Metabolic Syndrome, Insulin Resistance and Adiponectin Level in Patients with Chronic Hepatitis C

Mircea Grigorescu[1], Corina Radu[1], Dana Crişan[1], Mircea Dan Grigorescu[1], Alexandru Şerban[2], Dana Neculoiu[3], Margareta Rusu[2], Monica Acalovschi[1]

1) 3rd Medical Clinic, University of Medicine and Pharmacy, Cluj-Napoca; 2) Emergency Universitary Hospital, Cluj-Napoca; 3) Emergency County Hospital, Braşov, Romania

Abstract

Aims. To assess insulin resistance and adiponectin profile in patients with chronic hepatitis C (CHC), according to the presence or absence of metabolic syndrome (MS). **Patients and methods**. One hundred and fifty-two patients with histologically proven CHC, genotype I were prospectively studied. Parameters of MS according to the IDF criteria were evaluated. Insulin resistance was established by homeostasis model assessment (HOMA-IR]. An index ≥ 2.0 was designated as IR and ≥ 4 as prediabetic state. Serum adiponectin levels were measured by ELISA. **Results**. MS was found in 61.48% of cases. HOMA-IR was significantly higher in patients with CHC and MS vs those without MS (7.88±1.11 vs 4.29 ± 0.5, p=0.023]. Adiponectin levels had an inverse behaviour (9,946.1 ± 5,811 ng/ml vs 13,215.5 ± 815.5 ng/ml, p< 0.001]. By multiple linear regression analysis the independent predictors associated with HOMA-IR ≥ 4 in patients with CHC and MS were visceral obesity, adiponectin levels, activity and degree of steatosis. Only visceral obesity and HOMA-IR were independently associated with adiponectin. A significant negative correlation was established between adiponectin and insulin (r = - 0.169, p=0.003] and between adiponectin and HOMA-IR (r = - 0.188, p=0.02]. **Conclusions.** CHC with MS was associated with a higher insulin resistance and lower adiponectin level. Adiponectin level and insulin resistance were significantly correlated.

Key words

Chronic hepatitis C – metabolic syndrome – insulin resistance – serum adiponectin

J Gastrointestin Liver Dis
June 2008 Vol.17 No 2, 147-154
Address for correspondence: Prof. Mircea Grigorescu
3rd Medical Clinic
Croitorilor Str 19-21
400162, Cluj-Napoca, Romania
Email: mdgrigorescu@yahoo.com

Introduction

The pathogenesis of chronic hepatitis C (CHC] is a challenging issue due to the complex interplay between host and viral factors. Hepatitis C virus (HCV] infection is not confined to the liver, but can induce disturbances in many other organs and systems [1, 2].

Chronic hepatitis C has many features which suggest that this disease must be viewed not only as a viral disease, but also as a metabolic liver disease which implies: insulin resistance (IR] [3], high prevalence of steatosis [4], increased prevalence of impaired glucose tolerance [5], type 2 diabetes mellitus [6], changes in lipid metabolism [7]. These findings together suggest that chronic HCV infection is closely related to the metabolic syndrome (MS]. Accordingly, CHC should be divided in CHC with and CHC without MS.

Insulin resistance is the main feature of the MS. In CHC, there is a close association between IR, hepatic steatosis [3, 8, 9], progression of fibrosis [10] and a lower rate of sustained virological response [11].

Viral genotype is a major factor associated with steatosis in HCV infection. Traditionally, two types of hepatic steatosis are distinct in HCV infection: virus-induced steatosis and metabolic steatosis. Virus–induced steatosis is related to genotype 3 infection, the degree of steatosis is related to viral load [12], the antiviral therapy leads to complete reversal of lipid alterations and steatosis [13], but the virological relapse leads to the reappearance of steatosis [14]. Numerous studies indentified the metabolic intracellular pathways that mediate "viral" steatosis" [7, 15]. Metabolic steatosis occurs in non-3 genotype HCV infection and is associated with host metabolic factors: elevated body mass index (BMI] and central adiposity [12]. This type of steatosis is either not or very little susceptible to improvement following HCV eradication [16]. The pathogenetic mechanisms of metabolic steatosis are the IR induced by direct action of HCV on the insulin signaling pathways [1, 2] as well as the host factors, especially obesity [9].

The adipocytokine profile seems to play a distinct role, together with IR, in the pathogenesis of CHC [12]. The cytokines produced by adipose tissue have a pivotal

role in modulating inflammatory response and insulin sensitivity and contribute to the development of metabolic abnormalities. Adiponectin modulates hepatic fat content and has an anti-steatotic effect on the liver [17, 18]. Adiponectin is also a hepatic insulin sensitizer and has the opposite effect in comparison with TNF-α on lipid metabolism, insulin sensitivity and inflammation [19]. In CHC, adiponectin levels are associated with the degree of steatosis and insulin resistance [20].

