

Prevalence of insulin resistance in chronic hepatitis C genotype 1 and 3 patients

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ABSTRACT

Background and rationale. Chronic infection with the hepatitis C virus (HCV) is associated with a higher prevalence of insulin resistance compared to the general population. This finding is associated with hepatic steatosis, increased liver fibrosis and lower rates of sustained virological response to interferon based therapy. The relationship of insulin resistance and HCV genotype is controversial. Our aim was to compare the prevalence of insulin resistance between patients with HCV genotype 1 and 3. The association of insulin resistance, hepatic steatosis and liver fibrosis was also investigated. **Results.** Forty four consecutive treatment naïve patients with HCV genotypes 1 or 3, without cirrhosis and without risk factors for metabolic syndrome were prospectively included. Insulin resistance was defined as a homeostasis model assessment for insulin resistance (HOMA-IR) above 2.0. Steatosis and fibrosis were assessed histologically. Insulin resistance was found in 27 (61%) patients and significant steatosis in 37 (84%) patients. Comparison between patients with HCV genotype 1 and 3 showed insulin resistance in 15 (65%) vs. 12 (57%), respectively ($P = 0.81$) and steatosis in 19 (83%) vs. 18 (86%), respectively ($P = 0.93$). Comparison between patients with and without insulin resistance showed, respectively, a higher prevalence of significant fibrosis (56% vs. 6%; $P = 0.0001$), and a higher mean degree of steatosis (1.3 ± 0.72 vs. 0.76 ± 0.56 ; $P = 0.01$). **Conclusions.** Prevalence of insulin resistance was not different between HCV infected patients with genotype 1 vs. 3. Nevertheless, independent of HCV genotype, there was a statistically significant relationship between insulin resistance and a higher amount of liver fibrosis and steatosis.

Key words. Insulin resistance. Hepatic steatosis. Liver fibrosis. Hepatitis C virus.

INTRODUCTION

Hepatitis C virus (HCV) infection is currently one of the main causes of cirrhosis and hepatocellular carcinoma in most developed countries, with an estimated world prevalence of 185 million people chronically infected.¹ There is unequivocal association between chronic HCV infection, insulin resistance (IR) and type 2 diabetes.² Moreover, patients with HCV infection and IR may experience a more rapid progression of liver fibrosis and a lower rate of sustained virological response to interferon based

therapy.³⁻⁶ Compelling data indicates that HCV can induce IR directly, mainly by increased tumor necrosis factor alpha (TNF α) production together with over-expression of suppressor of cytokine signaling 3 (SOC-3).^{2,5,7} However, the relationship between IR and HCV genotype is still controversial.^{2,7-9} Thus, the aim of this study was to compare the prevalence of IR, estimated by the homeostasis model assessment for IR (HOMA-IR) in patients infected with HCV genotypes 1 and 3 without cirrhosis and without risk factors for the metabolic syndrome. The association of IR with hepatic steatosis and liver fibrosis was also investigated.

MATERIAL AND METHODS

For this prospective cross-sectional study, 44 patients (30 men, 14 women) with chronic hepatitis C were included between March 2010 and March

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2011 at Hospital de Clínicas de Porto Alegre, a tertiary care center located in Porto Alegre, Southern Brazil. The study was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki, and was approved by the local Ethics Committee. Written informed consent was obtained from all patients. All authors had access to the data, were actively involved in its analysis and interpretation, and approved the final manuscript. Eligible patients were 18 years or older, and had chronic hepatitis C without cirrhosis or hepatocellular carcinoma. All individuals had a liver biopsy of adequate size (above 1.5 cm, and more than nine portal tracts) obtained within 24 months of study entry. Liver tissue sample was analyzed by an experienced liver pathologist. Steatosis was classified histologically according to the percentage of hepatocytes with fat using previously defined criteria¹⁰:

- **Grade 0.** Less than 5%.
- **Grade 1.** Between 5 and 33%.
- **Grade 2.** Between 33 and 66%.
- **Grade 3.** More than 66%.

