, there was a significant negative correlation between serum leptin levels, and serum AST, ALT, albumin, ALP, and creatinine ($r = -0.78, -0.39, -0.37, -0.70, -0.38$, and $P < 0.001$, 0.05, 0.05, 0.001, 0.05, respectively). However, there was a significant positive correlation with BMI, as well as serum total bilirubin ($r = 0.43$, 0.64, and $P < 0.05$, $P < 0.001$, respectively). There was no significant correlation detected with age, PT, serum

Table 1 Clinical and laboratory data of patients studied and controls (mean ± SD)

Parameter	Group Ⅰ (*n* = 30)	Group Ⅱ (*n* = 15)	Group Ⅲ (*n* = 15)	*F* test	*P* value
Age (yr)	39.87 ± 6.1	38.20 ± 6.09	39.40 ± 4.56	0.52	*P* > 0.05 NS
BMI (kg/m^2)	29.33 ± 1.92	28.20 ± 3.05	27.73 ± 1.62	1.71	*P* > 0.05 NS
AST (U/L)	62.40 ± 23.03	39.00 ± 8.7	27.00 ± 5.35	32.63	*P*1 < 0.001[1]
					*P*2 < 0.001[1]
					*P*3 < 0.001[1]
ALT (U/L)	42.60 ± 16.31	35.33 ± 7.67	25.67 ± 5.99	16.42	*P*1 > 0.05 NS
					*P*2 < 0.001[1]
					*P*3 < 0.001[1]
PT (%)	71.87 ± 8.92	61.33 ± 19.22	89.53 ± 5.85	22.04	*P*1 < 0.001[1]
					*P*2 < 0.001[1]
					*P*3 < 0.001[1]
Protein (gm/dL)	5.40 ± 1.03	5.00 ± 0.85	7.13 ± 0.88	5.59	*P*1 > 0.05 NS
					*P*2 < 0.001[1]
					*P*3 < 0.001[1]
Albumin (gm/dL)	2.46 ± 0.47	2.37 ± 0.24	4.35 ± 0.28	33.14	*P*1 < 0.001[1]
					*P*2 > 0.05 NS
					*P*3 < 0.001[1]
Total bilirubin (mg/dL)	1.30 ± 0.92	1.90 ± 0.97	0.53 ± 0.35	20.85	*P*1 < 0.001
					*P*2 < 0.001
					*P*3 < 0.001
ALP (U/L)	7.21 ± 3.99	4.30 ± 1.08	2.4 ± 0.82	24.97	*P*1 > 0.05 NS
					*P*2 < 0.001[1]
					*P*3 < 0.001
Urea (mg/dL)	28.2 0± 8.49	28.33 ± 6.45	23.27 ± 4.86	2.65	*P*1 > 0.05 NS
					*P*2 < 0.05[1]
					*P*3 < 0.05[1]
Creatinine (mg/dL)	1.10 ± 0.36	0.83 ± 0.34	0.89 ± 0.24	3.77	*P*1 > 0.05 NS
					*P*2 > 0.05 NS
					*P*3 > 0.05 NS
Leptin (ng/mL)	34.69 ± 7.69	35.17 ± 1.06	10.69 ± 0.84	107.28	*P*1 > 0.05 NS
					*P*2 < 0.001[1]
					*P*3 < 0.001[1]
Adiponectin (pg/mL)	131.40 ± 7.91	436.00 ± 9.75	342.40 ± 44.48	374.77	*P*1 < 0.001[1]
					*P*2 < 0.001[1]
					*P*3 < 0.05[1]

[1]Significant. Group Ⅰ: Patients with hepatic steatosis; Group Ⅱ: Patients with hepatic fibrosis; Group Ⅲ: Controls. *P*1: Comparison between group Ⅰ and group Ⅱ; *P*2: Comparison between group I and group Ⅲ; *P*3: Comparison between group Ⅱ and group Ⅲ. NS: Non significant; BMI: Body mass index; AST: Aspartate transaminase; ALT: Alanine transaminase; PT: Prothrombin activity; ALP: Alkaline phosphatase.