The objective of the present study was to assess the insulin resistance and the adiponectin profile in patients with CHC according to the presence or absence of MS.

Patients and methods

A number of 152 consecutive patients with CHC, who had underwent liver biopsy were prospectively evaluated.

CHC infection was defined by the presence of anti-HCV for at least 6 months and a positive HCV-viremia.

Patients with other etiology of chronic liver disease: hepatitis B, autoimmune liver disease, Wilson disease, hemochromatosis, α1-antitripsin deficiency, HIV infection, patients with a history of hepatotoxic or steatosis-inducing drug use, patients having an alcohol consumption more than 20 g/day for women and 30 g/day for men, as well as those with a history of diabetes mellitus were excluded from the study.

All patients were naïve for the antiviral treatment.

The study was performed according to the Declaration of Helsinki and was approved by the local Ethics Committee. A written informed consent was obtained from each patient.

Anthropometric data

Height, weight and waist circumference were determined. Body mass index (BMI) was calculated (kg/m2). According to the IDF criteria, visceral obesity was considered when waist circumference was ≥ 94 cm in men and ≥ 80 cm in women [21].

Laboratory investigations

A blood sample was obtained after 12h overnight fasting for routine investigations: plasma glucose, ALT, AST, gamma-glutamyltranspeptidase (GGT), total cholesterol, HDL-cholesterol, triglycerides. All the assessments were made on an automatic analyzer (Konelab 30 I – Thermo Electron Corp Finland).

Fasting insulin and C-peptide levels were measured by the ELISA method (Mercodia ELISA, Sweden]. Total serum adiponectin levels were measured by using specific ELISA tests (R and D systems) for the whole group and separately in men and women.

The degree of insulin resistance was calculated according to the homeostasis model assessment for insulin resistance (HOMA-IR] by the formula: fasting insulin level (mUI/l] x fasting glucose level (mg/dl) / 405 [22]. A HOMA-IR index value of more than 2.0 was considered as the criterion of insulin resistance, and that higher than 4.0 as a prediabetic state [23].

All patients were infected with genotype 1 HCV (HCV genotype assay: INNO-LIPA). Serum HCV-RNA was measured by PCR (Cobas Amplicor HCV 2.0 version, Roche).

Morphopathological study

Liver biopsies obtained under ultrasonographic guidance and stained with hematoxilin – eosin and Masson's trichrome were assessed blindly according to the METAVIR scoring system [24] by one expert pathologist (A.S.). According to the METAVIR system, fibrosis was staged on a scale from F0 to F4, as follows: F0: no fibrosis; F1: portal fibrosis, without septa; F2: few septa; F3: many septa without cirrhosis and F4: cirrhosis. F0 and F1 were considered as insignificant fibrosis while the scores F2-F4 were considered as significant fibrosis. Grading of histological activity that evaluated the necroinflammatory lesions was scored as: A0: no activity; A1: mild activity; A2: moderate activity, and A3: severe activity.

The degree of steatosis was expressed as the percent of affected hepatocytes using a five degree scale: 0: no steatosis; 1: minimal steatosis (1-10%); 2: moderate steatosis (11-30%); 3: severe steatosis (31-70%) and 4: very severe steatosis (70-100%) [25]. The degrees 0-2 (S0-2] were considered as insignificant steatosis, while degrees 3 and 4 (S3-4) as significant steatosis. The superimposed nonalcoholic steatohepatitis (NASH) on CHC was diagnosed according to histological features described by Kleiner et al [26].

Evaluation of the metabolic syndrome

The IDF consensus worldwide definition of the MS was used. It implies the presence of the central obesity (defined as waist circumference ≥ 94 cm for men and ≥ 80 cm for women] plus two of the following four features: raised triglyceride levels ≥ 150 mg/dl; reduced HDL-cholesterol < 40 mg/dl in males and < 50 mg/dl in females; raised blood pressure: systolic ≥ 130 or diastolic ≥ 85 mmHg; raised fasting plasma glucose ≥100 mg/dl or previously diagnosed type 2 diabetes [21].

Statistical analysis

Comparison between groups was performed using Student's t-test for continuous variables and $\chi 2$ test for categorical variables. All values were presented as mean ± SE. Correlations between HOMA-IR and adiponectin level with other variables were established by using Pearson or Spearman's rank correlation. Mann-Whitney U-test was used for the continuous ordinal data between two qualitative variables. Variables that achieved statistical significance with the univariate analysis were included in multiple regression analysis to evaluate the independent factors associated with HOMA-IR and adiponectin. P values < 0.05 were considered statistically significant.

Results

Patients' characteristics

The baseline characteristics of the studied group are shown in Table I. The values for adiponectin were obtained on the whole group and differentiated according to gender.

