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RESEARCH REPORT

Insulin resistance, adipokine profile and hepatic expression of *SOCS-3* gene in chronic hepatitis C

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Abstract

AIM: To analyze adipokine concentrations, insulin resistance and hepatic expression of suppressor of cytokine signaling 3 (SOCS-3) in patients with chronic hepatitis C genotype 1 with normal body weight, glucose and lipid profile.

METHODS: The study group consisted of 31 patients with chronic hepatitis C and 9 healthy subjects. Total levels of adiponectin, leptin, resistin, visfatin, omentin, osteopontin and insulin were measured using an ELISA kit. The hepatic expression of SOCS-3 was determined by the use of the reverse transcription polymerase chain reaction method.

RESULTS: Homeostasis model assessment for insulin resistance (HOMA-IR) values were significantly higher in hepatitis C virus (HCV) infected patients without metabolic disorders compared to healthy controls (2.24 *vs* 0.59, $P = 0.0003$). Hepatic steatosis was observed in 32.2% of patients with HCV infection and was found in patients with increased HOMA-IR index (2.81 *vs* 1.99, $P = 0.05$) and reduced adiponectin level (5.96 vs 8.37, $P = 0.04$). Inflammatory activity ($G \geqslant 2$) was related to increased osteopontin concentration (34.04 *vs* 23.35, $P = 0.03$). Advanced liver fibrosis ($S \geqslant 2$) was associated with increased levels of omentin and osteopontin (436.94 *vs* 360.09, $P = 0.03$ and 32.84 *vs* 20.29, $P = 0.03$) and reduced resistin concentration (1.40 *vs* 1.74, $P = 0.047$). No correlations were reported between adipokine profile, HOMA-IR values and hepatic expression of the *SOCS-3* gene.

CONCLUSION: We speculated that no relationship between adipokines and HOMA-IR values may indicate that HCV can induce insulin resistance itself. Some adipokines appear to be biochemical markers of steatosis, inflammation and fibrosis in patients with chronic HCV infection.

Key words: Adipokines; Steatosis; Chronic hepatitis C; Insulin resistance; *SOCS-3* gene

Core tip: To investigate the direct effect of hepatitis C virus (HCV) on insulin resistance we analyzed adipokines and homeostasis model assessment for insulin resistance (HOMA-IR) in selected chronic hepatitis C patients without metabolic disorders. In this group of patients we confirmed higher HOMA-IR values compared to healthy subjects. We speculated that no relationship between adipokines and HOMA-IR values may indicate that HCV can induce insulin resistance itself, regardless of metabolic disorders. This is the first study to determine the impact of a wide spectrum of adipokines and HOMA-IR index on histopathological changes in liver biopsy specimens and hepatic expression of suppressor of cytokine signaling 3 mRNA.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a worldwide health problem. HCV is a positive strand RNA virus that infects about 3% of the world population. The World Health Organization (WHO) estimates that 3-4 million new HCV infections occur every year[1]. Hepatitis C virus is an important risk factor for insulin resistance[2,3]. The prevalence of insulin resistance in chronic HCV infection is estimated to be between 30% to 70% of chronic HCV patients and is higher than in chronic hepatitis B (10%)[4-6]. The mechanism of insulin resistance in chronic hepatitis C is complex and bidirectional, and the exact pathogenesis is still unknown. Some clinical observations support a "fat independent" mechanism in the development of insulin resistance in HCV-infected subjects[7]. Koike[8] demonstrated that HCV can induce insulin resistance itself by disturbing the insulin signaling pathway in an animal model. It was found that the core HCV protein stimulates increased levels of the molecule suppressor of cytokine signaling 3 (SOCS-3), which leads to ubiquitination and proteasomal degradation of insulin signaling receptors 1 and 2[9]. The other mechanism through which HCV contributes to the development of insulin resistance might be its impact on adipokine concentration, although the role of adipokines in the pathogenesis of insulin resistance is not fully elucidated. Hence, this study analyzes both mechanisms of insulin resistance in patients with chronic hepatitis C without metabolic disorders.

The aim of this study was to analyze adipokine concentrations and homeostasis model assessment for insulin resistance (HOMA-IR) values in healthy subjects and in patients infected with chronic HCV genotype 1 and normal body weight, glucose and lipid profiles. It focuses on the relationship between adipokine profile, HOMA-IR index and the histopathological changes in liver biopsy specimens. It also attempts to determine whether adipokine serum concentrations and HOMA-IR values are associated with hepatitis C viremia. Moreover, adipokine concentrations and HOMA-IR index are determined according to interleukin 28B (IL28B) single-nucleoside polymorphism C/T (rs 12979860) and hepatic expression of suppressor of cytokine signaling 3 (SOCS-3) mRNA.