proteins, blood urea, and serum adiponectin (r = -0.24, 0.11, -0.28, -0.25, 0.09, and $P > 0.05$ for each). In group Ⅱ, there was a significant negative correlation between serum leptin levels, and age, serum AST, albumin, and ALP (r = -0.77, -0.99, -0.80, and -0.95, and $P < 0.001$ for each, respectively). However, there was a significant positive correlation with BMI, PT, serum total bilirubin, blood urea, and serum creatinine (r = 0.94, 0.93, 0.98, 0.95, 0.97, and $P < 0.001$ for each). There was no significant correlation detected with ALT, serum proteins, and adiponectin (r = -0.51, -0.93, -0.1 and $P > 0.05$ for each).

Table 3 shows the correlation between serum adiponectin and other parameters in the patients studied. In group Ⅰ, there was a significant negative correlation between serum adiponectin levels, and AST (r = -0.39, and $P < 0.05$). However, there was no significant correlation with all other parameters studied ($P > 0.05$, for each). In group Ⅱ, there was a significant negative correlation between serum adiponectin levels, and age (r = -0.55, and $P < 0.05$). However, there was a significant positive correlation with serum ALT, proteins, and albumin (r = 0.91, 0.95, 0.68 and $P < 0.001$ for each). There was no significant correlation detected with BMI, serum AST, PT, total bilirubin, ALP, blood urea, and serum creatinine (r = -0.45, 0.03, -0.46, 0.09, 0.41, -0.39, -0.35 and $P > 0.05$ for each).

DISCUSSION

The behavior of leptin concentrations in the course of liver disease due to HCV infection is still under investigation[23].

The relationship between leptin and hepatic fibrosis is controversial. Studies *in vitro* have clearly demonstrated a role in profibrogenic responses within the liver[24,25]. However, human studies describing the role of leptin in fibrosis are less convincing[26-28].

We found that serum leptin levels were significantly associated with fibrosis in patients with chronic HCV-4 infection. This is in agreement with many previous studies[23,29,30]. In contrast, several other studies have shown

Table 2 Correlation between serum leptin and other parameters in patients studied

Parameter	Group Ⅰ (n = 30)		Group Ⅱ (n = 15)	
	r	P value	r	P value
Age (yr)	-0.24	> 0.05 NS	-0.77	< 0.001[1]
BMI (kg/m^2)	0.43	< 0.05[1]	0.94	< 0.001[1]
AST (U/L)	-0.78	< 0.001[1]	-0.99	< 0.001[1]
ALT (U/L)	-0.39	< 0.05[1]	-0.51	> 0.05 NS
PT (%)	0.11	> 0.05 NS	0.93	< 0.001[1]
Protein (gm/dL)	-0.28	> 0.05 NS	-0.39	> 0.05 NS
Albumin (gm/dL)	-0.37	< 0.05[1]	-0.80	< 0.001[1]
Total bilirubin (mg/dL)	0.64	< 0.001[1]	0.98	< 0.001[1]
ALP (U/L)	-0.70	< 0.001[1]	-0.95	< 0.001[1]
Urea (mg/dL)	-0.25	> 0.05 NS	0.95	< 0.001[1]
Creatinine (mg/dL)	-0.38	< 0.05[1]	0.97	< 0.001[1]
Adiponectin (pg/mL)	0.09	> 0.05 NS	-0.10	> 0.05 NS

[1]Significant. Group Ⅰ: Patients with hepatic steatosis; Group Ⅱ: Patients with hepatic fibrosis. NS: Non significant; BMI: Body mass index; AST: Aspartate transaminase; ALT: Alanine aminotransferase; PT: Prothrombin activity; ALP: Alkaline phosphatase.