Table I. Baseline demographic, anthropometric, biochemical, virological, and histological characteristics of the 152 patients with CHC

Variables (units of measurement)	All patients
Age (years)	48.26 ± 0.77
Gender	
M (%)	53 (34.9%)
F (%)	99 (65.1%)
Visceral obesity (n, %)	103 (67.8%)
Elevated blood pressure (≥130/85 mmHg) (n, %)	73 (48)
ALT (IU/l)	92.9 ± 5.9
AST (IU/l)	62.23 ± 3.1
GGT (IU/l)	79.03 ± 6.3
Serum cholesterol (mg/dl)	191.5 ± 4.04
HDL-cholesterol	54.7 ± 1.5
F <50 mg/dl (n, %)	34 (34.3%)
M <40 mg/dl (n, %)	15 (28.3%)
Serum triglycerides (mg/dl)	123.4 ± 5.1
Fasting glucose (mg/dl)	108.5 ±-2.7
Fasting insulin (mU/l)	23.5 ± 2.5
C peptide (nmol/l)	1361.9 ± 72.2
HOMA-IR	6.51 ± 0.72
HOMA-IR ≥ (n, %)	109 (71.7%)
HOMA-IR ≥ (n, %)	58 (38.2%)
Adiponectin whole group (ng/ml)	11193.7 ± 491.1
Adiponectin in males (ng/ml)	9444.8 ± 785.6
Adiponectin in females (ng/ml)	12129.9 ± 607.8
Viral load (copies/ml)	1039248 ± 85,762
Histology (METAVIR) (n, %)	
Activity	
0	20 (13.2)
1	24 (15.8)
2	82 (53.9)
3	26 (17.1)
Fibrosis	
0	15 (9.9)
1	41 (27.0)
2	56 (36.8)
3	27 (17.8)
4	13 (8.5)
Steatosis	
0	77 (50.7)
1	26 (17.1)
2	26 (17.1)
3	20 (13.1)
4	3 (2.0)
NASH	14 (9,2)

HOMA-IR was calculated according to the level of significance for insulin resistance.

CHC patients' characteristics according to the MS

According to the presence or absence of MS the patients

Table II. Characteristics of CHC patients according to the presence or absence of metabolic syndrome

Variables	**Metabolic syndrome**		
	Absence	**Presence**	**p**
Number	58 (38.15%)	94 (61.84%)	0.000
Age (years)	47.09 ± 1.37	48.99 (0.92)	0.237
Gender			
M (n, %)	28 (52.8%)	25 (47.2%)	0.006
F (n, %)	30 (30.3%)	69 (69.7%)	
BMI (kg/m^2)	25.28 ± 1.73	28.69 ± 0.67	0.036
Visceral obesity			
Presence	9 (8.7)	94 (91.3)	
Absence	49 (100)	0 (0)	0.000
Blood pressure >130/85 mmHg (n, %)	14 (24.1)	59 (62.8)	
ALT (IU/l)	96.17 ± 8.18	90.97 ± 8.11	0.670
AST (IU/l)	66.26 ± 5.54	59.74 ± 3.85	0.332
GGT (IU/l)	89.56 ± 14.32	72.64 ± 5.26	0.196
Serum cholesterol (mg/dl)	186.6 ± 5.17	194.4 ± 5.7	0.351
HDL- cholesterol (F) (mg/dl)	62.5 ± 2.76	55.5 ± 2.28	0.080
HDL- cholesterol (M) (mg/dl)	50.61 ± 2.93	47.5 ± 3.45	0.491
Serum triglycerides (mg/dl)	107.38 ± 5.53	133.26±7.42	0.014
Fasting glucose (mg/dl)	98.09 ± 2.53	115.06±4.08	0.003
Fasting insulin (mU/l)	17.97 ± 2.05	26.93 ± 3.74	0.119
C-peptide (pmol/l)	1169.55±94.99	1480.4±99.40	0.011
HOMA-IR	4.29 ± 0.5	7.88 ± 1.11	0.023
HOMA-IR ≥ 2 (n, %)	34 (58.2)	75 (79.8)	0.000
HOMA-IR ≥ 4 (n, %)	18 (31.0)	40 (42.5)	0.000
Adiponectin whole lot	13215.5±815.5	9946.1±581.1	0.001
Adiponectin in females (ng/ml)	14836.6 ±1038.14	10953.1 ± 704.9	0.003
Adiponectin in males (ng/ml)	11478.6 ± 1205.1	7166.9 ± 771.9	0.005
Viral load (copies/ml)	920129.6 ± 146789.5	1112747 ± 104929.7	0.086
Histology (n, %)			
Activity			
A0-1	19 (32.8)	24 (25.5)	0.337
A2-3	39 (67.2)	70 (74.5)	
Fibrosis			
F0-1	18 (31.0)	38 (40.4)	0.244
F2-4	40 (69.0)	56 (59.6)	
F4	18 (31.0)	22 (23.4)	
Steatosis			
S0-2	55 (94.8)	74 (78.7)	0.007
S3-4	3 (5.2)	20 (21.3)	
NASH	0 (0)	14 (14.9)	0.000

were divided in two groups for comparison (Table II).

