

Increased serum leptin concentration is related to steatosis in hepatitis C virus-infected patients

Artmış serum leptin konsantrasyonu hepatit C virüsü ile enfekte hastalarda steatozla ilişkilidir

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Background and Aims: The mechanism that leads to steatosis in hepatitis C virus infection is complex. The aim of our study was to search the role of adipocytokines in hepatitis C virus-related steatosis. **Materials and Methods:** Sixty-five untreated genotype 1 chronic hepatitis C virus patients were included in the study to evaluate the effects of adipocytokines, body mass index, age, and insulin resistance on hepatic steatosis. **Results:** Steatosis was observed in 31 patients (47.7%). When adiponectin and leptin were grouped according to fibrosis, we did not determine a difference between adiponectin and leptin (p values 0.597 and 0.159, respectively). We did not determine a difference in adiponectin levels in patients with different degrees of steatosis ($p=0.674$), while there was a significant difference for leptin ($p=0.021$). **Conclusions:** Hyperleptinemia is related to the development of steatosis in patients with chronic hepatitis C virus.

Key words: Adiponectin, fibrosis, hepatitis C virus, insulin resistance, leptin, steatosis

Giriş ve Amaç: Hepatit C virüsü enfeksiyonunda steatoza yol açan mekanizma karmaşıktır. Bizim çalışmamızın amacı hepatit C virüsü ile ilgili steatozda adipositokinlerin rolünü araştırmaktır. **Gereç & Yöntem:** Tedavi edilmemiş genotip 1 kronik hepatit C virüslü 65 hasta adipositokinler, beden kitle indeksi, yaş, ve insulin direncinin karaciğer yağlanmasındaki etkilerini değerlendirmek için çalışmaya alındı. **Bulgular:** Yağlanma 31 (%47.7) hastada gözlemlendi. Fibroza göre adiponektin ve leptin gruplandırıldığında, adiponektin ve leptin arasında bir fark gözlemlenmedi (p sırasıyla 0.597 ve 0.159). Leptin için anlamlı bir fark ($p=0.021$) varken, steatozlu hastalarda adiponektin düzeyleri arasında bir fark saptamadık ($p=0.674$). **Sonuç:** Hiperleptinemi kronik hepatit C virüslü hastalarda steatozun gelişimiyle ilişkilidir.

Anahtar kelimeler: Adiponektin, fibroz, hepatit C virüsü, insulin direnci, leptin, steatoz

INTRODUCTION

Steatosis is a common histological feature of hepatitis C virus (HCV) infection (1). The prevalence of steatosis in liver biopsy specimens of chronic HCV patients is about 50% (1). Recent studies have found a role for steatosis in the progression of chronic HCV (1–3). In addition, hepatic steatosis is an independent risk factor for hepatocellular carcinoma in patients with chronic HCV infection (4).

The pathogenesis of steatosis in hepatitis C patients is not well understood. HCV genotype 3a has been linked to steatosis more strongly than other genotypes (5). Moreover, HCV-related steatosis is not always virally related, and other factors may coexist. Obesity is a well-recognized risk factor for the development of steatosis and of fibrosis in HCV-infected patients (1,3,6). Visceral fat distribution rather than body mass index (BMI) has been proven to be associated with HCV-related steatosis (3).

The mechanisms by which accumulation and anatomic distribution of adipose tissue may be related to the development of steatosis and fibrosis are under intense investigation. Recently, a new role has emerged for adipose tissue as an endocrine organ (7,8). Adipose tissue secretes a variety of hormones including adiponectin, leptin and inflammatory cytokines, for example, tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), which may contribute to the development of metabolic abnormalities (7,8). There are some controversial data about the relationship between serum leptin levels and HCV-related steatosis (9,10). Adiponectin levels are associated in healthy humans with plasma concentrations of various liver function tests (11). Although the deleterious association between obesity and HCV infection is well recognized, it has not been ascertained whether adipocytokines and, particularly, adiponectin, may have a role in the development of steatosis in chronic hepatitis C (CHC).

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We hypothesized that the relationship between obesity and HCV-related steatosis is the consequence of the endocrine function of adipose tissue. Therefore, the primary aim of our study was to investigate the role of adipocytokines in HCV-related steatosis.

