Association between leptin, metabolic factors and liver histology in patients with chronic hepatitis C

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BACKGROUND: Steatosis is common in hepatitis C virus (HCV)-infected patients and likely accelerates fibrosis progression. Leptin, the peptide product of the obesity gene (ob), has been implicated in hepatic fibrogenesis; circulating levels of leptin correlate with body fat mass. The objective of the present study was to determine the clinical and histological correlates of serum leptin in HCV-infected patients, and to determine its utility in predicting liver histological lesions.

PATIENTS AND METHODS: In 62 patients with chronic HCV, serum leptin was measured using a commercially available immunosay. Associations between leptin, metabolic parameters, and severe hepatic fibrosis (stages 2 to 4) and steatosis (30% or greater) were determined. The utility of leptin in predicting liver histology was determined using receiver operating characteristic (ROC) curves.

RESULTS: The median body mass index (BMI) was 23.2 kg/m² (range 17.7 kg/m² to 35.6 kg/m²); 16% of patients (n=10) had HCV genotype 3. Severe fibrosis and steatosis were present in 23% and 13% of patients, respectively. Leptin was strongly correlated with the BMI, and its levels were higher in women. BMI-corrected leptin levels were not independently associated with severe fibrosis but were significantly associated with steatosis (OR of 1.07; 95% CI 1.01 to 1.04). On its own, leptin was poorly predictive of severe steatosis (area under the ROC curve was 0.64; 95% CI 0.42 to 0.87). However, its accuracy improved with the addition of HCV genotype (area under the ROC curve was 0.86; 95% CI 0.72 to 1.00; P=0.07).

CONCLUSIONS: As observed in the non-HCV setting, serum leptin correlates with BMI; higher leptin levels are found in women than men with chronic HCV. Serum leptin is a poor predictor of HCV-related fibrosis but may play a role in predicting steatosis when combined with HCV genotype.

Key Words: Fibrosis; Hepatitis; Leptin; Noninvasive; Prediction; Steatosis

Chronic hepatitis C virus (HCV) infection is a major cause of cirrhosis, end-stage liver disease and hepatocellular carcinoma (1). Approximately 40% to 80% of patients have steatosis on liver biopsy (2). The majority of patients (approximately 75%) have mild steatosis that affects less than 30% of hepatocytes. Factors associated with steatosis in chronic HCV-infected patients include genotype 3 infection, alcohol consumption, obesity, hyperlipidemia and insulin resistance (3-5). The impact of steatosis on the course of HCV is controversial. Accelerated fibrosis progression (4-7) and reduced response to interferon-based therapy (5,6,8) have been reported in some, but not all, studies (9,10).

Leptin is a 16 kDa peptide product of the obesity (ob) gene initially identified by Friedman in 1994 (11). Leptin was identified as the hormone whose absence resulted in morbid obesity and hepatic steatosis in the ob/ob mouse, thus acquiring its name from the Greek word ‘leptos’ (thin). Rare cases of functional leptin deficiency due to mutations in the gene for leptin (or its receptor) have been reported in humans (12,13). These patients develop morbid obesity that is responsive to leptin replacement.

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Received for publication May 11, 2006, Accepted August 14, 2006
The use of clinical data and serum for research purposes. Included hepatitis B or HIV coinfection, coexistent nonviral liver lesions, and to evaluate serum leptin as a potential predictor of fibrosis in hepatic fibrosis is less clear. Experimental models have shown that leptin production by culture-activated hepatic stellate cells and suggest a profibrogenic role, likely mediated by the upregulation of transforming growth factor-beta 1 (19-26). In patients with chronic HCV infection, data regarding the association between serum leptin and liver histological lesions are conflicting (27-31).

The objectives of the present study were to determine predictors of serum leptin in patients with chronic HCV, to examine the association between serum leptin levels and liver histological lesions, and to evaluate serum leptin as a potential predictor of fibrosis and steatosis for use in clinical practice.

PATIENTS AND METHODS

Patients and study design

The patients in the present cross-sectional study belong to a cohort of HCV-infected patients evaluated in the liver unit of the Pitié-Salpêtrière Hospital (Paris, France) (32). Chronic HCV was defined as a positive serological test for HCV by at least a second-generation ELISA, positive HCV RNA results by polymerase chain reaction assay and compatible liver biopsy. To be eligible for the study, patients had to be naïve to interferon-based treatment, and have an interpretable liver biopsy and fasting serum sample drawn within six months of the biopsy and stored at –80°C. Exclusion criteria included hepatitis B or HIV coinfection, coexistent nonviral liver disease and prior organ transplantation. All patients consented to the use of clinical data and serum for research purposes.