In the univariate analysis, 9 variables were significantly related to the MS associated with CHC: female gender, increased BMI, visceral obesity, serum triglycerides, fasting glucose, C-peptide, HOMA-IR, adiponectin (both in males and females] and steatosis degree (Table II). In the multivariate analysis, only BMI was an independent predictor of MS (p=0.002, OR: 1.678, 95% CI 1.545-2.045).

Correlations between IR and other variables

Data on the relationship between HOMA-IR and the other variables are shown in Table III. In the univariate analysis, HOMA-IR was positively associated with visceral obesity, ALT, GGT, glucose and insulin levels and negatively associated with adiponectin (r = -0.207, p< 0.0011).

Table III. Univariate analysis of correlations between HOMA-IR and clinical and biological variables

Variables	HOMA-IR	
	r	p
Age (years)	-0.076	0.355
Gender		
F	0.920	0.488
M	1.140	0.488
BMI (kg/m^2)	0.078	0.342
Waist circumference (cm)	0.201	0.013
GGT(IU/l)	0.236	0.004
Cholesterol (mg/dl)	-0.150	0.066
Triglycerides (mg/dl)	0.058	0.477
Fasting glucose (mg/dl)	0.467	0.000
Insulin (mU/l)	0.967	0.000
C-peptide (pmol/l)	0.825	0.000
Adiponectin (ng/ml)	-0.207	0.011
Viremia (copies/ml)	0.141	0.084
Histology		
Activity	0.179	0.027
Fibrosis	0.148	0.069
Steatosis	0.257	0.001

For HOMA-IR ≥ 2, the multiple linear regression analysis revealed that visceral obesity and adiponectin were the only independent factors associated with IR at this threshold (Table IV).

Table IV. Multiple linear regression analysis for factors associated with HOMA-IR ³ 2 in patients with CHC and metabolic syndrome

Variables	p	OR	95%C.I. for OR
Waist circumference	0.015	1.204	1.037-1.398
Adiponectin	0.029	0.999	0.999-0.999

By multiple linear regression analysis, the independent predictors associated with HOMA-IR ≥ 4 in patients with CHC and MS were visceral obesity, adiponectin level, activity and degree of steatosis (Table V).

Table V. Multiple linear regression analysis for factors associated with HOMA-IR ≥ 4 in patients with CHC and metabolic syndrome

Variables	p	OR	95% C.I. for OR
Waist circumference	0.010	1.237	1.018-1.532
Adiponectin	0.001	0.999	0.999-0.999
Activity	0.025	2.841	1.143-7.064
Fibrosis	0.892	0.946	0.428-2.092
Steatosis	0.007	2.048	0.707-5.937

Correlation between adiponectin and other parameters

In the univariate analysis, adiponectin was positively correlated with age and negatively with BMI, visceral obesity, serum triglycerides, insulin, C-peptide, HOMA-IR and steatosis (Table VI).

Table VI. Univariate analysis of the correlations between adiponectin level and clinical and biological variables

Variables	Serum adiponectin	
	r	p
Age	0.197	0.015
Gender	0.340	0.001
BMI	-0.275	0.001
Waist circumference	-0.387	0.000
ALT	-0.025	0.755
GGT	-0.051	0.537
Cholesterol	0.129	0.112
Triglycerides	-0.219	0.007
Glycaemia	-0.098	0.231
Insulin (mU/l)	-0.212	0.009
C-peptide (pmol/l)	-0.266	0.001
HOMA-IR	0.207	0.011
Viremia	-0.76	0.352
Histology		
Activity	-0.096	0.241
Fibrosis	0.113	0.165
Steatosis	-0.179	0.027

Multiple linear regression analysis showed that only visceral obesity and HOMA-IR were independently associated with adiponectin level (Table VII).

Table VII. Multiple linear regression analysis for factors associated with adiponectin level in patients with chronic hepatitis C

Variables	Coefficient B	SE of coefficient B	95% C.I.	p
Visceral obesity	-77.13	-0.198	-131.06/ 23.20	0.005
HOMA-IR	-72.45	-0.204	-91.34/ 8644	0.004

A significant negative correlation was established between adiponectin and insulin levels (Fig.1a), and between adiponectin and HOMA-IR (Fig.1b).