Table 3 Correlation between serum adiponectin and other parameters in patients studied

Parameter	Group Ⅰ (n = 30)		Group Ⅱ (n = 15)	
	r	P value	r	P value
Age (yr)	-0.09	> 0.05 NS	-0.55	< 0.05[1]
BMI (kg/m^2)	-0.24	> 0.05 NS	-0.45	> 0.05 NS
AST (U/L)	-0.39	< 0.05[1]	0.03	> 0.05 NS
ALT (U/L)	-0.15	> 0.05 NS	0.91	< 0.001[1]
PT (%)	-0.35	> 0.05 NS	-0.46	> 0.05 NS
Protein (gm/dL)	-0.09	> 0.05 NS	0.95	< 0.001[1]
Albumin (gm/dL)	-0.17	> 0.05 NS	0.68	< 0.001[1]
T.Bilirubin (mg/dL)	-0.16	> 0.05 NS	0.09	> 0.05 NS
ALP (U/L)	0.04	> 0.05 NS	0.41	> 0.05 NS
Urea (mg/dL)	-0.08	> 0.05 NS	-0.39	> 0.05 NS
Creatinine (mg/dL)	-0.10	> 0.05 NS	-0.35	> 0.05 NS

[1]Significant. Group Ⅰ: Patients with hepatic steatosis; Group Ⅱ: Patients with hepatic fibrosis. NS: Non significant; BMI: Body mass index; AST: Aspartate transaminase; ALT: Alanine aminotransferase; PT: Prothrombin activity; ALP: Alkaline phosphatase.

no association between serum leptin and fibrosis in HCV infection[31-33]. The reason for this discrepancy is not clear. It is possible that leptin levels intrahepatically, rather than in the serum, are more important determinants of hepatic fibrosis[9].

Meanwhile, we found that serum leptin was increased in patients with steatosis. In line with our work, patients with NAFLD[8,26], and alcoholic liver disease have increased levels of circulating leptin in their bodies[34].

In the present study, we observed that serum leptin levels correlated with BMI in our overweight HCV-4 patients. This is in accordance with Myers RP, study[9].

Hepatic steatosis is defined as excessive lipid accumulation within the hepatocyte cytoplasm. The prevalence of steatosis among different HCV genotypes is quite variable[35].

In the current study, hepatic steatosis was detected in about half of our patients with chronic HCV-4 associated steatosis, with predominance of mild steatosis in 63.5% of them. These findings are in accordance with other, similar, studies conducted by El-Zayadi *et al*[36], and Tsochatzis *et al*[37]. The mechanisms underlying the development of parenchymal steatosis in HCV infection are not precisely known[38].

Regarding serum adiponectin, we found that it was significantly elevated in patients with HCV-4 associated hepatic fibrosis versus controls. This coincides with other studies[39,40].

In contrast, the results of our study indicate that serum adiponectin was decreased with the presence of steatosis in patients with chronic HCV-4 infection. This is in accordance with other studies conducted in patients with HCV-4[41,42] and HCV of different genotypes[43]. However, Tiftikci *et al*[40], found an increase in serum adiponectin levels in patients (about 57% were females) with HCV (mostly with genotype 1). Also, Kara *et al*[44], found that serum adiponectin levels in HCV genotype 1 were similar to healthy control subjects. This difference might be due to the effect of gender and different HCV genotypes. In addition, circulating adiponectin concentrations may also be affected by renal clearance, as adiponectin levels are elevated in states characterized

by impaired renal function, such as macroalbuminuria[45].

We include only male patients in our study for two reasons: firstly, it is known that serum leptin and adiponectin levels are higher in females than males[27,46]; secondly, to avoid the confirmed negative role of menopause on steatosis, and the potential benefit of hormone replacement therapy on hepatic fibrosis in HCV patients[47].

In conclusion, serum leptin levels were elevated in male patients with both HCV-4 related hepatic steatosis and fibrosis, so it has a poor predictive value for either alone. In contrast, serum adiponectin levels were elevated in those patients with hepatic fibrosis, but decreased in hepatic steatosis, therefore, hypoadiponectinemia is a good predictor of hepatic steatosis in those patients.