MATERIALS AND METHODS

Patient Selection

A total of 65 consecutive prospectively enrolled patients with chronic HCV infection due to genotype 1 who underwent liver biopsy at Mersin University Faculty of Medicine Hospital comprised the study cohort. The study protocol was approved by the Human Ethics Committee of our hospital, and subjects gave written informed consent to participate in the present study. All subjects had antibodies against HCV (Abbott, AxSYM) and detectable HCV RNA by polymerase chain reaction (PCR) (Amplicor HCV; Roche Diagnostics, Branchburg, NJ).

Exclusion criteria were; concurrent active hepatitis B virus (positive for hepatitis B surface antigen) or human immunodeficiency virus infection, autoimmune hepatitis, drug-induced steatohepatitis, alcohol consumption more than 20 g/day, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, Wilson's disease, and α -1-antitrypsin deficiency (diagnosed by appropriate history and confirmed by biochemical and serological tests). Patients with type 2 diabetes mellitus (T2DM) according to the American Diabetes Association guidelines or a history of previous antiviral therapy were also excluded (12). Patients with T2DM were not included since the majority of them were maintained on insulin-sensitizing agents, which could influence the levels of the adipocytokines. No patient had clinical evidence of hepatic decompensation (jaundice, hepatic encephalopathy, ascites, hepatorenal syndrome, or variceal bleeding) at the time of biopsy.

A complete clinical evaluation was performed on each patient. Baseline characteristics collected at the time of liver biopsy included the age, height, weight, and BMI. The BMI was calculated as weight in kg/(height in meters)². Information regarding the average current daily alcohol intake (g/day) in the past six months and past alcohol intake (g/day) before the last six months was obtained.

Laboratory Tests

On the morning of the liver biopsy, venous blood was drawn after a 12-hour overnight fasting to determine the serum levels of alanine aminotransferase (ALT), albu-

min, bilirubin, platelet count, international normalized ratio, glucose, insulin, lipids, leptin, and adiponectin.

Insulin resistance (IR) was calculated by the homeostasis model (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (μ U/ml) X plasma glucose (mmol/L)/22.5 (13). Serum adiponectin and serum leptin concentrations were measured by using a commercial ELISA (human adiponectin ELISA kit, LINCO Research Inc., Missouri, USA and human leptin ELISA kit, Biosource, Europe S.A., Nivelles, Belgium).

All other biochemical tests were performed using a conventional automated analyzer within the Department of Clinical Chemistry at Mersin University Faculty of Medicine Hospital.

Histological Data

In liver biopsy, necroinflammatory activity was scored based on Knodell's Histological Activity Index (HAI): 0-4 corresponded to portal inflammation, 0-4 to lobular degeneration and necrosis and 0-10 to periportal necrosis (14). The disease stage was determined based on Scheuer classification: 0 corresponded to absence of fibrosis, 1 to enlarged fibrotic portal tracts, 2 to periportal or portoportal septa but regular architecture, 3 to fibrosis of irregular architecture without overt cirrhosis, and 4 to possible or confirmed cirrhosis (15). Liver steatosis was graded as follows: 0, steatosis in less than 5% of hepatocytes; 1, steatosis in 5-34% of hepatocytes; 2, steatosis in 35-69% of hepatocytes; and 3, steatosis in more than 69% of hepatocytes (16). It was a slightly modified version of the grading reported in the literature.

Statistical Analyses

Chi-square test was used to analyze frequencies, Student's t test or Mann-Whitney U test to analyze demographic data and laboratory results when appropriate, Spearman's rank correlation to determine the correlation between the variables, and ANOVA or Kruskal-Wallis test to determine differences between the groups. Data were analyzed with SPSS 10.0 (SPSS for Windows, Chicago, IL). A value of $p < 0.05$ was considered significant.

RESULTS

Characteristics of Patients

The main clinical and laboratory data are summarized in Table 1. There was no significant difference in fibrosis and steatosis between genders. No patient was considered an excessive drinker (more than 20 g per day). No significant relationship was determined between IR and gender ($p = 0.678$).