Data collection

For all patients in the cohort, a questionnaire of 129 items, including sociodemographic, clinical, virological, histological and treatment-related information, was completed (32). For the purpose of the present study, demographics, daily alcohol consumption (average intake over the preceding five years), anthropometric measurements (weight, height and body mass index [BMI]) and diabetes mellitus history were recorded. Fasting serum samples were tested for glucose, total cholesterol, triglycerides and leptin levels. Leptin was measured by a commercially available ELISA (Quantikine, R&D Systems Inc, USA). The sensitivity of this assay is 7.8 pg/mL; reported intra- and interassay coefficients of variation do not exceed 5.4% (Quantikine package insert, catalog #DLP00). All patients had HCV genotyping by the Inno-LiPA line probe assay (Innogenetics, Belgium).

Liver histology

Liver biopsies were fixed, paraffin embedded and stained for collagen with hematoxylin-eosin-safran, and Mason’s trichrome or picrosirius red. A single pathologist blinded to patient characteristics and laboratory results analyzed the biopsies according to the METAVIR classification (33,34). Fibrosis was staged from F0 to F4: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Necroinflammatory activity was graded from A0 to A3: A0, no activity; A1, mild activity; A2, moderate activity and A3, severe activity. Fibrosis stages F2 to F4 and activity grades A2 to A3 were considered severe. Steatosis was graded as mild if less than 30% of hepatocytes were affected; otherwise, it was considered severe (2).

Statistical analysis

Quantitative data are expressed as medians (ranges) or proportions. Univariate associations were determined using Pearson’s correlations, logistic regression, and Mann-Whitney U and Fisher’s exact tests, as appropriate. Multivariate logistic regression analyses were also used. Receiver operating characteristics curves (ROCs) were constructed to determine the predictive utilities of various models for the identification of severe steatosis and fibrosis. The area under the ROC (AUROC) curves was compared using DeLong et al’s (35) method. P≤0.05 was considered significant. STATA 8.0 software (Stata Corporation, USA) was used for all analyses.

RESULTS

Patients

Sixty-two patients were included in the study (Table 1). The median age was 47 years (range 27 to 70 years); 56% were men. Three patients (5%) were diabetic; all three were on hypoglycemic medication and had normal fasting glucose levels. The median BMI was 23.2 kg/m² (17.7 kg/m² to 35.6 kg/m²); only 6% (n=4) were obese (BMI of 30 kg/m² or greater). The majority (60%) were infected with genotype 1; 10 patients (16%) had genotype 3. The prevalences of severe fibrosis, inflammation and steatosis were 45%, 23% and 13%, respectively.

Serum leptin in HCV and its determinants

Leptin levels varied over a wide range (median 11.3 ng/mL, range 1.0 ng/mL to 99.3 ng/mL) and correlated significantly with the BMI (r=0.57, P<0.0005). This correlation was more pronounced in women than men (women: r=0.83, P<0.0005; men: r=0.49, P=0.003; Figure 1). Due to the strong association between serum leptin and BMI, further analyses consider leptin levels adjusted for BMI unless otherwise indicated. The leptin/BMI ratio was significantly higher in women than men (median 0.78 ng/mL·m²/kg

<table>
<thead>
<tr>
<th>TABLE 1 Characteristics of the 62 patients</th>
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<tbody>
<tr>
<td>Characteristic</td>
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<tr>
<td>Clinical factors</td>
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<tr>
<td>Median age, years (range)</td>
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<tr>
<td>Men, n (%)</td>
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<tr>
<td>Alcohol consumption &gt;50 g/day, n (%)</td>
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<td>Diabetes, n (%)</td>
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<td>Median BMI, kg/m² (range)</td>
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<tr>
<td>Obese (BMI ≥30 kg/m²), n (%)</td>
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<tr>
<td>HCV genotypes, n (%)</td>
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<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<tr>
<td>4, 5, 6</td>
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<tr>
<td>Liver histology, n (%)</td>
</tr>
<tr>
<td>Severe fibrosis (F2–F4)</td>
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<tr>
<td>Cirrhosis (F4)</td>
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<td>Severe necroinflammatory activity (A2–A3)</td>
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<td>Severe steatosis (≥30%)</td>
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BMI Body mass index; HCV Hepatitis C virus.