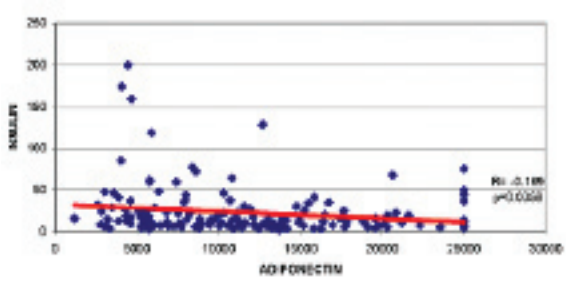

Fig1a. Correlation between adiponectin and insulin levels

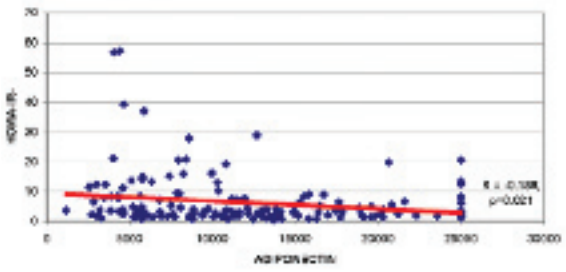

Fig1b. Correlation between adiponectin level and HOMA-IR

Discussion

Metabolic syndrome represents a constellation of risk factors for cardiovascular diseases, having IR as the major underlying pathogenetic factor [27]. For this reason, the term "insulin resistance syndrome" is often used instead of MS [28].

Hepatitis C and MS are common conditions worldwide and both have IR as a key pathogenetic factor.

We found that MS, according to the IFD definition [13] was present in 61.84% (94 out of 152) patients having CHC. All of them had visceral obesity, evaluated by waist circumference and a significantly higher BMI as compared with patients without MS. Low HDL-cholesterol level (68.4%), raised plasma glucose (59.8%), elevated blood pressure (48%) and high triglyceride levels (30.2%) were also present in these patients.

Chronic hepatitis C and MS may coexist in the same individual [29], but chronic HCV infection can also generate by itself some metabolic abnormalities characteristic for the MS.

It is known that chronic HCV infection is specifically associated with IR even in the absence of type 2 diabetes mellitus and irrespective of the presence of cirrhosis [30], and that high serum glucose levels have a fibrogenetic effect [31]. In a large study, HCV infection was not associated with MS, but was associated with HOMA-IR [32].

Insulin resistance in chronic HCV infection could be caused by an interplay between viral and host factors. HCV infection per se generates multiple defects in hepatic insulin-signaling pathways [33-35].

The major role of HCV in IR development is also supported by the identification of IR in patients with normal BMI and without significant fibrosis [36]. Recently, some studies were able to demonstrate a direct role of viral replication in IR development, establishing a significant correlation between HOMA-IR and HCV-RNA levels [36, 37] even after adjustment for age, gender and BMI as known factors which might be confounders [38].

Similarly to other investigations [11, 39, 40], we did not find an association between viremia and the MS or HOMA-IR. Insulin resistance was higher in patients with CHC and MS than in those without MS, and in the univariate analysis HOMA-IR was correlated with BMI and visceral obesity. Visceral obesity estimated by waist circumference is viewed as the phenotypic expression of IR [40] and we found that HOMA-IR was almost two-fold higher in patients with CHC and MS than in those with CHC alone.

In the univariate analysis, HOMA-IR significantly correlated with BMI, with histological activity and with degree of steatosis. In patients with CHC and MS, the multivariate analysis found a correlation of HOMA-IR ≥ 2 with visceral obesity and adiponectin, but not with steatosis. In the multivariate analysis, the factors independently associated with a HOMA-IR ≥ 4 were: visceral obesity, adiponectin levels, activity and steatosis, but not fibrosis. Our results suggest an association between the degree of IR and steatosis.

Hepatic steatosis usually occurs in about 50% of patients with CHC, a figure significantly higher than the prevalence of hepatic steatosis in the general population [9, 41]. Moreover, the prevalence of HCV-associated steatosis is 2.5 times higher than that expected by simple coexistence of nonalcoholic liver disease (NAFLD] and CHC, and 30-40% of patients with CHC and steatosis have no risk factors for NAFLD [4]. Steatosis and IR are two linked conditions and association between steatosis and fibrosis has been documented [42].

Insulin resistance is undoubtedly the key factor in the development of hepatic steatosis, especially in genotype 1 infection, generating the metabolic steatosis [9].

Our study revealed, surprisingly, a lack of correlation between IR and fibrosis, and a positive correlation between IR and activity. Most investigators have demonstrated that IR has developed before the stage of cirrhosis and that it is higher in patients with CHC and mild fibrosis than in healthy controls [30]. Despite the large body of evidence demonstrating the association between IR and liver fibrosis in CHC [10], some studies do not support this association. They suggest that IR enhances progression to fibrosis by inducing steatosis, implying a complex mechanism in which inflammatory activity and a modified cytokine profile have a distinct role [40]. This is supported by some studies that were not able to demonstrate an association between IR and liver fibrosis [37, 39, 40] and suggested that the link between IR and fibrosis was represented by hepatic steatosis. In concordance with these studies, we found that IR correlated with hepatic steatosis, but not with fibrosis.