Table 1. Clinical and epidemiological characteristics of HCV patients

	All patients Mean±SD (median)	Men Mean±SD (median)	Women Mean±SD (median)	P (Men vs. Women)
n	65	36	29	
Age (year)	50±10.32 (50.00)	49.14±11.52 (50.00)	51.07±8.68 (51.00)	0.458
BMI (kg/m ²)	27.05±3.97 (27.00)	25.94±3.86 (26.00)	28.41±3.71 (28.00)	0.012*
Adiponectin (µg/ml)	43.71±3.43 (44.00)	43.56±3.63 (44.00)	43.90±3.21 (45.00)	0.694
Leptin (pg/ml)	14.50±14.27 (11.00)	6.74±8.38 (4.00)	21.11±2.89 (18.00)	<0.0001***
Total cholesterol	167.20±38.51 (162.00)	168.03±40.80 (166.00)	166.17±36.15 (162.00)	0.849
HDL cholesterol	53.80±20.409 (50.00)	50.86±23.258 (44.50)	57.45±15.844 (51.00)	0.198
Triglyceride	118.23±51.76 (112.00)	137.22±57.39 (129.00)	94.66±31.115 (95.00)	0.001**
Insulin	19.48±10.022 (17.00)	20.72±10.743 (18.00)	17.93±8.992 (17.00)	0.268
HOMA	5.9812±5.753 (4.1136)	6.8638±7.087 (4.1556)	4.8855±3.257 (4.1136)	0.170
IR (n(%))	53 (81.5)	30 (83.3)	23 (79.3)	0.678
Fasting blood glucose	113.20±52.65 (95.00)	114.89±52.03 (95.00)	111.10±54.27 (95.00)	0.776

Student's t test or Mann-Whitney U test was used to analyze demographic data and laboratory results.

Adipocytokine Concentrations

Women had significantly higher leptin levels than men ($p < 0.0001$), while adiponectin levels of women and men were similar ($p > 0.05$). Leptin levels were weakly correlated with steatosis ($r = 0.256$; $p = 0.039$), whereas no correlation was found with others. No correlation was found with adiponectin levels. No correlation was determined between the levels of adiponectin with the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, or BMI (p levels 0.869, 0.934, 0.537, 0.219, respectively). A correlation was determined only between leptin levels with BMI ($p = 0.0001$). No correlation was determined between leptin with total cholesterol, HDL cholesterol or triglyceride (p levels 0.653, 0.627, 0.223, respectively).

Factors Associated with Steatosis and Fibrosis

Grade 1 steatosis was present in 12 patients, grade 2 steatosis in 16 patients and grade 3 steatosis in 3 patients. Fibrosis grade 0–1 was present in 23 patients (35.4%), grade 2 in 23 patients (35.4%) and grades 3–4 in 19 patients (29.3%). There was a weak correlation

between fibrosis and ALT ($r = 0.251$; $p = 0.044$), aspartate aminotransferase (AST) ($r = 0.328$, $p = 0.008$), gamma-glutamyl transpeptidase (GGT) ($r = 0.264$, $p = 0.033$), and platelet count ($r = -0.372$, $p = 0.002$). There was also a weak correlation between steatosis and hemoglobin level ($r = 0.245$, $p = 0.049$).

There was a significant difference in leptin levels ($p = 0.021$), but no significant difference in adiponectin levels ($p = 0.674$) between the patients with different degrees of steatosis. In fact, leptin levels of the patients with grade 2 steatosis were significantly different from leptin levels of the patients with grade 0 steatosis. P value should have been < 0.0125 , but it was found to be $p = 0.014$ (mean 11.21 vs. 21.06 pg/ml; $p = 0.044$). However, there was no significant difference in leptin and adiponectin levels between patients with and without steatosis ($p = 0.428$ for adiponectin; $p = 0.066$ for leptin) (Table 2). Adiponectin and leptin levels were not different between the patients with different degrees of fibrosis (without fibrosis=F0, moderate=F1 & F2 and severe=F3 & F4) ($p = 0.682$ for adiponectin; $p = 0.082$ for leptin) (Table 3).

Tablo 2. Adipocytokine levels according to steatosis grade

	Without steatosis Mean±SD (median)	With steatosis Mean±SD (median)	P
n	34	31	
Age (year)	47.91±11.14 (48.00)	52.29±8.967 (52.00)	0.088
BMI (kg/m ²)	26.24±4.432 (26.00)	27.94±3.235 (27.00)	0.085
Adiponectin (µg/ml)	43±3.861 (44,00)	44.06±2.909 (44.00)	0.428
Leptin (pg/ml)	11.21±11.272 (7.00)	18.68±16.714 (13.50)	0.066

Student's t test or Mann-Whitney U test was used to analyze demographic data and laboratory results.

