Original Article

THAI J GASTROENTEROL 2008
Vol. 9 No. 1
Jan. - Apr. 2008
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Correlation of Leptin and Severity of Hepatic Fibrosis in Thai Patients with Chronic Hepatitis C

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ABSTRACT

Background: Leptin is a peptide hormone that mainly regulates food intake, energy expenditure, body weight and reproductive function. Leptin also releases from activated hepatic stellate cells and may have a role in the regulation of fat deposition, fibrogenesis and inflammation. In human chronically infected by HCV (Hepatitis C virus), the role of leptin-associated steatosis and fibrosis of the liver is still unclear. There is no data in Thai patients with chronic HCV infection by regarding leptin level and its correlation with hepatic histology and fibrosis.

Objective: The purpose of this study was to evaluate the relationship between leptin level and severity of liver fibrosis, steatosis in Thai patients who chronically infected by HCV.

Methods: Sixty-six patients (31 men and 35 women) with chronic HCV infection diagnosed on the basis of biochemical data and positive for both anti-HCV antibody and HCV PCR were enrolled. Liver biopsies were done within 3 months after enrollment. Fasting blood samples were obtained and serum leptin levels were measured by using ELISA technique. BMI, blood sugar, liver function test, lipid profile, HCV RNA viral load and HCV genotype were also measured and correlated with histological findings.

Results: Mean serum leptin levels were significantly higher in women than in male (19.3 ± 10.5 ng/mL VS 6.5 ± 4.9 ng/mL, $p < 0.001$). There was a significantly correlation between serum leptin and BMI ($r = 0.469$, $p < 0.001$). Leptin levels were not associated with hepatic fibrosis ($r = 0.166$, $p = 0.183$), necroinflammation ($r = 0.203$, $p = 0.102$) and steatosis ($r = 0.231$, $p = 0.062$). Steatosis was significantly associated with severe necroinflammation ($r = 0.261$, $p = 0.034$), but not fibrosis ($r = 0.22$, $p = 0.076$).

Conclusions: These findings failed to demonstrate any usefulness of serum leptin in detecting more advanced liver disease and predicting the presence of hepatic steatosis in Thai patients who chronically infected with HCV.

Key words : Leptin, Hepatic fibrosis, Chronic hepatitis C

[Thai J Gastroenterol 2008; 9(1): 5-11.]

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INTRODUCTION

Hepatitis C virus (HCV) is a major health problem worldwide including Thailand[1]. It is the major cause of chronic hepatitis, which frequently leads to cirrhosis and also hepatocellular carcinoma. Liver histology in chronic hepatitis C (CHC) is characterized by several histological features ranging from bile duct damages, lymphoid follicles, steatosis and fibrosis.

Standard treatment of CHC is the combination of pegylated interferon and ribavirin dosed according to HCV genotype. The results of treatment in term of sustained virological response are also varied depending on treatment regimen and HCV genotypes, from just about 40% in genotype 1 to almost 90% in genotype 2, 3. These treatment regimens also have significant side effects, many of which may severely impair quality of life of the patients. So before treatment initiation, one must balance between risk and benefit of the treatment especially for those with HCV genotype 1 that poorly responds to the treatment. Liver biopsy will provide more information on histological stage of the disease, extent of parenchymal inflammation and fibrosis. In the presence of active histology or moderate to advanced fibrosis i.e. using METAVIR; necroinflammation and fibrosis score of 2 or greater, the likelihood of disease progression is high and treatment will be most beneficial. Steatosis found in histology is identified as another factor that may associate with progressive hepatic fibrosis[2]. Liver fatty change has been reported in approximately 30-70% of patients with CHC[3-6]. Recent observations in humans chronically infected with HCV indicate that steatosis is an important cofactor in accelerating both liver necroinflammatory activity and fibrosis[7-9].