The lack of correlation between IR and fibrosis in our and in the above mentioned studies, might be explained by a higher contribution of the metabolic versus viral factors in this cohort of patients with genotype 1 infection [43]. The cases of NASH superimposed on CHC could have influenced our results, but due to their limited number we did not perform a separate analysis of this group.

The debate regarding the role of steatosis versus IR in liver fibrosis and its progression is still on-going. The progression of fibrosis might be due to a complex mechanism, implying the inflammation, the steatosis and a subtle, and not yet understood, interplay between proinflammatory and antiinflammatory cytokines [44- 46]. Probably an integrated approach would be more reliable. The most accepted opinion is that steatosis and fibrosis are linked together, and the role of steatosis is not direct and might be confounded by the underlying IR [47].

Another unexpected finding of our study was the correlation of IR with the necroinflammatory activity. This is not a singular finding. Another study found an association between IR, high serum viral load and moderate-severe necroinflammation in patients with CHC infected with genotype 1 or 4 [36]. The inflammatory process generated by HCV implies the intervention of proinflammatory (TNF-α, IL-6] and antiinflammatory (adiponectin] cytokines. It was found that the serum levels of TNF-α were associated with portal/periportal inflammation independently of host factors, including BMI [20]. TNF-α can induce IR by direct and indirect mechanisms, including the regulation of expression of some adipocyte genes known to modulate insulin sensitivity [48]. In CHC, serum TNF-α levels and circulationg soluble TNF receptors (sTNFR] were increased, and sTNFR significantly correlated with aminotransferase levels and the degree of inflammation [49]. The role of HCV itself was revealed in a transgenic mouse model that specifically expressed the HCV core protein in hepatocytes. This model suggested that HCV could induce IR through a proinflammatory cytokine mediated pathway, including the elevated intrahepatic TNF-α [35].

Despite the major role played by HCV in the development of IR and hepatic steatosis, host metabolic factors might have a great contribution in chronic HCV infection.

A significant number of our patients had MS, and visceral obesity was the constant criterion for the definition of MS. The adipose tissue is no longer considered only as a storage organ, but rather a very active neuroendocrine organ, that produces and secretes a large number of active peptides, collectively named adipocytokines or adipokines [45], with significant implications in several metabolic processes. The imbalance between the increased proinflammatory cytokines and the decreased antiinflammatory adipokines may generate metabolic disturbances, including IR, that have a pivotal role in the configuration of MS [50, 51]. Among these cytokines, the role of adiponectin in NAFLD and CHC has been largely studied.

Adiponectin, an adipocytokine secreted by adipocytes, exerts a number of protective effects: it increases insulin sensitivity [52], and has antisteatotic [53], antiinflammatory and antifibrotic effects [54]. Because chronic HCV infection often shows features of MS, the serum adiponectin, as well as its receptors have been extensively studied in CHC [46, 55, 56]. We found significantly lower levels of adiponectin in patients with CHC and MS both in males and females. As in other reports, serum adiponectin levels negatively correlated with the metabolic parameters exploring IR: BMI, visceral obesity, serum insulin, C-peptide and HOMA-IR (Fig. 1). These findings support the hypothesis that IR is not only the result of a direct action of the virus, but also of an imbalance of adipocytokines, mainly in patients with MS [57, 58], confirming the role of the metabolic factors in modulating insulin sensitivity. By multivariate regression analysis the only independent predictors of adiponectin level, in our study, were visceral obesity and HOMA-IR.

The link between viral factors and adiponectin was recently established. A positive association of serum adiponectin and HCV load, independent of IR, was found. Patients with HCV genotype 1 infection had a higher IR than those with genotype 2 infection and lower serum adiponectin levels. These findings suggest HCV genotype specific differences in inducing IR and adiponectin levels [37, 59]. There are two possible explanations: the influence of the degree of hepatic pathological changes (inflammation or fibrosis] on serum adiponectin levels [37], or the existence of HCV specific differences in AdipoR1 and AdipoR2 gene expression [59].

Although we did not study the response of adiponectin to antiviral treatment, two findings must be mentioned: the basal levels of adipocytokines are independent from treatment outcome, and the kinetic analysis of adipocytokines during and after therapy revealed an increase in serum adiponectin levels at the and of follow-up [40]. Insulin resistance evaluated by HOMA-IR also decreased at the end of follow-up in patients with virological response [60].

The main limitation of our study is the evaluation of visceral obesity based only on waist circumference criterion. We did not use objective methods to evaluate body composition and visceral obesity. It is possible that the adherence to the IDF criteria for diagnosing MS, especially the waist circumference could be too restrictive as compared to the WHO criteria [27] or NCEP III (Adult Treatment Panel III] [61]. This might either overestimate the prevalence of MS or underestimate the real prevalence and importance of visceral fat. Then, in order to explain the correlation between IR and the degree of activity, the assessment of serum TNF-α levels would be helpful. By increasing the number of patients, a distinct analysis of patients with CHC, CHC with superimposed NASH and pure NASH would better discriminate between viral and host factors.