MATERIALS AND METHODS

Patient population

The study group consisted of 31 patients with chronic hepatitis C and 9 healthy subjects. The diagnosis of chronic hepatitis C was based on detectable HCV viremia in serum and liver biopsy examination.

The exclusion criteria included diabetes mellitus and glucose intolerance, being overweight [body mass index (BMI) > 25], lipid disorders, other causes of liver disease, previous antiviral treatment and consumption of more than 20 g alcohol per day. Pregnant women and patients receiving concomitant medication, including lipid-lowering drugs, were also excluded from this analysis.

All subjects enrolled in this study provided their written informed consent. This study was approved by the local Bioethics Committee.

Liver histology

The grade of inflammation and necrotic changes, as well as stage of fibrosis, were assessed according to the 1995 Batts and Ludwig scale. The hepatic expression of SOCS3 was determined by the use of the reverse transcription polymerase chain reaction (RT-PCR) method.

Virology assays

Plasma HCV viremia was measured within 3 mo of liver biopsy and was determined by reverse transcription polymerase chain reaction (RT-PCR method; Cobas AmpliPrep/Cobas TaqMan/HCV Terst, Roche Diagnostic), HCV genotypes were determined by second generation test [(VERSANT HCV Genotype 2.0 Assay (LiPA)].

Measurements of adipokines, glucose and insulin

Serum glucose concentrations were estimated by glucose hexokinase enzymatic assay (Olympus Beckman Coulter, Switzerland). Total levels of adiponectin, leptin, resistin, visfatin, omentin, osteopontin and insulin were measured using an ELISA kit (Bio Vendor, NC, United States) in fasting venous blood samples collected from the patients.

Routine laboratory investigations

Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR) index, which was calculated according to the following formula: [fasting insulinemia (μU/mL) × fasting glucose (mmol/L)]/22.5. HOMA-IR values higher than 2.5 were considered as significant for insulin resistance. Alanine aminotransferase (ALT) activity was measured enzymatically 1-3 d before liver biopsy without the addition of pyridoxal-5'-phosphate, the reference ranges were 0-41 U/L and 0-31 U/L for men and women, respectively.

Determination of anthropometric parameters

Height and weight were determined. Body mass index was calculated as weight (kg)/height (m^2).

Total RNA extraction

Total RNA was extracted using the mirVana™ miRNA isolation kit (Ambion) according to the manufacturer's instructions. Briefly, frozen samples were homogenized in 300 μL of Lysis/Binding Solution using a TissueRuptor homogenizer (Qiagen). RNA was eluted in 100 μL

RNase-free water and quantified using a PicoDrop spectrophotometer. The quality of RNA samples was analyzed by measuring the ratio of absorptions at 260/280 nm. The purified total RNA was immediately used for cDNA synthesis or stored at -80 ℃.

Analysis of IL28B single-nucleotide polymorphism C/T (rs12979860)

Genomic DNA was isolated from 200 μL of blood using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol. DNA was quantified using a PicoDrop spectrophotometer (Picodrop Limited). The *IL-28B* single-nucleotide polymorphism C/T (rs12979860) was analyzed using Custom® SNP Genotyping Assays (Applied Biosystems). Primer and probe sequences were Forward Primer 5'-GCCTGTCGTGTACTGAACCA, Reverse Primer 5'-GCGCGGAGTGCAATTCAAC, Probe (C allele) 5'-VIC-TGGTTCGCGCCTTC and Probe (T allele) 5'-FAM-CTGGTTCACGCCTTC. Genotyping was performed using the ABI7900HT Real-Time PCR System (Applied Biosystems) in a 25 μL reaction volume containing 10 ng DNA, 12.5 μL TaqMan® Universal PCR Master Mix and 1.25 μL (40 ×) Custom® SNP Genotyping Assays and analyzed using Sequence Detection System 2.3 Software.

Quantitative real-time PCR

mRNA expression: Homo sapiens-specific TaqMan Gene Expression Assay (Applied Biosystems) for *SOCS3* (Hs02330328_s1) mRNA was used for gene expression assays. cDNA generation was performed using 250 ng of total RNA with High Capacity cDNA Reverse Transcription Kits according to the manufacturer's protocols (Applied Biosystems). Ten repeated dilutions of first-strand cDNA were made in nuclease-free water before addition to the RT-PCR reaction mixture. mRNA expression levels were analyzed using beta actin (ACTB) as an endogenous control.