Degree of activity and stage of fibrosis was classified using Metavir.¹¹ Patients were excluded based on the following findings: co-infection with hepatitis B or D, co-infection with Human Immunodeficiency Virus; other causes of liver disease, risk factors for the metabolic syndrome such as arterial hypertension, body mass index ≥ 30 kg/m², dyslipidemia, fasting plasma glucose ≥ 100 mg/dL and/or a previous diagnosis of type 2 diabetes. HCV RNA was detected by COBAS Amplicor HCV PCR test version 2.0 (Roche Diagnostic Systems Inc., Nutley, NJ, USA) with a detection limit of 50 IU/mL and quantified using COBAS Amplicor HCV Monitor (Roche Diagnostic Systems Inc., Nutley, NJ, USA) with a detection limit of 200 IU/mL. IR was determined by HOMA-IR using the following formula: fasting glucose (mmol/L) multiplied by fasting insulin (mIU/L) divided by 22.5. For statistical analysis, continuous variables were expressed as mean \pm standard deviation (SD). T-test or Mann-Whitney was used for comparison of variables with or without normal distribution, respectively. Qualitative data was expressed as proportions. Chi-square or Fisher's Exact Test were used for comparison of variables depending on the number of results obtained in each observation. P-value was based on two-sided test, and alpha < 0.05 was considered statistically significant.

RESULTS

Study sample comprised 44 consecutive patients, with ages between 28 and 68 years, and body mass index ranging from 20.8 to 29.7 kg/m². There was no clinically significant difference between the 23 (52%) patients with HCV genotype 1 and the 21 (48%) patients with genotype 3. Overall, IR was found in 27/44 (61%) patients. Comparison between individuals with HCV genotypes 1 and 3, showed IR in 15/23 (65%) and 12/21 (57%), respectively (P = 0.81). Main baseline characteristics and comparison between HCV genotype 1 and 3 patients are depicted in table 1. There was a higher prevalence of fibrosis Metavir \geq F2 among the 27 patients with IR *vs.* the 17 patients without IR (56 *vs.* 6%, respectively; P = 0.0001). The mean degree of liver steatosis was also higher among patients with IR versus those without IR (1.3 \pm 0.72 *vs.* 0.76 \pm 0.56, respectively; P = 0.01). There was a numerically higher prevalence of steatosis $\geq 5\%$ among patients with *vs.* without IR, however the difference was not statistically significant (93 *vs.* 71%, respectively; P = 0.09). Other characteristics such as age, gender, ALT level, HCV viral load, HCV genotype, lipid profile, and estimated duration of infection were not significantly different among groups.

DISCUSSION

Chronic HCV infection is currently considered a systemic disease.¹² Indeed, several extra hepatic manifestations have been already proven to be related to HCV, including abnormalities in glucose and lipid metabolism, which can be associated with liver steatosis. Many observational studies reported a higher prevalence of type 2 diabetes mellitus or impaired fasting glucose among patients with chronic hepatitis C as compared to matched non-HCV infected controls.¹³⁻¹⁷ Although the specific mechanisms involved in the pathogenesis of diabetes associated with HCV have not yet been fully elucidated, IR seems to play a key role.^{6,18-20}

IR is a situation in which several cell types (particularly muscle, fat, and liver) fail to take up and utilize glucose from the blood stream, leading to a state of compensatory hyperinsulinemia.²¹ In its early stages, the condition is asymptomatic, but later on it may develop into the metabolic syndrome, with or without hyperglycemia.²¹ Several studies indicate that patients with HCV infection have an increased risk of IR compared to the general population.^{3,18,22,23}

Table 1. Characteristics of the sample.

	Total n = 44	HCV genotype 1 n = 23	HCV genotype 3 n = 21	P value*
	Mean ± SD or n (%)			
Patients characteristics				
Age (years)	47.5 ± 9.1	46.5 ± 9.9	48.6 ± 8.3	0.44
Male gender	30 (68)	17 (74)	13 (62)	0.60
Body Mass Index (kg/m ²)	25 ± 1.8	25.2 ± 1.7	24.7 ± 1.9	0.31
Laboratory results				
Alanine aminotransferase (IU/mL)	104 ± 51	103 ± 58	105 ± 43	0.9
Total cholesterol (mg/dL)	161 ± 28	161 ± 27	161 ± 30	1
HDL cholesterol (mg/dL)	49.5 ± 14	49 ± 16	50 ± 13	0.94
Tryglicerides (mg/dL)	100 ± 8	106 ± 38	93 ± 37	0.26
Fasting glycemia (mg/dL)	92 ± 8	92 ± 8	93 ± 9	0.82
Fasting insulinemia (mmol/L)	11.9 ± 7	11.7 ± 6.9	12 ± 7.2	0.9
Insulin resistance (HOMA-IR > 2.0)	27 (61)	15 (65)	12 (57)	0.8
HCV RNA log ₁₀ (IU/mL)**	6.1 ± 0.6	6.3 ± 0.6	5.9 ± 0.5	0.02
Hepatic biopsy results				
Steatosis ≥ 5%	37 (84.1)	19 (82.6%)	18 (85.7%)	1
Degree of steatosis	1.1 ± 0.71	1.04 ± 0.77	1.14 ± 0.66	0.39
Metavir fibrosis score	1.39 ± 1.15	1.52 ± 1.08	1.24 ± 1.22	0.33
Metavir activity score	1.52 ± 0.85	1.52 ± 0.85	1.52 ± 0.87	0.89
Metavir fibrosis score F2/F3	16 (36)	10 (44%)	6 (29%)	0.48

* Between HCV genotype 1 and 3 patients. ** Data available in 39/44 patients.