Leptin is a 16 kDa protein was initially identified in 1994 as a product of adipocyte obese (ob) gene[10]. Several recent studies suggest that leptin regulates food intake, energy expenditure, body weight and reproductive function[11]. Leptin also releases from activated hepatic stellate cells and may have a role in the regulation of fat deposition, fibrogenesis and inflammation[12,13]. In animal models, leptin has been proposed as a mediator of liver histological changes such as steatosis and fibrosis[13,14]. In human chronically infected by HCV, the role of leptin-associated steatosis and fibrosis of the liver is still unclear. Some studies have found no links between leptin and steatosis or fibrosis[15] while the others have noted elevated levels of leptin in CHC associated with steatosis and fibrosis[16,17]. There is no data in Thai patients who chronically infected by HCV regarding leptin level and its correlation with hepatic histology and fibrosis.

The aim of this study was to evaluate the relationship between leptin level and severity of liver fibrosis, steatosis in Thai patients with chronic HCV infection.

MATERIAL AND METHODS

Study Population

This cross-sectional study included 66 patients who chronically infected by HCV at liver clinic, Siriraj Hospital, Mahidol University during January 2006 to January 2007. Patients would be assessed for enrollment into the present study if they fulfilled the following criteria: men or women at least 18 years old, chronic HCV infection diagnosed on the basis of biochemical data (serum aminotransferase greater than or equal 1.5 times the upper normal value for at least 6 months) and positive for both anti-HCV antibody and HCV PCR. All patients were negative for anti-HIV antibody in serum and liver biopsies were performed within 3 months before entry. Exclusion criteria included previous or current antiviral therapy, other causes of chronic liver disease including; alcohol, chronic hepatitis B, hepatotoxic drugs or herbs, autoimmune hepatitis, hemochromatosis, Wilson's disease, pregnancy or lactation and contraindication for liver biopsy.

This study was approved by institutional ethic committee and all subjects gave written informed consent.

Methods

Body mass index (BMI) was calculated using weight (kilograms) divided by height (meters) squared. Alcohol consumption history was independently assessed by physicians in liver clinic by interviewing patients and family members if possible.

Anti-HCV antibodies were detected by third generation enzyme immunoassays. HCV RNA was detected in serum by RT-PCR method (Amplicor; Roche Diagnostic Systems, Branchburg, NJ, USA). HCV viral loads were determined by PCR-based technique (Cobas Amplicor Monitor HCV Test Version # 2.0; Roche Molecular Systems, Branchburg, NJ, USA). All biochemical data were collected after an overnight fasting at the day of performing liver biopsy. Serum leptin levels were measured by ELISA for human leptin

(BioVendor Laboratory Medicine, Inc., Czech Republic). The limit of detection was 0.17 ng/mL with the intra and inter-assay coefficients of variation of 5.4% and 6.8%, respectively.

A liver biopsy was performed in all patients. Liver biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin for staining with haematoxylin-eosin and Masson trichrome. All specimens were examined by a single pathologist with blind to the clinical and laboratory finding of patients. Liver fibrosis was assessed semi-quantitatively using the METAVIR score which classifies into five stages (F0 = no fibrosis, F1 = portal fibrosis and no septa, F2 = portal fibrosis and a few septa, F3 = septal fibrosis and no cirrhosis, F4 = cirrhosis). Necroinflammatory activity of liver was determined by semi-quantitatively using the METAVIR score which classifies into four stages (A0 = none, A1 = mild inflammation, A2 = moderate inflammation, A3 = severe inflammation). Steatosis was assessed semi-quantitatively by Brunt grading systems which classifies into four grades (grade 0 = no steatosis, grade 1 = steatosis up to 33%, grade 2 = steatosis from 33% up to 66%, grade 3 = steatosis more than 66%).