The lack of correlation between IR and fibrosis in our study should be further investigated.

In **conclusion**, our study, performed on a large number of patients with CHC having underwent liver biopsy and a thorough biological and metabolic evaluation, supports the association of CHC with the MS, confirming the presence

of IR in chronic HCV infection, especially in patients with concomitant MS, as well as the role of the modified adiponectin profile in generating IR.

Conflicts of interest

None to declare.

Acknowledgment

This study was supported by the Romanian National Authority for Scientific Research as a part of the CEEX program FINALISM 94/31.07.2006.

References

1. Koike K. Hepatitis C as a metabolic disease: Implication for the pathogenesis of NASH. Hepatol Res 2005; 33: 145-150.
2. Narita R, Abe S, Kihara Y, Akiyama T, Tabaru A, Otsuki M. Insulin resistance and insulin secretion in chronic hepatitis C virus infection. J Hepatol 2004; 41: 132-138.
3. Fartoux L, Poujol-Robert A, Guéchot J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut 2005; 54: 1003-1008.
4. Adinolfi LE, Durante-Mangoni E, Zampino R, Ruggiero G. Review article: hepatitis C virus-associates steatosis – pathogenic mechanisms and clinical implications. Aliment Pharmacol Ther 2005; 22(suppl 2]: 52-55.
5. Lecube A, Hernandez C, Simó R, Esteban JI, Genesca J. Glucose abnormalities are an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. Am J Gastroenterol 2007; 102: 2189-2195.
6. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med 2000; 133: 592-599.
7. Perlemuter G, Sabile A, Letteron P, et al. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral – related steatosis. FASEB J 2002; 16: 185-194.
8. Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. Gastroenterology 2004; 126: 586-597.
9. Camma C, Bruno S, DiMarco V, et al. Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis C. Hepatology 2006; 43: 64-71.
10. Hui JM, Sud A, Farrell GC, et al. Insulin resistance is associated with chronic hepatitis C infection and fibrosis progression. Gastroenterology 2003; 125: 1695-1704.
11. Conjeevaram HS, Kleiner DE, Everhart JE, et al. Race, insulin resistance and hepatic steatosis in chronic hepatitis C. Hepatology 2007; 45: 80-87.
12 Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001; 33: 1358-1364.
13. Kumar D, Farrell GC, Fung C, George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: Reversal of hepatic steatosis after sustained therapeutic response. Hepatology 2002; 36: 1266-1272.
14. Rubbia-Brandt L, Giostra E, Mentha G, Quadri R, Negro F. Expression of liver steatosis in hepatitis C virus infection and pattern of response to alpha- interferon. J Hepatol 2001; 35: 307.
15. Serfaty L, Andreani T, Giral P, Carbonell N, Chazouillères O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. J Hepatol 2001; 34: 428-434.
16. Poynard T, Ratziu V, McHutchinson J, et al. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 2003; 38: 75-85.
17. Matsuzawa Y, Funahashi T, Kihara S, Shimowura I. Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol 2004; 24: 29-33.
18. Tsochatzis E, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. Am J Gastroenterol 2006; 101: 2629-2640.
19. Diehl AM, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. Gut 2005; 54: 303-306.
20. Cua IHY, Hui JM, Bandara P, et al. Insulin resistance and liver injury in hepatitis C is not associated with virus-specific changes in adipocytokines. Hepatology 2007; 46: 66-73.
21. International Diabetes Federation. The IDF consensus worldwide definition of the metabolic syndrome. http://www.idf.org.May 2005
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.
23. Romero-Gómez M. Insulin resistance and hepatitis C. World J Gastroenterol 2006; 12: 7075-7080.
24 Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperation Study Group. Hepatology 1994; 20: 15-20.
25. Cholet F, Nousbaum JB, Richecoeur M, et al. Factors associated with liver steatosis and fibrosis in chronic hepatitis C patients. Gastroenterol Clin Biol 2004; 28: 272-278.
26. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41: 1313-1321.
27. WHO consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva: World Health Organization, 1999, report No WHO/NCD/NCS/99.2.
28. Grundy SM. Metabolic syndrome: part II. Endocrinol Metab Clin North Am 2004; 33: xi-xiii.
29. Sanyal AJ. Review article: non-alcoholic fatty liver disease and hepatitis C – risk factors and clinical implications. Aliment Pharmacol Ther 2005; 22(suppl 2]: 48-51.
30. Petit JM, Bour JB, Galland-Jos C, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol 2001; 35: 279-283.
31 Ratziu V, Munteanu M, Charlotte F, Bonyhay L, Poynard T; LIDO Study Group. Fibrogenic impact of high serum glucose in chronic hepatitis C. J Hepatol 2003; 39: 1049-1055.
32. Shaheen M, Echeverry D, Oblad MG, Montoya MI, Teklehaimanot S, Akhtar AJ. Hepatitis C, metabolic syndrome, and inflammatory markers: results from the Third National Health and Nutrition Examination Survey (NHANES III]. Diabetes Res Clin Pract 2007; 75: 320-326.
33. Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology 2003; 38: 1384-1392.
34. Kawaguchi T, Yoshida T, Harada M, et al. Hepatitis C virus down-

regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 2004; 165: 1499-1508.
35. Shintani Y, Fujie H, Miyoshi H, et al. Hepatitis C virus and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 2004; 126: 840-848.
36. Moucari R, Asselah T, Cazals-Hatem D, et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level and liver fibrosis. Gastroenterology 2008; 134: 416-423.
37. Hsu CS, Liu CJ, Liu CH, et al. High hepatitis C viral load is associated with insulin resistance in patients with chronic hepatitis C. Liver Int 2008; 28: 271-277.
38. Yoneda M, Saito S, Ikeda T, et al. Hepatitis C virus directly associated with insulin resistance independent of the visceral fat area in nonobese and nondiabetic patients. J Viral Hepat 2007; 14: 600-607.
39. Durante-Mangoni E, Zampino R, Marrone A, et al. Hepatic steatosis and insulin resistance are associated with serum imbalance of adiponectin/tumour necrosis factor-alpha in chronic hepatitis C patients. Aliment Pharmacol Ther 2006; 24: 1349-1357.
40. Lo Iacono O, Venezia G, Petta S, et al. The impact of insulin resistance, serum adipocytokines and visceral obesity on steatosis and fibrosis in patients with chronic hepatitis C. Aliment Pharmacol Ther 2007; 25: 1181-1191.
41. Negro F. Mechanisms and significance of liver steatosis in hepatitis C virus infection. World J Gastroenterol 2006; 12: 6756-6765.
42. Ratziu V, Trabut JB, Poynard T. Fat, diabetes and liver injury in chronic hepatitis C. Curr Gastroenterol Rep 2004; 6: 22-29.
43. Liu CJ, Jeng YM, Chen PJ, et al. Influence of metabolic syndrome, viral genotype and antiviral therapy on superimposed fatty liver disease in chronic hepatitis C. Antivir Ther 2005; 10: 405-415.
44. Czaja MJ. Liver injury in the setting of steatosis: crosstalk between adipokine and cytokine. Hepatology 2004; 40: 19-22.
45. Vettor R, Milan G, Rossato M, Federspil G. Review article: adipocytokines and insulin resistance. Aliment Pharmacol Ther 2005; 22(suppl 2]: 3-10.
46. Jonsson JR, Moschen AR, Hickman IJ, et al. Adiponectin and its receptors in patients with chronic hepatitis C. J Hepatol 2005; 43: 929-936.
47. Ratziu V, Sabouri M, Poynard T. Worsening of steatosis and fibrosis progression. Gut 2003; 53: 1386-1387.
48. Ruan H, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor-alpha suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor-kappaB activation by TNF-alpha is obligatory. Diabetes 2002; 51: 1319-1336.
49. Zylberberg H, Rimaniol AC, Pol S, et al. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. J Hepatol 1999; 30: 185-191.
50. Iannucci CV, Capoccia D, Calabria M, Leonetti F. Metabolic syndrome and adipose tissue: new clinical aspects and therapeutic targets. Curr Pharm Des 2007; 13: 2148-2168.
51. Guerre-Millo M. Adiponectin: an update. Diabetes Metab 2008; 34: 12-18.
52. Pittas AG, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. J Clin Endocrinol Metab 2004; 89: 447-452.
53. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver disease in mice. J Clin Invest 2003; 112: 91-100.
54. Bastard JP, Maachi M, Lagathu C, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006; 17: 4-12.
55. Petit JM, Minello A, Jooste V, et al. Decreased plasma adiponectin concentrations are closely related to steatosis in hepatitis C virus infected patients. J Clin Endocrinol Metab 2005; 90: 2240-2243.
56. Siagris D, Vafiadis G, Michalaki M, et al. Serum adiponectin in chronic hepatitis C and B. J Viral Hepat 2007; 14: 577-583 .
57. Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. Hepatology 2005; 42: 987-1000.
58. Tilg H, Hotamisligil GS. Nonalcoholic fatty liver disease: Cytokine-adipokine interplay and regulation of insulin resistance. Gastroenterology 2006; 131: 934-945.
59. Liu CJ, Chen PJ, Jeng YM, et al. Serum adiponectin correlates with viral characteristics but not histologic features in patients with chronic hepatitis C. J Hepatol 2005; 43: 235-242.
60. Tai TY, Lu JY, Chen CL, et al. Interferon-alpha reduces insulin resistance and beta-cell secretion in responders among patients with chronic hepatitis B and C. J Endocrinol 2003; 178: 457-465.
61. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP] Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III]. JAMA 2001; 285: 2486-2497.