Identification of individuals with hepatic fibrosis in chronic HCV-4 may be important for a number of reasons. Firstly, it can decrease the need for liver biopsy in those patients. Secondly, pharmacological treatments are currently being evaluated in NAFLD, but if successful agents are found it will be important to have identified a target population that can potentially be treated. Finally, once fibrosis is identified it may increase the imperative for patients to implement major lifestyle changes and clinicians to monitor the response to intervention[48].

On the other hand, serum adiponectin can be considered as a non-invasive marker for hepatic steatosis, and might decrease the need for liver biopsy in patients with chronic HCV-4 infection. Also, therapy to increase circulating adiponectin concentration, such as overweight reduction or thiazolidinediones, might represent a novel strategy to improve steatosis in those patients.

Limitations of the present study include the small sample size studied, which consisted of Egyptian male patients with chronic HCV-4, and thus, applicability to other populations requires further work and, the lack of data related to serum HCV load, which may not significantly impact the results. Indeed, HCV quantity is not an independent predictor of pathology[47]. Finally, we measured total serum leptin levels (which is composed of free and protein-bound components) and total serum adiponectin levels (which is composed of three forms); so, we cannot exclude the beneficial role(s) for measurement of these specific components for each one[1,49].

COMMENTS

Background

Leptin and adiponectin are the main metabolic products of adipose tissue. Recently, there is an increasing interest in the role of these adipokines in the development of hepatic steatosis and fibrosis, particularly in patients with nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis C virus (HCV) infection. Chronic HCV genotype-4 (HCV-4) is known to be endemic in Egypt, Central Africa, Middle East, and recently, increasing in Europe. The role of leptin in hepatic steatosis and fibrosis is not clear. Moreover, the levels of adiponectin in patients with different stages of liver diseases, particularly in those with NAFLD and chronic HCV (especially genotype-4) infection, has been partly unraveled. Hence, the aim of this study was to measure serum leptin and adiponectin levels, as potential predictors of liver steatosis and fibrosis, for use in clinical practice.

Research frontiers

Liver biopsy is still the standard method for evaluation of liver steatosis and fibrosis. However, it is an invasive procedure and may carry some complications. So, authors tried to discover a simple blood test to measure a substance/substances which can address liver steatosis and fibrosis.

Innovations and breakthroughs

Many noninvasive methods for the evaluation of liver fibrosis and steatosis, in patients with HCV-4, have been proposed, but none are easy nor simple methods. The present study shows that estimation of serum adiponectin , in patients may potentially be used for identification of hepatic steatosis and fibrosis.

Applications

Diagnosis of hepatic fibrosis in chronic HCV-4 is important because it can decrease the need for liver biopsy. Also, pharmacological treatments are currently being evaluated in NAFLD. Furthermore, once fibrosis is identified it may increase the imperative for patients to implement major lifestyle changes. In contrast, serum adiponectin can be considered as a non-invasive marker for hepatic steatosis, and might decrease the need for liver biopsy in patients with chronic HCV-4 infection. Also, therapy to increase circulating adiponectin concentration, such as overweight reduction or thiazolidinediones, might represent a novel strategy to improve steatosis in those patients.

Terminology

Serum leptin and adiponectin are produced mainly by adipose tissue. However, recently, it was found that they may have an important role in NAFLD and HCV.

Peer review

This study focuses on the potential use of serum adiponectin in identification of liver steatosis and fibrosis in people with HCV-4. Although this study is primary, but avoiding liver biopsy through estimation of these substances in the serum is promising. So, this study may be interesting for the readers, particularly those with HCV.