Statistical analysis

Quantitative variables were expressed as mean ± SD and were compared using the Mann-Withney U-test. Categorical variables were tested by the χ^2 -test and the 2-tailed Fisher exact test. Liver fibrosis was classified as a categorical variable either being none to moderate (F0-F2) or severe (F3-F4). Liver necroinflammatory activity was classified as a categorial variable either being none to mild (A0-A1) or moderate to severe (A2-A3). Multivariate analysis was performed through logistic regression to determine independent metabolic predictors of liver fibrosis, necroinflammation and steatosis. The relationship between leptin and metabolic parameters were assessed by linear regression. A p-value <0.05 was considered significant.

RESULTS

Patient Demographic Data

The demographic data and virological characteristics of patients are presented in Table1. The mean age of the patients was 49.6 ± 9.8 years with an estimated age at infection and duration of infection in those with known onset of 28.3 ± 9.1 and 21 ± 9.5 years, respectively. The study population comprised 31 men and 35 women. The possible sources of infection were intravenous drug abuse (N = 5), blood transfusion (N = 30), tattoo (N = 2), health worker occupation (N = 1) and unknown etiology (N = 28). The mean BMI was 24.9 ± 3.5 kg/m^2. Genotype 1 was found in 37 patients (56.1%), genotype 3 in 26 patients (39.4%), genotype 4 in 1 patient (1.5%) and un-identified in 2

Table 1 Demographic data and virological data of the study population

Variables	Patients (N = 66)
Age (years)	49.6 ± 9.8
Sex (male/female)	31/35
BMI (kg/m^2)	24.9 ± 3.5
Alcohol consumption (g/day)	0.9 ± 6.6
Age at infection (years)	28.3 ± 9.1
Duration of infection (years)	21 ± 9.5
Source of infection (%)	
Intravenous drug abuse	5 (7.6)
Blood transfusion	30 (45.5)
Tattoo	2 (3)
Health worker occupation	1 (1.5)
Unknown	28 (42.4)
Genotype (%)	
Genotype 1	37 (56.1)
Genotype 2	0 (0)
Genotype 3	26 (39.4)
Genotype 4	1 (1.5)
Un-identified genotype	2 (3)
Viral load (× 10^6 IU/mL)	4.65 ± 10.91

BMI = body mass index.

Table 2 Laboratory data of the study population

Variables	Patients (N = 66)
Blood sugar (mg/dL)	96.4 ± 18.2
Cholesterol (mg/dL)	190.2 ± 33.5
Triglycerides (mg/dL)	110.9 ± 44.3
Prothrombin time (seconds)	12.0 ± 0.9
Liver chemistry	
Albumin (g/dL)	4.2 ± 0.3
Globulin (g/dL)	3.9 ± 0.7
AST (U/L)	83.8 ± 55.7
ALT (U/L)	110 ± 68.3
Total bilirubin (mg/dL)	0.8 ± 0.3
Leptin (ng/mL)	13.3 ± 10.5

AST = aspatate aminotransferase, ALT = alanine aminotransferase

patients (3%). The mean HCV viral load was $4.65 \times 10^6 \pm 10.9 \times 10^6$ IU/mL. The laboratory data of patients are presented in Table 2. Due to the sex dependency of plasma leptin levels, the severity of liver fibrosis and all metabolic data were divided into two groups (males and females). Demographic, virological and biochemical data of the study population according to the severity of liver fibrosis are presented in Table 3. Globulin, AST, ALT, and necroinflammation were significantly higher in patients with severe liver fibrosis while albumin/globulin ratio was significantly higher in patients with none to moderate liver fibrosis (Table 3). Demographic, virological and biochemical data of the study population according to the severity of liver necroinflammation are presented in Table 4. Globulin, AST, ALT and steatosis were significantly more pronounced in patients with moderate to severe necroinflammation while albumin/globulin

Table 3 Demographic, virological and biochemical data of the study population according to the severity of liver fibrosis

