Original Article

Preliminary Study of the Level of Visfatin and the Relationship with Insulin Resistance in Chinese Patients with Chronic Hepatitis C

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Abstract

Background: Many studies have suggested that visfatin expression is closely related to the occurrence of insulin resistance (IR), while the precise role of visfatin in the regulation of IR in chronic hepatitis C (CHC) is not clear.

Methods: We investigated fasting glucose, fasting insulin (FINS), C peptide, visfatin, visfatin mRNA, interleukin (IL)-6, tumor necrosis factor (TNF)-α, C-reactive protein (CRP) and other parameters of 315 patients with CHC and 150 control cases in China. Meanwhile we collected clinical and other laboratory data for further analysis.

Results: Compared with the control group, the CHC group had a significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), the AST to platelet ratio index (APRI), ratio of AST to ALT (AAR), gammaglutamyl trans-peptidase, IL-6, TNF-α, visfatin, visfatin mRNA, FINS, fasting C peptide, and IR index. The visfatin, visfatin mRNA, insulin, IR index, Homaβ cell function index (HBCI), and fasting β-cell function index (FBCI) of the subjects with high body mass index (BMI) from the CHC sub-group were significantly higher than the normal BMI sub-group of CHC patients. We found a positive correlation between visfatin, visfatin mRNA and BMI, IL-6, TNF-α, and IR index.

Conclusion: Our data suggest that visfatin may be related to IR in Chinese CHC patients.

Keywords: Chronic hepatitis C, insulin resistance, liver, visfatin

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Introduction

Insulin resistance (IR) is a state in which a given concentration of insulin produces a less-than-expected biological effect. It is closely related to glucose metabolism, lipid metabolism, hypertension, and obesity. The liver plays a key role in nutrient and hormone metabolism,[1,2] and hepatogenous diabetes is used to describe the state of hyperglycemia in advanced cirrhosis. Some reports confirm a close relation between chronic hepatitis C (CHC) and IR.[3,4] IR not only accelerates the development of liver fibrosis and liver cancer but also interferes with anti-viral treatment. IR significantly improves following antiviral therapy in CHC patients, indicating that early diagnosis and treatment of IR not only reduces viral replication, but also prevents disease progression.[5–8] Hepatitis C virus (HCV) may lead to IR either directly via its core protein, or indirectly via the induction of cytokines,[9] while the mechanism is complicated and not clear.[9–11]

Visfatin is a fat factor that plays an insulin-like role; it decreases blood glucose by binding the insulin receptor, promoting the differentiation and synthesis of adipose tissue. Many studies have found a close relation between visfatin expression and the occurrence of IR.[12–16] Therefore, we speculate that visfatin may play a role in IR in CHC. Tumor necrosis factor (TNF)-α and interleukin (IL)-6 play an important role in IR development[17,18] and are significantly higher in CHC.[19,20]

Approximately 40 million people in China are infected with hepatitis C, according to a report at the 62nd Annual Meeting of the American Society of Study of Liver Diseases (AASLD). Huang et al.[21] have reported a relationship between serum visfatin, disease severity and metabolic syndrome in Chinese CHC patients. The precise role of visfatin in the regulation of IR in CHC is not definite. To address this question, we have attempted to detect serum visfatin and visfatin mRNA in peripheral blood mononuclear cells and investigate the role of visfatin in the occurrence of IR in CHC.

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Materials and Methods

Patient selection

This study was approved by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical College and all patients provided written informed consent to participate.

CHC group (group 1)

The study was performed on 315 patients with CHC (120 females/195 males) that were infected with chronic hepatitis C (HCV). Patients had persistently elevated alanine aminotransferase (ALT) levels for at least six months. Participants were between the ages of 23 and 81 years (average: 40.9 ± 13.9) and inpatients at the First Affiliated Hospital of Wenzhou Medical College during June 2009 to December 2011. Diagnosis of CHC was confirmed by the presence of serum HCV-RNA and viral load assayed by real-time fluorescent quantitative RT-PCR (ABI7000 Automated Fluorescence Quantitative PCR Analyzer, USA). Exclusion criteria included: drug or alcohol abuse; autoimmune, neoplastic, thyroid and psychiatric diseases; other hepatitis or HIV co-infections; diabetes mellitus; hyperlipidemia; high blood pressure; and renal or heart failure. There was no significant difference between men and women with regards to age ($t = 0.261$, $P > 0.05$). According to

"the knowledge of maintaining a healthy weight" announced by the Chinese Ministry of Health in 2009 and the normal BMI range is 18.5 to 24 kg/m^2 In China. Therefore we subdivided the CHC group into normal BMI (< 24 kg/m^2, n = 200) and elevated BMI (≥ 24 kg/m^2, n = 115) sub-groups.