REFERENCES

1 **Chan JL**, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet* 2005; **366**: 74-85 [PMID: 15993236]

2 **Saxena NK**, Fu PP, Nagalingam A, Wang J, Handy J, Cohen C, Tighiouart M, Sharma D, Anania FA. Adiponectin modulates C-jun N-terminal kinase and mammalian target of rapamycin and inhibits hepatocellular carcinoma. *Gastroenterology* 2010; **139**: 1762-1773, 1773. e1-e5 [PMID: 20637208]

3 **Tsochatzis E**, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. *Am J Gastroenterol* 2006; **101**: 2629-2640 [PMID: 16952281]

4 **Chamberlain RW**, Adams N, Saeed AA, Simmonds P, Elliott RM. Complete nucleotide sequence of a type 4 hepatitis C virus variant, the predominant genotype in the Middle East. *J Gen Virol* 1997; **78**: 1341-1347 [PMID: 9191927]

5 **Matera G**, Lamberti A, Quirino A, Focà D, Giancotti A, Barreca GS, Guadagnino V, Liberto MC. Changes in the prevalence of hepatitis C virus (HCV) genotype 4 in Calabria, Southern Italy. *Diagn Microbiol Infect Dis* 2002; **42**: 169-173 [PMID: 11929687]

6 **van Asten L**, Verhaest I, Lamzira S, Hernandez-Aguado I, Zangerle R, Boufassa F, Rezza G, Broers B, Robertson JR, Brettle RP, McMenamin J, Prins M, Cochrane A, Simmonds P, Coutinho RA, Bruisten S. Spread of hepatitis C virus among European injection drug users infected with HIV: a phylogenetic analysis. *J Infect Dis* 2004; **189**: 292-302 [PMID: 14722895]

7 **Giannini E**, Ceppa P, Botta F, Mastracci L, Romagnoli P, Comino I, Pasini A, Risso D, Lantieri PB, Icardi G, Barreca T, Testa R. Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C. *Am J Gastroenterol* 2000; **95**: 3211-3217 [PMID: 11095344]

8 **Romero-Gómez M**, Castellano-Megias VM, Grande L, Irles JA, Cruz M, Nogales MC, Alcón JC, Robles A. Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C. *Am J Gastroenterol* 2003; **98**: 1135-1141 [PMID: 12809839]

9 **Myers RP**, Messous D, Poynard T, Imbert-Bismut F. Association between leptin, metabolic factors and liver histology in patients with chronic hepatitis C. *Can J Gastroenterol* 2007; **21**: 289-294

10 **Berg AH**, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; **7**: 947-953 [PMID: 11479628]

11 **Pagano C**, Soardo G, Esposito W, Fallo F, Basan L, Donnini D, Federspil G, Sechi LA, Vettor R. Plasma adiponectin is decreased in nonalcoholic fatty liver disease. *Eur J Endocrinol* 2005; **152**: 113-118 [PMID: 15762194]

12 **Kuczmarski RJ**, Flegal KM. Criteria for definition of overweight in transition: background and recommendations for the United States. *Am J Clin Nutr* 2000; **72**: 1074-1081 [PMID: 11063431]

13 **Scherwin JE**. Liver function. In: Kaplan LA, Pesce AJ, Kazmierc-zak SC. Clinical Chemistry: Theroy, Analysis, Corrolation. 4th edition. St Louis: Mosby inc, 2003: 492 and appendix

14 **Doumas BT**, Perry BW, Sasse EA, Straumfjord JV. Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. *Clin Chem* 1973; **19**: 984-993 [PMID: 4744825]

15 **Pinnell AE**, Northam BE. New automated dye-binding method for serum albumin determination with bromcresol purple. *Clin Chem* 1978; **24**: 80-86 [PMID: 618669]

16 **Rej R**. Effect of incubation with Mg2+ on the measurement of alkaline phosphatase activity. *Clin Chem* 1977; **23**: 1903-1911 [PMID: 902417]

17 **Henry RJ**. Clinical Chemistry, Principles and Technique. In: Canon DC, Winkelman JW. Harper and Row. 2nd edition. New York: 1974: 407

18 **Fawcett JK**, Scott JE. A rapid and precise method for the determination of urea. *J Clin Pathol* 1960; **13**: 156-159 [PMID: 13821779]