Total RNA isolation: Total RNA was extracted using the mirVana™ miRNA isolation kit (Ambion Europe) according to the manufacturer's instructions. Briefly, frozen samples were homogenized in 300 μL of Lysis/Binding Solution using a TissueRuptor homogenizer (Qiagen). RNA was eluted in 100 μL RNase-free water and quantified using a PicoDrop spectrophotometer. The quality of the RNA samples was analyzed by measuring the ratio of absorptions at 260/280 nm. The purified total RNA was immediately used for cDNA synthesis or stored at -80 ℃.

mRNA expression: A total of 250 ng of tissue RNA was reverse transcribed to cDNA with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. Homo sapiens-specific TaqMan Gene Expression Assays (Applied Biosystems) for *SOCS-3* (Hs02330328_s1) as well as endogenous control beta-actin (*ACTB*, Hs99999903_m1) were used for the gene expression assays.

Real time PCR analysis

TaqMan PCR assays were performed in 96-well optical plates in a 7900HT Fast Real-Time PCR System (Applied Biosystems) and were analyzed using Sequence Detection System 2.0 Software. Fold induction values were calculated according to the equation 2ΔΔCt, where ΔCt represents the differences in cycle threshold numbers between the target gene and endogenous control, and ΔΔCt represents the relative change in these differences between examined and control groups.

Statistical analysis

The Mann-Whitney test was used to evaluate differences between the examined groups. Correlations between adipokine levels or HOMA-IR and hepatic *SOCS-3* expression were analyzed with the Spearman rank correlation coefficient. Values of $P < 0.05$ were considered to be statistically significant. All statistical analyses were performed using Statistica software version 10.0 (StatSoft Inc., 2011).

RESULTS

Study population

Thirty-one patients with chronic hepatitis C with a mean age of 32 years (range: 23-48) were enrolled in the study: 23 male, 8 female. The fasting levels of adiponectin, leptin, resistin, visfatin, omentin and osteopontin and BMI and HOMA-IR of the study groups and healthy controls are shown in Table 1.

Differences in HOMA-IR and adipokine profile between patients infected with HCV genotype 1 and healthy controls

Among chronic hepatitis C patients, 83.8% (26/31) had insulin resistance defined by a HOMA-IR index > 2.5. The mean HOMA-IR index in HCV infected patients was 2.87 (+/- 2.13), median 2.24 and was significantly higher compared to healthy controls (2.24 *vs* 0.59, $P = 0.0003$) (Table 1). However, there was no significant difference in adipokine concentrations between patients with chronic hepatitis C and healthy subjects (Table 1). Moreover, adipokine levels were not related to HOMA-IR index (Table 2).

Association of insulin resistance index and adipokine profile with hepatic steatosis

Hepatic steatosis was observed in 32.2% (10/31) of patients with HCV and was related to increased HOMA-IR index (2.81 *vs* 1.99, $P = 0.05$) and reduced adiponectin level (5.96 *vs* 8.37, $P = 0.04$) (Table 3). Fatty changes involved macrovesicular steatosis of 5%-8% liver cells in zone 1. Lobular inflammation and fibrosis in zone 3 were not described.

Table 1 Characteristics of the study population

	HCV			Healthy subjects			*P* value
	Median	LQ-UQ	Min-Max	Median	LQ-UQ	Min-Max	
Age	32	23-48	18-61	32	23-48	26-56	$P > 0.05$
BMI	23.6	23.2-24.7	20.1-24.9	23.3	22.4-24.7	19.5-24.9	$P > 0.05$
ALT U/L	52	43-79	23-339				
HCV viremia (IU/mL)	1230000	382000-3880000	112000-8950000				
SOCS-3	5.17	2.09-9.78	0.5-24.53				
HOMA	2.24	1.50-3.36	0.78-9.67	0.56	0.34-1.02	0.25-2	$P = 0.0003$
Visfatin (ng/mL)	2.64	1.17-3.58	0.45-9.37	2.75	2.47-3.8	1.26-5.27	$P > 0.05$
Omentin (ng/mL)	385.07	340.33-522.77	115.7-757.34	375.55	278.02-477.33	115.68-587.51	$P > 0.05$
Adiponectin (μg/mL)	7.23	5.48-8.72	3.51-18.48	9.335	6.275-11.20	5.89-15.33	$P > 0.05$
Resistin (ng/mL)	1.54	1.11-1.74	0.73-2.92	1.48	1.31-1.71	1.04-2.7	$P > 0.05$
Leptin (ng/mL)	4.85	2.425-13.6	0.77-54.65	5.785	3.81-17.06	1.85-42.76	$P > 0.05$
Osteopontin (ng/mL)	28.71	18.06-36.19	5.33-59.15	20.56	15.47-21.2	6.14-32.42	$P > 0.05$
	Number of patients (*n*)			Number of patients (*n*)			
Age > 40	9			4			$P > 0.05$
Age < 40	22			5			
Women	10			4			$P > 0.05$
Men	21			5			