(14). Leptin is expressed primarily by adipocytes, but also in the stomach, placenta and mammary gland (15). Circulating leptin levels correlate closely with the amount of body fat mass. Leptin plays a crucial role in the regulation of appetite and energy expenditure through its effect on the hypothalamus.

Numerous in vitro and in vivo studies (16-18) have shown that leptin plays a role in hepatic steatosis. Patients with alcoholic (16,17) and nonalcoholic fatty liver disease (18) have increased circulating leptin levels in their bodies. The role of leptin in hepatic fibrosis is less clear. Experimental models have shown leptin production by culture-activated hepatic stellate cells and suggest a profibrogenic role, likely mediated by the upregulation of transforming growth factor-beta 1 (19-26). In patients with chronic HCV infection, data regarding the association between serum leptin and liver histological lesions are conflicting (27-31).
Similar in diabetics and nondiabetics (0.32 ng/mL•m²/kg versus 0.50 ng/mL•m²/kg; P=0.31). The median leptin/BMI ratios were shown) and total cholesterol (r=0.05, P=0.72) or fasting glucose versus 0.23 ng/mL•m²/kg; P=0.0002). There were no associations patients with mild/severe lesions

TABLE 2
Leptin/body mass index ratio according to the severity of histological lesions and genotype

<table>
<thead>
<tr>
<th>Histological lesion*</th>
<th>Mild, median (range)</th>
<th>Severe, median (range)</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>Necroinflammatory activity</td>
<td>All patients (n=62; 48/14) 0.48 (0.04–2.79) 0.61 (0.05–1.47) 0.27</td>
<td>Genotype 3 (n=10; 8/2) 0.31 (0.04–0.60) 0.46 (0.33–0.60) 0.79</td>
<td>Nongenotype 3 (n=52; 40/12) 0.48 (0.04–2.79) 0.74 (0.05–1.47) 0.36</td>
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<tr>
<td>Fibrosis</td>
<td>All patients (n=62; 34/28) 0.46 (0.04–2.25) 0.59 (0.04–2.79) 0.23</td>
<td>Genotype 3 (n=10; 4/6) 0.76 (0.29–0.60) 0.23 (0.04–0.60) 0.06</td>
<td>Nongenotype 3 (n=52; 30/22) 0.42 (0.04–2.25) 0.81 (0.76–2.79) 0.03</td>
</tr>
<tr>
<td>Steatosis</td>
<td>All patients (n=62; 54/8) 0.45 (0.04–1.76) 0.76 (0.04–2.79) 0.19</td>
<td>Genotype 3 (n=10; 6/4) 0.33 (0.07–0.60) 0.51 (0.04–0.78) 1.0</td>
<td>Nongenotype 3 (n=52; 48/4) 0.48 (0.04–1.76) 1.65 (0.51–2.79) 0.03</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate total number of patients and number of patients with mild/severe lesions versus 0.23 ng/mL•m²/kg; P=0.0002). There were no associations between the leptin/BMI ratio (or absolute leptin; data not shown) and total cholesterol (r=0.05, P=0.72) or fasting glucose levels (r=0.13, P=0.31). The median leptin/BMI ratios were similar in diabetics and nondiabetics (0.32 ng/mL•m²/kg versus 0.51 ng/mL•m²/kg; P=0.30). The leptin/BMI ratio was weakly correlated with serum triglycerides (r=0.26, P=0.04). There was no association with HCV genotype (χ² [five degrees of freedom] = 4.73; P=0.45); leptin/BMI ratios were similar in patients with genotype 3 and nongenotype 3 (0.33 ng/mL•m²/kg versus 0.50 ng/mL•m²/kg; P=0.31).

Association between leptin, fibrosis and necroinflammation
The association between BMI-corrected leptin levels and liver histological lesions is summarized in Table 2. In univariate analysis including all patients, irrespective of genotype, the leptin/BMI ratio was not associated with the severity of necroinflammation (P=0.27) or fibrosis (P=0.23). In nongenotype 3 patients, median leptin/BMI ratios were higher in those with severe (n=52) versus mild (n=10) fibrosis (P=0.03). This association was not observed in genotype 3 patients (Table 2; Figure 2). Moreover, in a multivariate logistic regression analysis controlling for age, sex, daily alcohol consumption and BMI (known factors associated with fibrosis), serum leptin was not independently associated with severe fibrosis (OR=1.03; 95% CI 0.98 to 1.09; P=0.28). The AUROC of serum leptin as a predictor of severe fibrosis was only 0.58 (95% CI 0.44 to 0.73), not significantly different from chance alone. Only age at biopsy was significant in this analysis (OR=1.10; 95% CI 1.04 to 1.17; P=0.002).