DISCUSSION

The results of our study indicate that hyperleptinemia is associated with the presence of steatosis in patients with chronic HCV. These findings were independent of age, gender, viral characteristics, adiponectin or insulin concentration, and BMI. In the present study, it was found that leptin level was higher in women than men. Leptin concentrations have been reported to be up to four times higher in women than in men, a finding associated with the higher body fat content observed in women (17). Men and women differ in regard to body composition, IR and energy balance. For a given BMI, men have higher lean mass and more visceral and hepatic adipose tissue, whereas women have elevated general adiposity. These differences in adipose tissue distribution may contribute to a more insulin-sensitive environment in women, as visceral and hepatic adiposity is associated with increased IR. Estrogen may also play a role in these gen-

der differences because it has a favorable effect on insulin and glucose homeostasis, adipose tissue distribution and proinflammatory markers (17).

Adipose tissue is regulated by hormonal, neural and nutrient stimuli. In fasting states, adipocytes release non-esterified fatty acids (NEFAs) as a result of lipolysis, whereas the postprandial increase in glucose and lipids promotes lipogenesis in the presence of insulin (18). However, adipose tissue apparently also regulates metabolism in addition to its role in fuel storage. In addition to NEFAs, adipocytes secrete a number of bioactive proteins, collectively termed the adipocytokines (18–33). Adiponectin, leptin, IL-6, and TNF- α are among the best characterized of the adipocytokines, and are attracting increasing attention due to the important role they are proposed to play in IR. Secretion of the adipocytokines is altered in obese individuals. Indeed, obesity has been proposed to be a chronic inflammatory state, indicated by increased plasma concentrations of C-reactive protein (CRP) and the adipocytokine IL-6 (23,34,35). Furthermore, plasma CRP concentration has been demonstrated to predict cardiovascular disease in apparently healthy individuals and is elevated in insulin-resistant prediabetic subjects (36,37). Hepatic synthesis and secretion of CRP is predominantly regulated by the adipocytokine IL-6 (34–36). Therefore, there is currently great interest in the role of the adipocytokines in the pathogenesis of T2DM and cardiovascular risk as well as hepatosteatosis. Increased adiposity is a major determinant of IR, and altered adipocytokine regulation is a key common mechanism. Furthermore, weight loss improves IR (38).

The associations among HCV, IR, liver steatosis, and fibrosis have driven research into the evaluation of the roles of leptin and adiponectin. Increased serum leptin levels were higher in 30 CHC patients than in 30 controls

Tablo 3. Adipocytokine levels according to fibrosis grade

	Without fibrosis (F0) Mean±SD (median)	Moderate fibrosis (F1-F2) Mean±SD (median)	Severe fibrosis (F3-F4) Mean±SD (median)	P
n	5	41	19	
Age (year)	51.40±7.829 (51.00)	47.34±10.382 (47.00)	55.37±8.864 (58.00)	0.016*
BMI (kg/m ²)	27.20±5.404 (29.00)	26.93±3.784 (27.00)	27.26±4.201 (27.00)	0.952
Adiponectin (µg/ml)	42.60±5.320 (42.00)	43.66±3.497 (44.00)	44.11±2.807 (44.00)	0.682
Leptin (pg/ml)	9.33±7.767 (7.00)	12.09±11.437 (7.50)	22±19.447 (19.00)	0.082

ANOVA or Kruskal-Wallis test was used to determine differences between the groups.

(39). In two studies, leptin levels were found independently associated with fibrosis severity, while a possible relation with steatosis was not evaluated or not found (9,41). In 131 CHC patients, leptin levels were found to be associated with steatosis in genotype 1 but not in genotype 3 patients, and this association remained unchanged after exclusion of 37 heavy drinkers (10). A weak association between leptin levels and fibrosis severity was also reported (10). In 48 CHC patients with steatosis but without diabetes, obesity, hyperlipidemia, and alcohol abuse, leptin levels did not correlate with fibrosis or steatosis severity (42). In a multicenter study including 221 nondiabetic CHC patients, there was no correlation between leptin levels and histological features regardless of genotype, while IR was associated with fibrosis in the 152 non-3 genotype patients (43). In another study, leptin levels were found to be associated with steatosis in 74 CHC patients only in the univariate analysis, while no correlation was observed between leptin levels and necroinflammation or fibrosis (44). Two additional studies failed to show any association of leptin in CHC, although they had significant findings for adiponectin (45,46). Finally, in a Spanish study, IR, fibrosis severity and genotype, but not leptin levels, were considered as the only predictors of sustained response to therapy (47).