19 **Wallander M**, Söderberg S, Norhammar A. Leptin: a predictor of abnormal glucose tolerance and prognosis in patients with myocardial infarction and without previously known Type 2 diabetes. *Diabet Med* 2008; **25**: 949-955 [PMID: 18959608]

20 **Lele RD**. Pro-insulin, C peptide, glucagon, adiponectin, TNF alpha, AMPK: neglected players in type 2 diabetes mellitus. *J Assoc Physicians India* 2010; **58**: 30, 35-40 [PMID: 20649096]

21 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864]

22 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010]

23 **Manolakopoulos S**, Bethanis S, Liapi C, Stripeli F, Sklavos P, Margeli A, Christidou A, Katsanika A, Vogiatzakis E, Tzourmakliotis D, Theocharis S. An assessment of serum leptin levels in patients with chronic viral hepatitis: a prospective study. *BMC Gastroenterol* 2007; **7**: 17 [PMID: 17540037]

24 **Saxena NK**, Ikeda K, Rockey DC, Friedman SL, Anania FA. Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology* 2002; **35**: 762-771 [PMID: 11915021]

25 **Otte C**, Otte JM, Strodthoff D, Bornstein SR, Fölsch UR, Mönig H, Kloehn S. Expression of leptin and leptin receptor during the development of liver fibrosis and cirrhosis. *Exp Clin Endocrinol Diabetes* 2004; **112**: 10-17 [PMID: 14758566]

26 **Angulo P**, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease. *J Hepatol* 2004; **41**: 943-949 [PMID: 15582127]

27 **Piche T**, Vandenbos F, Abakar-Mahamat A, Vanbiervliet G, Barjoan EM, Calle G, Giudicelli J, Ferrua B, Laffont C, Benzaken S, Tran A. The severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C. *J Viral Hepat* 2004; **11**: 91-96 [PMID: 14738564]

28 **Krawczyk K,** Szczesniak P, Kumor A, Jasinska A, Omulecka A, Pietruczuk M, Orszulak-Michalak D, Sporny S, Malecka-Panas E. Adipohormones as prognostric markers in patients with nonalcoholic steatohepatitis (NASH). *J Physiol Pharmacol* 2009; **60:** 71-75 [PMID:19996485]

29 **Testa R**, Franceschini R, Giannini E, Cataldi A, Botta F, Fasoli A, Tenerelli P, Rolandi E, Barreca T. Serum leptin levels in patients with viral chronic hepatitis or liver cirrhosis. *J Hepatol* 2000; **33**: 33-37 [PMID: 10905583]

30 **Lin SY**, Wang YY, Sheu WH. Increased serum leptin concentrations correlate with soluble tumour necrosis factor receptor levels in patients with cirrhosis. *Clin Endocrinol (Oxf)* 2002; **57**: 805-811 [PMID: 12460331]

31 **Comlekci A**, Akpinar H, Yesil S, Okan I, Ellidokuz E, Okan A, Ersoz G, Tankurt E, Batur Y. Serum leptin levels in patients with liver cirrhosis and chronic viral hepatitis. *Scand J Gastroenterol* 2003; **38**: 779-786 [PMID: 12889566]

32 **Zografos TA**, Rigopoulou EI, Liaskos C, Togousidis E, Zachou K, Gatselis N, Germenis A, Dalekos GN. Alterations of leptin during IFN-alpha therapy in patients with chronic viral hepatitis. *J Hepatol* 2006; **44**: 848-855 [PMID: 16530290]

33 **Muzzi A**, Leandro G, Rubbia-Brandt L, James R, Keiser O, Malinverni R, Dufour JF, Helbling B, Hadengue A, Gonvers JJ, Müllhaupt B, Cerny A, Mondelli MU, Negro F. Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. *J Hepatol* 2005; **42**: 41-46 [PMID: 15726693]

34 **Henriksen JH**, Holst JJ, Møller S, Brinch K, Bendtsen F. Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. *Hepatology* 1999; **29**: 1818-1824 [PMID: 10347125]