Control group (group 2)

The group encompassed 100 healthy volunteers (50 females/50 males) who lacked serum HCV-RNA and anti-HCV antibodies and were HIV and HBsAg negative. Controls had alcohol consumption of less than 20 g/day and normal ALT levels. They ranged in age from 24–80 years (average: 38.3 ± 10.3) and had a body mass index (BMI) of 21.7 ± 1.9 kg/m^2 (range: 18.5–24 kg/m^2). The control group had normal fasting glucose levels, oral glucose tolerance test results and lipid profiles. Exclusion criteria included heart, lung, liver, kidney and endocrine diseases. Healthy volunteers were chosen from a large number of people who underwent physical examinations.

Serological assays

Blood from groups 1 and 2 were collected after more than 12 hr fasting. All samples were centrifuged and serum was frozen at -70ºC for further processing. The levels of fasting blood glucose (FBG), ALT, aspartate aminotransferase (AST), albumin (Alb), total protein (TP), and the lipid profile were detected by a Hitachi 7600 Automatic Biochemical Analyzer (Hitachi Corporation, Japan). Fasting insulin (FINS) and fasting C peptide were assayed by a Roche E170 analyzer (Roche Corporation, Germany); C-reactive protein (CRP) was measured in a Beckman IMMAGE8000 Analyzer (Beckman Coulter Corporation, USA). Visfatin serum concentrations were assessed by a commercially available EILSA Assay Kit (BioVision Corporation, USA). IL-6 and TNF-α were detected by the radioimmunoassay method (Beijing Puerweiye Biotechnology Co., Ltd., China) and HCV-RNA was detected by quantitative RT-PCR (ABI7000 Automated Fluorescence Quantitative PCR Analyzer, USA). Insulin parameters were calculated as follows: Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) = FINS × FBG/22.5; Homaβ cell function index (HBCI) = 20 × FINS/(FBG-3.5); fasting β-cell function index (FBCI) = FINS/FBG; and insulin sensitivity index (IAI) = 1/(FBG × FINS). The parameters that reflected changes in liver tissue specimens were the AST/ALT ratio (AAR) = AST/ALT and AST to platelet ratio index (APRI) = [(AST/ULN)/platelet count (10^9)] × 100.

SYBR green real-time PCR

RNA was extracted from peripheral blood mononuclear cells of both groups by Trizol reagent (Invitrogen, USA). A total of 1 μg RNA was reverse transcribed to first-strand DNA with the SuperScript® II system (Invitrogen, USA). The primers for visfatin were designed with Primer Premier 5.0 as follows: forward 5'-ATGTTCTCTTCACGGTCGAAAAC-3' and reverse 5'-GGCCACTTTGATTGGATACCA-3'. We performed real-time PCR with the SYBR Green PCR Master Mix (Perkin-Elmer Applied Biosystems, Warrington, United Kingdom) and amplified cDNA with an ABI7000 Automated Fluorescence Quantitative PCR Analyzer (Perkin-Elmer Applied Biosystems) under universal thermal cycling conditions. Experimental results were normalized to the threshold cycle (CT) of ß-actin, according to the appropriate standard curves. The CT values were converted to relative transcript copy numbers.

Statistical analysis

All values are expressed as mean ± SD. The Shapiro-Wilk test was used to evaluate the distribution. Differences in studied variables between groups were tested using the t-test for independent groups. Correlations were analyzed with the Spearman rank correlation coefficient. We considered $P < 0.05$ to be statistically significant. The statistical analysis was performed with SPSS 13.0 (Statistical Package for the Social Sciences Corporation, USA).