Variables	F0-F2 (N = 48)	F3-F4 (N = 18)	p-value
Age (years)	49.0 ± 10.2	51.1 ± 8.7	0.445
Sex (male/female)	23/25	8/10	0.801
BMI (kg/m^2)	24.6 ± 3.4	25.7 ± 3.7	0.291
Alcohol consumption (g/day)	1.4 ± 7.7	0	0.426
Age at infection (years)	28.9 ± 9.3	26.6 ± 8.8	0.501
Duration of infection (years)	19.4 ± 8.6	24.9 ± 10.8	0.125
Source of infection (%)			
Intravenous drug abuse	3	2	
Blood transfusion	22	8	0.669
Tattoo	2	0	
Health worker occupation	1	0	
Unknown	20	8	
Genotype (%)			
Genotype 1	27	10	
Genotype 3	19	7	0.78
Genotype 4	1	0	
Un-identified genotype	1	1	
Viral load ($\times 10^6$ IU/mL)	5.35 ± 12.59	2.86 ± 40.78	0.416
Leptin (ng/mL)	12.1 ± 10.3	16.4 ± 10.8	0.147
Blood sugar (mg/dL)	94.2 ± 14.0	100.6 ± 24.2	0.249
Cholesterol (mg/dL)	191.8 ± 32.9	186.6 ± 36.0	0.656
Triglycerides (mg/dL)	106.0 ± 45.7	122.5 ± 40.4	0.286
Prothrombin time (seconds)	11.9 ± 0.9	12.4 ± 0.9	0.071
Liver chemistry			
Albumin (g/dL)	4.2 ± 0.3	4.0 ± 0.3	0.085
Globulin (g/dL)	3.9 ± 0.6	4.3 ± 0.7	0.019
Albumin/Globulin	1.1 ± 0.2	0.9 ± 0.5	0.045
AST (U/L)	68.2 ± 43.1	125.5 ± 64.6	<0.001
ALT (U/L)	96.5 ± 58.6	146.1 ± 80.2	0.007
AST/ALT	0.8 ± 0.3	0.9 ± 0.3	0.112
Total bilirubin (mg/dL)	0.7 ± 0.3	0.9 ± 0.5	0.184
Steatosis (with/without)	7/41	1/17	0.564
Necroinflammation (with/without)	3/45	0/18	<0.0001

BMI = body mass index, AST = aspatate aminotransferase, ALT = alanine aminotransferase, AST/ALT = AST/ALT ratio, Albumin/Globulin = Albumin/Globulin ratio.

ratio and albumin were significantly higher in patients with none to mild necroinflammation (Table 4).

Leptin levels

Mean fasting serum leptin levels were significant higher in females (19.3 ± 10.5 ng/mL) than in males (6.5 ± 4.9 ng/mL, p <0.001) (Table 5). When compared with previous study in Thai patients with NAFLD[18], leptin levels were higher in patients chronically infected with HCV (both males and females) than in controls (control males = 6.1 ± 1.4 ng/mL, control females = 8.4 ± 2.1 ng/mL).

Liver Histology

The liver histology of patients is presented in Table 6. Steatosis was detected in 58 of 66 patients

Table 4 Demographic, virological and biochemical data of the study population according to the severity of liver necroinflammatory activity