The mean HOMA-IR index in HCV infected patients was significantly higher compared to healthy controls (2.24 *vs* 0.59, $P = 0.0003$) (Table 1). However, there was no significant difference in adipocytokine concentration between patients with chronic hepatitis C and healthy subjects. LQ: Lower quartile; UQ: Upper quartile; BMI: Body mass index; ALT: Alanine aminotransferase; HCV: Hepatitis C virus; SOCS-3: Suppressor of cytokine signaling 3; HOMA: Homeostasis model assessment.

Table 2 Adipokines and homeostasis model assessment of insulin resistance in chronic hepatitis C

	Median (LQ-UQ)		*P* value
	HOMA-IR > 2.5 (*n* = 15)	HOMA-IR < 2.5 (*n* = 16)	
Omentin (ng/mL)	381.24 (332.79- 483.33)	399.91 (367.16-541.72)	$P > 0.05$
Adiponectin (μg/mL)	6.24 (5.48-7.92)	8.37 (5.63-12.18)	$P > 0.05$
Resistin (ng/mL)	1.56 (1.42-1.74)	1.24 (1.03-1.56)	$P > 0.05$
Leptin (ng/mL)	6.79 (2.34-13.11)	3.04 (2.51-17.2)	$P > 0.05$
Visfatin (ng/mL)	2.66 (1.09-3.12)	2.05 (1.17-3.3)	$P > 0.05$
Osteopontin (ng/mL)	28.71 (15.52-37.1)	31.18 (20.29-36.07)	$P > 0.05$

Adipokine levels were not related to HOMA-IR index in patients with chronic hepatitis C. HOMA-IR: Homeostasis model assessment of insulin resistance; LQ: Lower quartile; UQ: Upper quartile.

Table 3 Adipokines and liver steatosis in chronic hepatitis C

	With steatosis (*n* = 10)		Without steatosis (*n* = 21)		*P* value
	Median (LQ-UQ)	Min-max	Median (LQ-UQ)	Min-max	
SOCS3	6.553 (3.393-11.777)	1.042-24.537	5.106 (2.095-8.713)	0.5-17.95	$P > 0.05$
HOMA	2.815 (1.99-4.84)	1.25-9.67	1.995 (1.21-2.51)	0.78-7.25	$P = 0.05$
Adiponectin (μg/mL)	5.985 (4.83-7.23)	3.55-8.43	8.37 (5.71-9.07)	3.51-18.48	$P = 0.047$
Osteopontin (ng/mL)	33.51 (15.52-49.12)	5.33-59.15	24.7 (18.26-34.58)	11.57-47.85	$P > 0.05$
Leptin (ng/mL)	5.705 (3.04-17.2)	2.34-54.65	3.825 (1.33-13.11)	0.77-39.04	$P > 0.05$
Resistin (ng/mL)	1.65 (1.42-1.84)	1.17-2.36	1.51 (1.03-1.58)	0.73-2.92	$P > 0.05$
Omentin (ng/mL)	374.875 (332.79-483.33)	227.99-757.34	393.92 (360.09-522.77)	115.7-735.84	$P > 0.05$
Visfatin (ng/mL)	2.465 (1.37-3.12)	0.45-3.97	2.64 (1.17-4.08)	0.53-9.37	$P > 0.05$

Hepatic steatosis was related to increased HOMA-IR index (2.81 *vs* 1.99, $P = 0.05$) and reduced adiponectin level (5.96 *vs* 8.37, $P = 0.04$). SOCS-3: Suppressor of cytokine signaling 3; HOMA-IR: Homeostasis model assessment of insulin resistance; LQ: Lower quartile; UQ: Upper quartile.