Results

Comparison of parameters between groups 1 and 2

The levels of ALT, AST, apoptosis-related protein 1 (APRI), AAR, gamma-glutamyl transpeptidase (GGTP), IL-6, TNF-α, visfatin, visfatin mRNA, FINS, fasting C peptide, and HOMA-IR were significantly higher in group 1 compared with group 2 (Table 1). High-density lipoprotein cholesterol (HDL-C) and FBG concentrations were significantly lower ($P = 0.013$). No significant difference was observed in HBCI, FBCI, and IAI levels ($P > 0.05$; Table 1).

Comparison of parameters in subgroups with different body mass indices (BMIs) from group 1

In CHC patients, the levels of visfatin, visfatin mRNA, and FINS, and the values of HOMA-IR, HBCI, and FBCI were significantly higher in the sub-group with increased BMI compared to those with normal BMI (Table 2). All other parameters showed no statistical differences. The visfatin level of the normal BMI sub-group of CHC patients was higher than the control group ($P = 0.027$; Table 2).

Correlations of visfatin in CHC to other parameters

Pearson correlation analysis revealed a positive correlation between visfatin and visfatin mRNA levels to BMI, HOMA-IR, IL-6, and TNF-α. Other parameters showed no statistically significant correlations (Table 3).

Discussion

In recent years, based on epidemiological and clinical data, numerous researchers have shown that the incidence of diabetes in HCV patients is significantly higher than in those with chronic hepatitis B. CHC patients with diabetes and impaired glucose tolerance (IGT) have been shown to have higher HOMA-IR results than those with normal glucose tolerance (NGT), which suggested the possibility of IR in CHC patients who were glucose intolerant. Serum insulin, C peptide, and HOMA-IR values of CHC patients with stage 0 or stage 1 fibrosis and no diabetes were higher than those of healthy controls.[22–27] In the current study, serum insulin, C peptide, and HOMA-IR values were higher in CHC patients compared to the control group, which supported previous reports. The results also showed significantly higher visfatin and visfatin mRNA levels compared to the control group, which positively correlated with HOMA-IR. CHC patients had higher BMI, plasma visfatin, and visfatin mRNA values, which suggested that the protein and mRNA of visfatin increased in CHC, thus these patients were more susceptible to IR. Our data found that visfatin had an important relationship with glucose metabolism disorders

Table 1. Patient information and research data for both study groups.

Parameter	CHC group (n = 315)	Control group (n = 100)	P-value*
Age (years)	40.9 ± 13.9	38.3± 10.3	0.562
Weight (kg)	61.1 ± 10.9	65.1 ± 12.6	0.714
Height (m)	1.65 ± 0.04	1.67 ± 0.08	0.631
BMI (kg/m^2)	22.4 ± 3.8	23.9 ± 3.3	0.723
ALT (IU/L)	85.6 ± 14.4	18.6 ± 4.1	0.002
AST (IU/L)	68.3 ± 26.2	21.3 ± 5.3	0.011
APRI	0.64 ± 0.08	0.11 ± 0.04	0.014
AAR	1.37 ± 0.71	0.87 ± 0.45	0.036
GGTP (IU/L)	60.6 ± 68.7	27.0 ± 5.3	0.002
Viral load (copies/mL)	16137741.0 ± 15684382.0	—	—
PLT (10^9/L)	176.5 ± 79.3	201.9 ± 52.0	0.334
Alb (g/L)	42.7 ± 6.5	45.2 ± 3.4	0.235
TP (g/L)	74.6 ± 7.5	77.5 ± 4.0	0.239
TG (mmol/L)	4.09 ± 0.98	4.24 ± 0.54	0.561
HDL-C (mmol/L)	1.10 ± 0.34	1.41 ± 0.32	0.013
LDL-C (mmol/L)	2.24 ± 0.87	2.39 ± 0.37	0.609
TG(mmol/L)	1.31 ± 0.85	0.96 ± 0.41	0.229
FBG (mmol/L)	5.56 ± 1.77	5.28 ± 0.45	0.619
FINS (mmol/L)	83.92 ± 17.11	52.29 ± 8.01	0.031
Fasting C peptide (mmol/L)	866.92 ± 64.11	523.37 ± 30.80	0.027
HOMA-IR	2.34 ± 0.21	1.12 ± 0.59	0.017
HBCI	776.25 ± 83.41	592.93 ± 78.39	0.922
FBCI	11.55 ± 0.71	9.81 ± 0.98	0.618
IAI	0.0061 ± 0.0072	0.0046 ± 0.0022	0.510
CRP (mmol/L)	8.31 ± 2.86	2.71 ± 0.81	0.468
IL-6 (pg/mL)	50.72 ± 18.76	15.38 ± 8.08	0.003
TNF-α (pg/mL)	1.84 ± 0.30	1.071 ± 0.16	0.003
Visfatin (pg/mL)	7553.62 ± 165.25	2268.48 ± 133.46	0.011
Visfatin mRNA	188.4 ± 15.8	23.6 ± 2.8	0.001