As mentioned above, although serum leptin levels are increased in CHC patients, the data on their associations with histological lesions are rather conflicting. This may be due to the heterogeneity in the study populations. Leptin, however, is suggested to have no association with fibrosis or steatosis in cases without metabolic risk factors for hepatic steatosis (42). Given the more frequent development of hepatic steatosis due to metabolic factors in genotype 1 infection, there may be an association between leptin levels and fibrosis and/or steatosis in genotype 1 but not in genotype 3 CHC patients (48). It should be noted that no study suggesting an independent association between leptin levels and fibrosis severity evaluated IR, which therefore might have been a hidden confounding factor (40,41). Thus, more detailed studies are required to further clarify this issue.

Currently, there is increasing interest in the role of adiponectin in CHC. In a relatively small study without information on steatosis and HCV genotypes, no correlation between serum adiponectin levels and liver histology was found (49). Low adiponectin levels correlated with steatosis in 71 CHC patients, and with viral load, IR and genotype 2, but not with histological parameters or BMI, in 95 CHC patients (45,46). Baseline adiponectin levels did not predict treatment outcome, and posttreatment

steatosis changes did not correlate with changes in adiponectin levels. Intrahepatic AdipoR1 and AdipoR2 gene expression correlated inversely with serum adiponectin levels in a subgroup of 10 patients (50). In the largest cohort of 194 untreated CHC patients, hypo adiponectinemia was associated with steatosis only in males, while adiponectin levels increased with increasing inflammation but had no association with fibrosis severity (51). The lower BMI and milder steatosis in females may be responsible for the gender difference in the association between adiponectin and steatosis, whereas the increasing adiponectin levels with hepatic necroinflammation implies a possible secondary adiponectin response to liver injury (51).

Adiponectin, an insulin-sensitizing hormone, is also significantly higher in women compared with men, and whether this is due to differences in sex hormones or differences in adipose tissue distribution is not clear (17). However, in our study, we found that adiponectin levels of women and men were similar. The elevated visceral and hepatic adipose tissue reported in men, in conjunction with the lack of a possible protective effect of estrogen and lower adiponectin levels, may contribute to their higher IR compared with women (17). Gender-specific avenues of research into IR should consider these sex differences in adipose distribution and adipokine secretion. In addition, gender-tailored treatment of IR may benefit from focusing on visceral and hepatic adiposity and hypo adiponectinemia, which are more prominent in men than in women.

Finally, in a small study of 30 male CHC patients and 22 controls, the ratio of high molecular weight (HMW) to total adiponectin levels correlated inversely with steatosis in genotype 1 but not in genotype 3 patients, while no such relation was found when total adiponectin was used in the analysis (46). Patients with genotype 1 were less insulin-sensitive than those with genotype 3, while IR was associated with a decrease in total and HMW adiponectin in both patients and controls (46). From the above studies, an inverse association between serum adiponectin and steatosis in CHC seems rather likely, while no relation appears to exist between adiponectin and fibrosis. The possible associations of adiponectin with necroinflammation, the role of genotype variations in adiponectin levels, and the possible enhanced sensitivity of HMW forms merit further evaluation.

In our study, the fact that the plasma levels of leptin directly correlated with steatosis in HCV-infected subjects suggests that hyperleptinemia was at least partly responsible for hepatic steatosis and liver injury in this populati-

on. The mechanism of this association needs to be clarified; however, it is probably related to the effect of leptin on lipid metabolism.

In conclusion, this study demonstrates that hyperleptinemia in HCV-infected patients correlates with hepatic ste-

atosis. One practical implication is that therapy to decrease circulating leptin concentration, such as peroxisome proliferator-activated nuclear receptor- γ (PPAR- γ) agonists, might provide the potential to improve steatosis in CHC infection.

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