Association of adipokine profile with hepatic inflammation

Inflammatory activity (G ⩾ 2) was described in 30% of patients (9/31) and was associated with increased osteopontin concentration (34.04 *vs* 23.35, $P = 0.03$) (Table 4). No relationships were found between other adipokine concentrations and the grade of inflammatory activity.

Association of insulin resistance index and adipokine profile with hepatic fibrosis

Advanced liver fibrosis (S ⩾ 2) was reported in 70% of subjects (22/31) and was more commonly observed in

Table 4 Adipokines and liver inflammation in chronic hepatitis C

	G ⩾ 2 (*n* = 9)		G < 2 (*n* = 22)		*P* value
	Median (LQ-UQ)	Min-max	Median (LQ-UQ)	Min-max	
Omentin (ng/mL)	381.24 (367.16-522.77)	115.7-757.34	389.46 (340.33-483.33)	227.99-735.84	$P > 0.05$
Adiponectin (μg/mL)	6.21 (5.71-7.56)	4.6-9.07	8 (5.48-9.06)	3.51-18.24	$P > 0.05$
Resistin (ng/mL)	1.24 (1.17-1.65)	0.91-2.36	1.55 (1.11-1.82)	0.73-2.92	$P > 0.05$
HOMA	2.1(1.59-3.99)	0.78-9.67	2.38 (1.25-2.84)	0.88-7.25	$P > 0.05$
Leptin (ng/mL)	10.83 (4.26-31.44)	1.69-54.65	3.43 (1.83-9.36)	0.77-39.04	$P > 0.05$
Visfatin (ng/mL)	2.05 (1.83-2.66)	0.65-8.89	2.78 (1.17-4.08)	0.45-9.37	$P > 0.05$
Osteopontin (ng/mL)	34.04 (32.7-36.19)	22.47-49.12	23.25 (15.05-36.07)	5.33-59.15	$P = 0.03$
SOCS-3	3.69 (2.67-7.05)	0.66-11.52	8.13 (1.74-11.78)	0.5-24.53	$P > 0.05$

Inflammatory activity (G ⩾ 2) was associated with increased osteopontin concentration (34.04 *vs* 23.35, $P = 0.03$). No relationships were found between other adipokine concentrations and the grade of inflammatory activity. *n*: Number of patients, in liver biopsy: G: Grade of inflammation and necrosis; HOMA-IR: Homeostasis model assessment of insulin resistance; LQ: Lower quartile; UQ: Upper quartile.

Table 5 Adipokines and liver fibrosis in chronic hepatitis C

	S ⩾ 2 (*n* = 22)		S < 2 (*n* = 9)		*P* value
	Median (LQ-UQ)	Min-max	Median (LQ-UQ)	Min-max	
Omentin (ng/mL)	436.94 (367.16-538.15)	115.7-757.34	360.09 (296.34-383.55)	257.55-396.92	$P = 0.03$
Adiponectin (μg/mL)	7.39 (5.71-9.06)	4.6-18.48	6.46 (4.52-8.37)	3.51-11.28	$P > 0.05$
Resistin (ng/mL)	1.4 (1.03-1.58)	0.73-2.36	1.74 (1.54-2.67)	0.76-2.92	$P = 0.047$
HOMA (mmol/L)	2.1 (1.33-3.36)	0.78-9.77	2.5 (1.5-2.79)	0.88-5.17	$P > 0.05$
Leptin (ng/mL)	4.09 (2.51-8.56)	1.00-54.65	11.94 (1.17-21.5)	0.77-39.04	$P > 0.05$
Visfatin (ng/mL)	2.25 (1.17-3.3)	0.53-8.89	3.12 (1.57-5.26)	0.45-9.37	$P > 0.05$
Osteopontin (ng/mL)	32.84 (22.47-37.1)	8.46-59.15	20.29 (15.05-24.33)	5.33-38.46	$P = 0.03$
SOCS-3	4.51 (1.92-9.24)	0.66-24.53	8.61 (4.40-11.777)	0.5-17.95	$P > 0.05$

Advanced liver fibrosis (S ⩾ 2) was more commonly observed in patients with increased serum levels of omentin and osteopontin (436.94 *vs* 360.09, $P = 0.03$ and 32.84 *vs* 20.29, $P = 0.03$) and reduced resistin concentration (1.40 *vs* 1.74, $P = 0.047$). Other adipokines were not related to the progression of liver fibrosis. No difference was found in HOMA-IR index according to stages of fibrosis in liver biopsy specimens. *n*: Number of patients; S: Stage of fibrosis; SOCS-3: Suppressor of cytokine signaling 3; HOMA-IR: Homeostasis model assessment of insulin resistance; LQ: Lower quartile; UQ: Upper quartile.