CHC = Chronic hepatitis C; TG = Triglycerides; TCH = Total cholesterol; FBG = Fasting blood glucose; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; Alb = Albumin; TP = Total protein; FINS = Fasting insulin; HBCI = Homaβ cell function index; FBCI = Fasting β-cell function index; IAI = Insulin sensitivity index; CRP = C-reactive protein; AAR = AST/ALT ratio; APRI = AST to platelet ratio index; BMI = body mass index; HOMA-IR = Homeostasis Model of Assessment - Insulin Resistance.*Values of $P < 0.05$ are considered statistically significant.

Table 2. Results of parameters in different body mass index (BMI) sub-groups of chronic hepatitis C (CHC) patients.

Parameters	Normal BMI group	Elevated BMI group	P-value*
Visfatin (pg/mL)	5551.05 ± 118.99	8254.62 ± 190.42	0.018
FINS (mmol/L)	74.80 ± 10.97	102.15 ± 21.5	0.014
HOMA-IR	1.16 ± 0.78	3.41 ± 1.79	0.018
HBCI	694.62 ± 273.79	2581.64 ± 770.39	0.002
FBCI	10.12 ± 5.64	31.50 ± 25.61	0.012
Visfatin mRNA	90.8 ± 10.7	214.5 ± 42.5	0.002

*Values of $P < 0.05$ are considered statistically significant.

Table 3. Correlations of visfatin in chronic hepatitis C (CHC) to other parameters.

		BMI	HOMA-IR	IL-6	TNF-α
Visfatin (pg/mL)	Pearson	0.170	0.427	0.514	0.354
	P-value	0.035	0.002	0.001	0.027
Visfatin mRNA	Pearson	0.223	0.512	0.702	0.431
	P-value	0.015	0.000	0.000	0.003

and obesity. Additionally, in the current study CHC patients had lower levels of HDL-C. The visfatin level in CHC patients with normal BMI was higher than the control group, which revealed that BMI did not affect the level of visfatin.

Levels of cytokines, such as TNF-α, and IL-6, CRP, and inhibitors of cytokine signaling closely correlated with IR. The levels of TNF-α and IL-6 significantly increased in HBV and HCV infections.[28,29] Moschen[30] reported that visfatin increased the levels of IL-6 and TNF-α. Our study showed higher TNF-α and IL-6 levels in CHC patients compared to the control group. There was a significant association between visfatin and the levels of IL-6 and TNF-α. Visfatin might contribute increased expressions of IL-6 and TNF-α *in vivo*, thereby cause an increased occurrence of IR in CHC patients. We concluded that visfatin might induce the synthesis of IL-6 and TNF-α, which would result in adverse effects on insulin sensitivity. However the exact mechanism needed additional study.

Conclusion

In the presence of CHC with IR and dyslipidemia in vivo, visfatin levels may be closely associated with IR. The synthesis of IL-6 and TNF-α would be induced, which adversely impacts insulin sensitivity. While visfatin appears to exert a variety of biological effects, its exact role and mechanism remain unclear and need further study. Overall, the discovery of visfatin has added new dimensions to the study of IR pathogenesis in CHC and may provide a new target in the treatment of hepatic diabetes.

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