Besides its role in the metabolic syndrome, IR could have other clinical implications among patients with HCV infection, namely an accelerated hepatic fibrosis progression^{3,4,7,22} and a lower sustained virological response rate to pegylated interferon plus ribavirin treatment.^{6,22} Interestingly, IR does not seem to have a significant effect on the efficacy of triple therapy with pegylated interferon, ribavirin and telaprevir, although data is scant and, so far, based on *post-hoc* analysis.²⁴

The primary aim of the present study was to verify the prevalence of IR among patients with chronic hepatitis C genotype 1 *vs.* 3. Only individuals without any of the usual risk factors associated to metabolic syndrome were included. The main reason for excluding patients with type 2 diabetes, glucose intolerance, obesity, dyslipidemia or hypertension was to allow the investigation of the specific role of HCV genotype in the pathogenesis of IR. A cohort of patients with a high prevalence of IR due to other risk factors could mask an existing link between HCV genotype and IR.

IR in our study was assessed using HOMA-IR, which has been shown to correlate closely with the hyperinsulinemic euglycemic clamp technique

(HEC).²⁵ HEC is considered the gold-standard for measuring IR, however it is too cumbersome and time-consuming to be applied in routine clinical practice.²⁵ It is important to note that there is not an absolute cut-off value for HOMA-IR to diagnose IR.^{25,26} Most studies involving patients with chronic hepatitis C used HOMA-IR cut-off values ranging from 1.5 to > 4. In the present study a HOMA-IR ≥ 2.0 was chosen, based on recent data suggesting that values above this level have a significant impact on sustained virological response to interferon based therapy and fibrosis progression in this population.^{19,20}

Thus, using a HOMA-IR ≥ 2.0, we found that almost two thirds of our patients (61.4%) had IR. Other studies, using HOMA-IR > 3.0, showed a lower prevalence of IR in similar cohorts of non-diabetic chronic HCV infected patients, ranging from 32 to 35%.^{28,29} Applying the same cut-off in our study, 15 (34%) of the 44 non-diabetic HCV patients would be considered to have IR. Among patients with IR, we found a significantly higher mean degree of steatosis and a higher prevalence of significant fibrosis, defined as Metavir ≥ F2, when compared to those without IR. There was an absolute difference in the

prevalence of steatosis between patients with and without IR in our study population; however it did not reach statistical significance.

In the present study, the prevalence of IR was similar between patients with HCV genotype 1 and 3. Also, comparing both genotypes, there was no difference in any demographic, laboratory or histological characteristics, except for HCV viral load, which was slightly higher among HCV genotype 1 patients. Our data contradicts some studies that have found a more severe degree of liver steatosis and fibrosis among HCV genotype 3 patients.²⁸⁻³⁵ Indeed, HCV genotype 3 seems to have a direct cytopathic effect, which has been shown to decrease and disappear upon successful treatment with antivirals.^{36,37} However, despite inducing more steatosis and fibrosis, it is not clear whether HCV genotype 3 is linked or not to a higher degree of IR. In this regard, data is controversial, with some authors showing higher IR among HCV genotypes 1 and 4,²⁷ while others found it to be increased in patients infected with HCV genotype 3.³ Finally, a more recent multicentre study found that HCV genotype does not affect IR in non diabetic patients with chronic hepatitis C.³⁸ Our results corroborate these later findings.

In conclusion, our study did not show a difference on the prevalence of IR between chronic hepatitis C patients infected with genotypes 1 or 3. Nevertheless, independent of HCV genotype, we found that IR was associated with greater steatosis and liver fibrosis. This finding should lead health care providers involved with the management of chronic hepatitis C patients to consider measuring IR as part of the routine clinical evaluation, independent of the HCV genotype.

ABBREVIATIONS

- **ALT:** alanine aminotransferase.
- **BMI:** body mass index.
- **HCV:** hepatitis C virus.
- **HOMA-IR:** homeostasis model assessment-insulin resistance.
- **HS:** hepatic steatosis.
- **PCR:** polymerase chain reaction.
- **TNF α :** tumor necrosis factor-alpha.

AUTHOR'S CONTRIBUTION TO THE PAPER

All authors had access to the data, were actively involved in its analysis and interpretation, and approved the final manuscript.

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