Table 6 Correlations between adipokines and hepatic expression of suppressor of cytokine signaling 3 in chronic hepatitis C

	SOCS-3	*P* value
	Spearman's rank correlation ρ	
Age	0.12	$P > 0.05$
HCV viral load (IU/mL)	0.15	$P > 0.05$
ALT (U/L)	0.41	$P > 0.05$
HOMA	0.39	$P > 0.05$
Adiponectin (μg/mL)	0.24	$P > 0.05$
Osteopontin (ng/mL)	0.30	$P > 0.05$
Visfatin (ng/mL)	0.23	$P > 0.05$
Resistin (ng/mL)	0.55	$P > 0.05$
Leptin (ng/mL)	0.14	$P > 0.05$
Omentin (ng/mL)	0.50	$P > 0.05$

No correlation was found between serum adipokine levels or HOMA-IR index and hepatic expression of SOCS-3. SOCS-3: Suppressor of cytokine signaling 3; HOMA-IR: Homeostasis model assessment of insulin resistance; ALT: Alanine aminotransferase.

patients with increased serum levels of omentin and osteopontin (436.94 *vs* 360.09, $P = 0.03$ and 32.84 *vs* 20.29, $P = 0.03$) and reduced resistin concentration (1.40 *vs* 1.74, $P = 0.047$). Other adipokines were not related to the progression of liver fibrosis (Table 5).

No differences were found in HOMA-IR index according to grades of inflammation or stages of fibrosis in liver biopsy specimens.

Adipokine profile and hepatic SOCS-3 gene expression

No correlation was found between serum adipokine levels or HOMA-IR index and hepatic expression of *SOCS-3* (Table 6).

Adipokine profile and viral factors

Serum adipokine concentrations and HOMA-IR index were not found to be related to HCV viremia (Table 7).

Adipokine profile and single nucleotide polymorphisms of IL28B

In this study, the presence of single nucleotide polymorphisms (SNPs) of *IL28B* (rs 12979860) were not associated with adipokine levels or HOMA-IR index (Table 8).

DISCUSSION

Although insulin resistance was found to be present in chronic hepatitis C patients, even with normal body weight, glucose and lipid profiles, HOMA-IR index was not associated with adipokine concentrations. The reason for this might be that adipokines are secreted mainly by visceral

Table 7 Adipokines and hepatitis C virus viremia

	VL ⩾ 600000 IU/mL (n = 12)		VL < 600000 IU/mL (n = 19)		P value
	Median (LQ-UQ)	Min-max	Median (LQ-UQ)	Min-max	
Age	32 (23-54)	18-61	32.5 (23.5-38)	18-61	$P > 0.05$
Adiponectin (μg/mL)	6.46 (5.48-8.72)	3.51-18.48	7.42 (5.48-8.75)	3.55-17.93	$P > 0.05$
Osteopontin (ng/mL)	31.18 (15.52-37.1)	11.57-59.15	26.84 (21.19-33.78)	5.33-54.98	$P > 0.05$
Leptin (ng/mL)	4.09 (1.59-21.5)	0.77-54.65	5.47 (2.51-13.11)	1.17-17.2	$P > 0.05$
Resistin (ng/mL)	1.42 (1.03-1.74)	0.73-2.92	1.56 (1.29-1.78)	0.87-2.71	$P > 0.05$
Omentin (ng/mL)	393.92 (342.46-534.21)	115.7-757.34	381.88 (312.96-497.43)	227.99-735.84	$P > 0.05$
HOMA	2.01 (1.5-3.99)	0.88-9.67	2.5 (1.25-3.36)	0.78-5.66	$P > 0.05$
Visfatin (ng/mL)	2.67 (1.17-3.97)	0.53-9.37	2.14 (1.23-3.11)	0.45-9.33	$P > 0.05$

Serum adipokine concentrations and HOMA-IR index were not found to be related to HCV viremia. VL: Viral load; SOCS-3: Suppressor of cytokine signaling 3: HOMA-IR: Homeostasis model assessment of insulin resistance; HCV: Hepatitis C virus; LQ: Lower quartile; UQ: Upper quartile.