Variables	A0-1 (N = 33)	A2-3 (N = 33)	p-value
Age (years)	47 ± 10.6	51.5 ± 8.7	0.111
Sex (male/female)	17/16	14/19	0.459
BMI (kg/m^2)	24.6 ± 3.7	25.3 ± 3.4	0.489
Alcohol consumption (g/day)	1.9 ± 9.3	0	0.237
Age at infection (years)	27.7 ± 9.8	28.8 ± 8.4	0.725
Duration of infection (years)	20.4 ± 8.1	21.6 ± 10.9	0.726
Source of infection (%)			
Intravenous drug abuse	2	3	
Blood transfusion	17	13	0.723
Tattoo	1	1	
Health worker occupation	1	0	
Unknown	12	16	
Genotype (%)			
Genotype 1	20	17	
Genotype 2	0	0	0.831
Genotype 3	12	14	
Genotype 4	1	0	
Unidentify genotype	0	2	
Viral load (× 10^6 IU/mL)	6.86 ± 14.84	2.45 ± 3.42	0.107
Leptin (ng/mL)	11.7 ± 10.7	14.9 ± 10.3	0.209
Blood sugar (mg/dL)	93.6 ± 13.0	98.7 ± 21.6	0.331
Cholesterol (mg/dL)	192.1 ± 31.8	185.0 ± 36.2	0.705
Triglycerides (mg/dL)	104.6 ± 46.9	117.9 ± 41.4	0.349
Prothrombin time (seconds)	11.9 ± 1.0	12.2 ± 0.9	0.172
Liver chemistry			
Albumin (g/dL)	4.3 ± 0.4	4.1 ± 0.3	0.014
Globulin (g/dL)	3.7 ± 0.7	4.2 ± 0.5	0.002
Albumin/Globulin	1.2 ± 0.3	0.9 ± 0.1	<0.0001
AST (U/L)	51.4 ± 25.9	116.3 ± 58.6	<0.0001
ALT (U/L)	71.2 ± 36.6	148.8 ± 70.9	<0.0001
AST/ALT	0.8 ± 0.4	0.8 ± 0.2	0.868
Total bilirubin (mg/dL)	0.7 ± 0.3	0.8 ± 0.4	0.278
Steatosis (with/without)	7/26	1/32	0.039

BMI = body mass index, AST = aspatate aminotransferase, ALT = alanine aminotransferase, AST/ALT = AST/ALT ratio, Albumin/Globulin = Albumin/Globulin ratio.

Table 5 Metabolic data in both male and female patients

Variable	Male	Female	p-value
Age (years)	48.6 ± 10.1	50.5 ± 9.6	0.421
Alcohol consumption (g/day)	2.1 ± 9.5	0	0.238
Age of infection (years)	25.9 ± 8.9	31.5 ± 8.4	0.068
Duration of infection (years)	22.2 ± 10.9	19.4 ± 7.1	0.366
BMI (kg/m^2)	25.1 ± 3.3	24.9 ± 3.8	0.824
Blood sugar (mg/dL)	95.8 ± 12.4	97.0 ± 22.7	0.814
Cholesterol (mg/dL)	188.1 ± 24.4	192.7 ± 42.3	0.692
Triglycerides (mg/dL)	118.3 ± 49.8	101.9 ± 35.9	0.250
AST (U/L)	77.4 ± 53.3	89.5 ± 58.6	0.383
ALT (U/L)	116.8 ± 71.5	103.9 ± 65.7	0.448
Leptin (ng/mL)	6.5 ± 4.9	19.3 ± 10.5	<0.0001

BMI = body mass index, AST = aspatate aminotransferase, ALT = alanine aminotransferase.

(87.9%); grade 1 in 48 patients (72.7%), grade 2 in 9 patients (13.6%) and grade 3 in 1 patient (1.5%). Stage of fibrosis was F0 in 4 patients (6.1%), F1 in 24 patients (36.4%), F2 in 20 patients (30.3%), F3 in 10 patients (15.2%) and F4 in 8 patients (12.1%). The grade of necroinflammation was A0 in 3 patients (4.5%), A1 in 30 patients (45.5%), A2 in 28 patients (42.4%) and A3 in 5 patients (7.6%).

Relationship between serum leptin, metabolic characteristics and liver fibrosis

The following variables were evaluated by univariate analysis: age, age at infection, duration of infection, BMI, HCV genotype, HCV viral load, triglycerides, cholesterol, blood sugar, leptin (Figure 1), AST, ALT, albumin, globulin, steatosis and necroinflammation. Only necroinflammation was significantly and independently associated with severe fibrosis (OR = 14.59, CI 95%: 2.99-71.14, p = 0.03). Leptin levels were not associated with hepatic fibrosis (r = 0.166, p = 0.183), necroinflammation (r = 0.203, p = 0.102) and steatosis (r = 0.231, p = 0.062). Steatosis was significantly associated with severe necroinflammation (r = 0.261, p = 0.034), but not fibrosis (r = 0.22, p = 0.076). Leptin levels were significantly associated with BMI (r = 0.469, p <0.001).