35 **Yoon EJ**, Hu KQ. Hepatitis C virus (HCV) infection and hepatic steatosis. *Int J Med Sci* 2006; **3**: 53-56 [PMID: 16614743]

36 **El-Zayadi A,** Attia M, Barakat EMF, Zalata K, Saeid A, Hamdy H, El-Nakeeb A. Hepatic steatosis in hepatitis C genotype 4 infected patients. *Arab J Gastroenterol* 2007; **8**: 5-9

37 **Tsochatzis E**, Papatheodoridis GV, Manesis EK, Chrysanthos N, Kafiri G, Petraki K, Hadziyannis E, Pandelidaki H, Zafiropoulou R, Savvas S, Koskinas J, Archimandritis AJ. Hepatic steatosis in genotype 4 chronic hepatitis C is mainly because of metabolic factors. *Am J Gastroenterol* 2007; **102**: 634-641 [PMID: 17222326]

38 **Adinolfi LE**, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364 [PMID: 11391523]

39 **Derbala M**, Rizk N, Al-Kaabi S, Amer A, Shebl F, Al Marri A, Aigha I, Alyaesi D, Mohamed H, Aman H, Basem N. Adiponectin changes in HCV-Genotype 4: relation to liver histology and response to treatment. *J Viral Hepat* 2009; **16**: 689-696 [PMID: 19486470]

40 **Tiftikci A**, Atug O, Yilmaz Y, Eren F, Ozdemir FT, Yapali S, Ozdogan O, Celikel CA, Imeryuz N, Tozun N. Serum levels of adipokines in patients with chronic HCV infection: relationship with steatosis and fibrosis. *Arch Med Res* 2009; **40**: 294-298 [PMID: 19608019]

41 **Latif HA**, Assal HS, Mahmoud M, Rasheed WI. Role of serum adiponectin level in the development of liver cirrhosis in patients with hepatitis C virus. *Clin Exp Med* 2011; **11**: 123-129 [PMID: 20714777]

42 **Ashour E**, Samy N, Sayed M, Imam A. The relationship between serum adiponectin and steatosis in patients with chronic hepatitis C genotype-4. *Clin Lab* 2010; **56**: 103-110 [PMID: 20476641]

43 **Petit JM**, Minello A, Jooste V, Bour JB, Galland F, Duvillard

L, Verges B, Olsson NO, Gambert P, Hillon P. Decreased plasma adiponectin concentrations are closely related to steatosis in hepatitis C virus-infected patients. *J Clin Endocrinol Metab* 2005; **90**: 2240-2243 [PMID: 15644404]

44 **Kara B**, Gunesacar R, Doran F, Kara IO, Akkiz H. Correlation of serum adiponectin levels and hepatic steatosis in hepatitis C virus genotype 1 infection. *Adv Ther* 2007; **24**: 972-982 [PMID: 18029322]

45 **Looker HC**, Krakoff J, Funahashi T, Matsuzawa Y, Tanaka S, Nelson RG, Knowler WC, Lindsay RS, Hanson RL. Adiponectin concentrations are influenced by renal function and diabetes duration in Pima Indians with type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 4010-4017 [PMID: 15292342]

46 **Chandran M**, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: more than just another fat cell hormone? *Diabetes Care* 2003; **26**: 2442-2450 [PMID: 12882876]

47 **Codes L**, Asselah T, Cazals-Hatem D, Tubach F, Vidaud D, Paraná R, Bedossa P, Valla D, Marcellin P. Liver fibrosis in women with chronic hepatitis C: evidence for the negative role of the menopause and steatosis and the potential benefit of hormone replacement therapy. *Gut* 2007; **56**: 390-395 [PMID: 17005762]

48 **Guha IN**, Parkes J, Roderick PR, Harris S, Rosenberg WM. Non-invasive markers associated with liver fibrosis in non-alcoholic fatty liver disease. *Gut* 2006; **55**: 1650-1660 [PMID: 17047111]

49 **Waki H**, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem* 2003; **278**: 40352-40363 [PMID: 12878598]

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