Table 8 Adipokines and single nucleotide polymorphisms of interleukin 28B in chronic hepatitis C

	CC (n = 6)		TT/CT (n = 25)		P value
	Median (LQ-UQ)	Min-max	Median (LQ-UQ)	Min-max	
SOCS-3	11.52 (3.164-15.738)	2.17-17.95	5.10 (1.75-8.713)	0.5-24.537	$P > 0.05$
HCV viremia (IU/mL)	1195000 (362000-3830000)	112000-9850000	3780000 (799000-4290000)	382000-7210000	$P > 0.05$
ALT (U/L)	54 (43-83)	23-339	45 (44-47)	41-48	$P > 0.05$
HOMA	(1.02-3.99)	0.78-5.66	2.44 (1.5-2.84)	0.88-9.67	$P > 0.05$
Adiponectin (μg/mL)	7.56 (6-8.37)	5.33-9.07	6.35 (5.34-8.89)	3.51-18.48	$P > 0.05$
Osteopontin (ng/mL)	24.33 (22.18-32.98)	13.14-34.58	29.94 (16.79-37.78)	5.33-59.15	$P > 0.05$
Visfatin (ng/mL)	2.64 (1.57-6.35)	0.65-9.33	2.56 (1.13-5.69)	0.45-9.37	$P > 0.05$
Leptin (ng/mL)	13.11 (8.56-17.2)	5.47-39.04	3.04 (1.69-11.94)	0.77-54.65	$P > 0.05$
Omentin (ng/mL)	367.16 (257.55-399.91)	152.74-522.77	390.95 (341.39-536.18)	115.7-757.34	$P > 0.05$
Resistin (ng/mL)	1.17 (0.91-1.65)	0.76-1.74	1.55 (1.205-1.83)	0.73-2.92	$P > 0.05$

The single nucleotide polymorphisms (SNPs) of IL28B (rs 12979860) were not associated with adipokine levels or HOMA-IR index. Genotypes of IL28B: CC and not CC (CT and TT); SOCS-3: Suppressor of cytokine signaling 3; HOMA-IR: Homeostasis model assessment of insulin resistance; ALT: Alanine aminotransferase; LQ: Lower quartile; UQ: Upper quartile.

adipose tissue and reflect metabolic disturbances and the present study was conducted only in non-obese patients without metabolic disorders. This observation supports the hypothesis that not only metabolic factors lead to the development of insulin resistance. It is well known that HCV directly affects insulin signaling pathways, promoting insulin resistance at a cellular level[10-12].

Moreover, our results indicate that insulin resistance and adipokines are involved in the pathogenesis of liver injury in patients with HCV infection. The results of the present study confirm those of others insofar that hepatic steatosis was positively related to HOMA-IR index and hypoadiponectinemia[13,14]. Interestingly, the reduced hepatic expression of adiponectin was also demonstrated in chronic hepatitis B patients with hepatic steatosis[13]. Numerous studies have indicated that adiponectin stimulates matrix metalloproteinase complexes[14,15]. However, this antifibrogenic effect of adiponectin was not observed in the presented study, probably due to the small number of patients with stage 1 liver fibrosis. The results of the presented study indicate an association between reduced levels of resistin and higher fibrosis score. Resistin regulates the secretion of fibrogenic molecules, including TGFβ-1 and TNF-α, in hepatic stellate cells[16]. However, the literature presents several contradicting opinions on the role of resistin in liver fibrosis. Our observations are confirmed by those of Tiftikci *et al*[17] and indicate that reduced concentrations of resistin correlate with progression of liver fibrosis. On the contrary, Baranova *et al*[18] report that severe fibrosis (Metavir score > F2) was predicted by increased resistin levels. Thus, further studies are warranted to establish the role of resistin in liver fibrosis in chronic hepatitis C. However, the role of other adipokines in inflammatory processes is well established. Osteopontin is a pro-inflammatory cytokine secreted from many cells, including activated macrophages and T-lymphocytes. Osteopontin is known to be a chemotactic factor for fibroblasts, which modulates secretion of metalloproteinases[19]. In our study, osteopontin values correlated positively with severity of liver inflammation and fibrosis. These results are in accordance with those of Bassyouni *et al*[19], showing the important role played by osteopontin in the liver changes which occur during HCV infection.