Discussion

This is the first study assessing correlation of leptin levels and hepatic fibrosis in Thai patients who chronically infected with HCV. From the present study steatosis was found 88% in the study population which

Table 6 Liver histology of the study population

Variables	Patients (N = 66)
Fibrosis (%)	
F0	4 (6.1)
F1	24 (36.4)
F2	20 (30.3)
F3	10 (15.1)
F4	8 (12.1)
Necroinflammatory activity	
A0	3 (4.5)
A1	30 (45.5)
A2	28 (42.4)
A3	5 (7.6)
Steatosis	
Grade 0	8 (12.1)
Grade 1	48 (72.8)
Grade 2	9 (13.6)
Grade 3	1 (1.5)

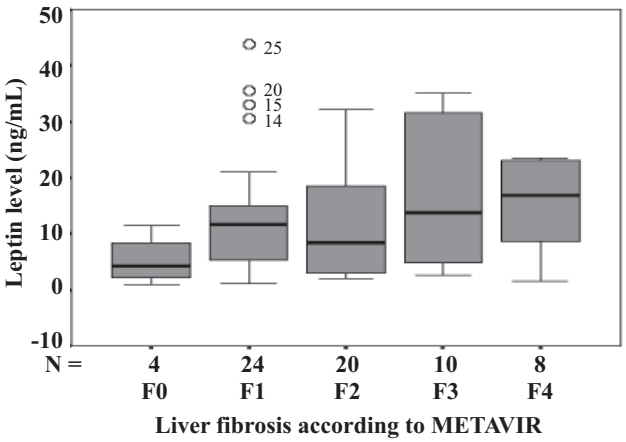

Figure 1 Leptin levels (mean ± SD) according to the severity of liver fibrosis

higher than previous finding in the literature[3-6]. The mechanism by which HCV induces steatosis remains unclear. The virus can increase glutathione turnover by eliciting free radical-mediated lipid peroxidation which would affect iron metabolism within the hepatocyte and which may promote fat droplet deposition[19]. Accordingly an increase in the concentration of monounsaturated fatty acid has been found in the liver of patients with CHC[20]. Thus, HCV may cause a change in lipid metabolism and promote triglyceride accumulation resulting in hepatocyte steatosis. Differently from previous finding in the literature, we found that steatosis correlate with hepatic necroinflammation, not hepatic fibrosis.

In the present study, leptin levels were significantly higher in females than males and significantly correlated with BMI. However, we failed to show any significant correlation between serum leptin levels and hepatic fibrosis, necroinflammation and steatosis. Only necroinflammation was significantly correlated with hepatic fibrosis. This might be against the potential role leptin in the pathogenesis of steatosis and hepatic fibrosis in CHC patients. One reason that we could not find any significant correlation may be due to small number of the patients in our study.

The limitation of this study was due to a small group of patients and we did not exclude the patient with higher BMI which may contribute to higher leptin level. Thus, some patients with lower stage of fibrosis but high BMI may have serum leptin levels as high as patients with higher stage of fibrosis. Thus, further studies are needed to determine more clearly the correlation of leptin and hepatic fibrosis.

In conclusion, we failed to demonstrate any usefulness of serum leptin in detecting more advanced liver disease and predicting the presence of hepatic steatosis in Thai patients with chronic HCV infection. This data did not support the role of leptin in the pathogenesis of hepatic fibrosis. However, serum leptin in CHC patients was higher than in controls. We need the further study to elucidate the significant of leptin in pathogenesis of hepatic steatosis, fibrosis and its usefulness for determining hepatic severity.

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