Predictors of hepatic steatosis
Clinical and biochemical predictors of severe steatosis are outlined in Table 3. In univariate analysis, only leptin, the leptin/BMI ratio and genotype 3 were significant. Severe steatosis was present in 40% (four of 10) of patients with genotype 3 versus 8% (four of 52) of nongenotype 3 patients (P=0.005). In multivariate analysis controlling for genotype and BMI, serum leptin was independently associated with severe steatosis (OR=1.07; 95% CI 1.01 to 1.04; P=0.01). Genotype 3 infection was significant (OR=28.8; 95% CI 3.18 to 261; P=0.003), whereas BMI was not (OR=0.87; 95% CI 0.66 to 1.16; P=0.36).

Due to the strong effect of genotype 3 on steatosis, a stratified analysis of leptin/BMI ratios according to genotype was performed (Table 2). In patients with genotype 3, no association was observed between the leptin/BMI ratio and steatosis (P=1.0). However, in patients with nongenotype 3, higher median leptin/BMI ratios were observed in the presence of severe steatosis (P=0.03) (Figure 3).

Serum leptin for the prediction of severe steatosis
To determine whether serum leptin could be used as a predictive tool for identifying severe steatosis, an index was created using the logistic regression model. The AUROC for this index was 0.64 (95% CI 0.42 to 0.87), indicative of poor predictive utility (Figure 4). At an optimally sensitive cut-off point of leptin (8.8 ng/mL or greater), the sensitivity, specificity and accuracy were only 87.5% (seven of eight patients), 50% (27 of 54 patients) and 54.8% (34 of 62 patients), respectively. The
positive and negative predictive values were 20.6% (seven of 34 patients) and 96.4% (27 of 28 patients), respectively. The AUROC for serum leptin was higher in patients with nongeno-type 3 (0.82; 95% CI 0.59 to 1.00) than in those with genotype 3 (0.50; 95% CI 0.05 to 0.95); however, this difference was not significant (P=0.21). A model including genotype in combination with leptin (0.86; 95% CI 0.72 to 1.00; P=0.07) had enhanced predictive accuracy (appendix shows formulas of predictive models).

**DISCUSSION**

In the present study, we describe the association between serum leptin, metabolic factors and liver histology in patients with chronic HCV. As observed in the non-HCV setting, leptin levels correlate strongly with BMI even in our relatively nonobese HCV patients. In a landmark study, Considine et al (36) demonstrated that this finding is due to the induction of ob gene expression, which signals the central nervous system to regulate caloric intake and energy expenditure in response to body fat stores. As previously reported (27,28,30,37), serum leptin levels were approximately twofold higher in women than men, even after adjustment for the BMI. The mechanism for this difference is unclear, but may relate to differential body fat distribution (ie, greater amounts of subcutaneous adipose tissue than visceral adipose tissue in women) or the effects of sex steroids (38-40).

We did not observe an association between leptin and features of the metabolic syndrome (total cholesterol levels, fasting glucose levels or diabetes), other than a weak correlation with serum triglycerides.

Our primary objective was to examine the association between leptin and HCV-related histological lesions. Overall, leptin was not associated with hepatic necroinflammation or fibrosis. The relationship between leptin and fibrosis is controversial; however, in vitro studies (19-26) have clearly...
Leptin and chronic HCV

Can J Gastroenterol Vol 21 No 5 May 2007

ACKNOWLEDGEMENTS: Dr Myers is supported by a Clinical Investigator Award from the Alberta Heritage Foundation for Medical Research.

CONFLICT OF INTEREST: Dr Poynard has a commercial interest in Biopredictive (Paris, France), the company marketing the SteatoTest.

APPENDIX

The logistic regression models for the steatosis indexes are as follows:

1) Leptinindex = 
   \[ \frac{1}{1 + \exp(- (2.71404 - 0.041667 \cdot \text{leptin}) \cdot \text{genotype})} \]

2) Leptin/geno = 
   \[ \frac{1}{1 + \exp(1.230441 - 0.066666 \cdot \text{leptin} - 3.132549 \cdot \text{genotype})} \]

Where leptin is measured in ng/mL, genotype 3=1 and nongenotype 3=0.
REFERENCES