A number of studies have indicated that intracellular factors are dysregulated by HCV and are responsible for insulin resistance. The core HCV protein stimulates increased SOCS-3 levels, which leads to ubiquitination and proteasomal degradation of insulin signaling receptor 1 and 2. Recently, Vanni *et al*[20] reported an increase in intrahepatic SOCS-3 mRNA expression with hepatic insulin

resistance. Some studies have suggested that adipokines may alter the expression of *SOCS-3* gene in many tissues but the mechanism is unclear[21,22]. Leptin induces the expression of *SOCS-3* gene in adipose tissue and liver, which in turn negatively regulates leptin signaling[23,24]. However, the present study does not reveal any correlations between hepatic expression of the *SOCS-3* gene, serum adipokine concentrations and systemic insulin resistance. As skeletal muscle and adipose tissue are key tissues contributing to peripheral insulin resistance, it would be worth assessing *SOCS-3* gene expression in these tissues in relationship to HOMA-IR levels and adipokine profiles to better elucidate systemic insulin resistance.

In the present study, adipokine levels and HOMA-IR index were not related to HCV viremia. It is well established that hyperinsulinemia stimulates viral replication *in vitro*, but it is still not clear whether insulin resistance directly effects HCV replication *in vivo*[25]. Some authors identify a correlation between HCV-RNA level and HOMA-IR index[26,27]. However, our results correspond with those of Huang *et al*[28] who do not identify different HCV viremia in chronic hepatitis C patients with or without insulin resistance. Other authors suggest that genetic factors may be significant factors in the pathogenesis of insulin resistance. Genetic variation in the *IL28B* gene has been associated with lipid disorders but also with hepatic steatosis[29]. However, in the presented study, similarly to Degasperi *et al*[30], single nucleotide polymorphisms (SNPs) of *IL28B* (rs 12979860) were not found to be associated with adipokine levels or HOMA-IR index.

In conclusion, in patients with chronic hepatitis C without metabolic disorders, we confirmed higher HOMA-IR values compared to healthy subjects. We speculated that no relationship between adipokines and HOMA-IR values may indicate that HCV can induce insulin resistance itself. Some adipokines appear to be biochemical markers of steatosis, inflammation and fibrosis. However, it is important to note that the present study is cross-sectional and does not elucidate the causal relationship between serum levels of adipokines and liver damage. The somewhat ambiguous nature of the results obtained from this analysis indicates that further studies are needed to precisely clarify the role of adipokines in pathogenesis of chronic hepatitis C.

COMMENTS

Background

The mechanism of insulin resistance in chronic hepatitis C is complex and bidirectional and the exact pathogenesis is still unknown. Some clinical observations support a "fat independent" mechanism in the development of insulin resistance in hepatitis C virus (HCV)-infected subjects. However, HCV can induce insulin resistance itself by stimulating the molecule suppressor of cytokine signaling 3 (SOCS-3), which leads to ubiquitination and proteasomal degradation of insulin signaling receptors 1 and 2.

Research frontiers

Adipokines are secreted mainly by visceral adipose tissue and reflect metabolic disturbances, although the role of adipokines in the pathogenesis of insulin resistance is not fully elucidated. The aim of this study was to analyze adipokine concentrations and homeostasis model assessment for insulin resistance (HOMA-IR) values in healthy subjects and in patients with chronic HCV genotype 1 and normal body weight, glucose and lipid profiles. Some studies have suggested that adipokines may alter the expression of *SOCS-3* gene in many tissues but the mechanism is unclear. In this study, adipokine concentrations and HOMA-IR index are determined according to hepatic expression of *SOCS-3* mRNA.

Innovations and breakthroughs

To investigate the direct effect of HCV on insulin resistance we analyzed adipokines and HOMA-IR in selected chronic hepatitis C patients without metabolic disorders. In this group of patients we confirmed higher HOMA-IR values compared to healthy subjects. The authors speculated that no relationship between adipokines and HOMA-IR values may indicate that HCV can induce insulin resistance itself, regardless of metabolic disorders. This is the first study to determine the impact of a wide spectrum of adipokines and HOMA-IR index on histopathological changes in liver biopsy specimens and hepatic expression of SOCS-3 mRNA.

Applications

By understanding the mechanism of insulin resistance, this study may represent a future strategy for therapeutic intervention in the treatment of patients with chronic hepatitis C.

Terminology

Adipokines reflect metabolic disturbances and may alter the expression of *SOCS-3* gene in many tissues. SOCS-3 (molecule suppressor of cytokine signaling 3) is protein which leads to development of insulin resistance with ubiquitination and proteasomal degradation of insulin signaling receptors 1 and 2.

Peer review

This study aims to investigate the association between adipokine concentration and HOMA-IR in patients with HCV. This is a topical study on insulin resistance and hepatic expression of *SOCS-3* gene in HCV infection. It is a well conducted study and deserves to be published.

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