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Insulin resistance and not steatosis is associated with modifications in oxidative stress markers in chronic hepatitis C, non-3 genotype

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Abstract

Background: Modifications of oxidative stress are reported in hepatitis C. The relationship between insulin resistance (IR), steatosis and oxidative stress is not established. *Materials and methods:* One hundred and eighty-seven HCV-RNA patients were assessed by determination of biochemical, metabolic and viral features, HOMA-IR and morphological alterations. In the 52-non-3 genotypes sub-group and 35 healthy individuals, thiobarbituric acid (TBARS), total glutathione (total-GSH), vitamins C and E, lycopene, β-carotene, glutathione peroxidase (GPx), catalase and superoxide dismutase were determined. *Results:* In non-3 genotype patients, steatosis was associated with higher values of BMI, HOMA-IR and triglycerides. In the 52-HCV sub-group, values of TBARS, GPx and total-GSH differ from the control group. Despite these, differences could not be observed according to the presence of steatosis, patients with IR presented significant differences regarding total-GSH ($p = 0.019$), β-carotene ($p = 0.006$), lycopene ($p = 0.005$) and GPx ($p = 0.009$). *Conclusion:* In non-3 genotype HCV carries, IR, and not steatosis, is associated with modifications in serum levels of oxidative stress.

Keywords: *Insulin resistance, chronic hepatitis C, hepatic steatosis, oxidative stress markers, fibrosis*

Introduction

A greater prevalence of type 2 diabetes mellitus (DM2) and glucose intolerance is found in patients with chronic hepatitis C (HCV) than in patients with other liver diseases or the general population, even when cirrhotic patients are excluded from the analysis [1–4]. These alterations in glucose homeostasis are related to the presence of insulin resistance (IR), an almost invariable finding in DM2 and the best predictor of the onset of the disease, preceding its onset by 10–20 years, as shown by some longitudinal studies [5,6]. IR has been related to the presence of steatosis in patients with hepatitis C and non-3 genotype [7,8]. These patients frequently have central obesity, hypertriglyceridemia, high blood pressure, decreased HDL cholesterol and diabetes or glucose intolerance [8,9] while in genotype-3 HCV infection, hepatic fat accumulation might depend mainly on viral hepatotoxicity [7–9].

Both insulin resistance and steatosis have been independently related to disease progression and with a lower response to interferon and ribavirin treatment in those patients [7,8,10–13]. Fatty liver and IR enhanced disease progression could be related to the development of oxidative stress, as it occurs in non-alcoholic fatty liver disease [14]. In chronic hepatitis C, several studies have shown a relationship between the activity of the disease and the presence of oxidative stress [15–18]. A reduction of serum and tissue levels of antioxidant elements and an increase in lipid peroxidation and protein oxidation have been shown in these patients, even in the initial stages of the disease [17,18]. However, the interaction between liver steatosis, insulin resistance and oxidative stress in chronic hepatitis C has not been comprehensively assessed.

The main goal of the present study was to establish a relationship between serum oxidative stress markers

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and insulin resistance and the presence of steatosis in the liver biopsy of chronic hepatitis C carriers.

Patients and methods

This study comprised 187 consecutive adult hepatitis C virus carriers who were submitted to liver biopsy in São Paulo Hospital of the Federal University of São Paulo. They were diagnosed by anti-HCV antibody detection using a 3rd generation ELISA immunoassay method (Abbott Laboratories®, IMX, USA) and confirmed by HCV-RNA test using the polymerase chain reaction technique (Amplicor-HCV Roche Diagnostics®, Switzerland). HCV genotyping was performed by sequencing of the 5′ non-coding segment and the quantitative measure of the viral load (mean HCV-RNA) by quantitative RT-PCR Amplicor Roche®. Patients with decompensated liver disease, co-infection with hepatitis B or HIV viruses, other associated liver diseases, alcohol use above 20 g/day for women and 40 g/day for men and previous anti-viral treatment were excluded.

Liver histology

A percutaneous biopsy of the liver was performed in all patients and the specimens were analysed, by an experienced pathologist (V.L.) blinded to the clinical data, for stage of fibrosis and grade of activity according to METAVIR scoring system [19]. Steatosis was assessed as the percentage of hepatocytes containing macrovesicular fat droplets [20]. It was graded as 0 (no steatosis), 1 (<33% of hepatocytes affected), 2 (33–66% of hepatocytes affected) or 3 (>66% of hepatocytes affected).

Biochemical and haematological measures

After an overnight fast of 12 h, venous blood was drawn to determined aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamil transpeptidase (GGT), total cholesterol, HDL-cholesterol and triglyceride levels. All those biochemical tests were quantified using the automated kinetic method. Serum ferritin levels were measured by chemiluminescence.

Metabolic profile

The body mass index (BMI) was calculated as weight in kilograms/height in square metres. Glucose, insulin and C-peptide serum levels were assessed by immunofluorimetry (Perkin Elmer BR-CS). Insulin resistance (IR) was determined using the homeostatic model assessment (HOMA-IR) [21].

Oxidative stress markers

In the last 52 consecutive non-3 genotype patients, oxidative stress markers were assessed, as it follows: blood samples were taken on heparin after 12 h fasting and were immediately processed. Plasma was separated from red blood cells by conventional centrifugation and assessed for nutritional antioxidants, lipid and protein oxidation. Red blood cells were further processed for Cu/Zn-superoxide dismutase, catalase and glutathione peroxidase activity measurements according to Junqueira et al. [22]. Lipid derived oxidation products plasma levels were measured as thiobarbituric acid reactants (TBARS) [22]. Plasma levels of oxidized proteins, used as an index of protein oxidation, were measured according to the method of Buss et al. [23]. Total glutathione (total GSH) was measured in erythrocytes according to Tietze [24]. Plasma concentrations of vitamin C, alpha-tocopherol (vitamin E), lycopene and beta-carotene were evaluated by HPLC according to Gomes et al. [25]. The activities of erythrocyte glutathione peroxidase (GPX), catalase and Cu/Zn-superoxide dismutase (SOD) were assessed using spectrophotometric methods [22].

Control group

Healthy individuals ($n = 35$) with body mass index (BMI) < 25 Kg/m^2, fasting glycemia < 100 mg/dl and 2-h post-prandial glycemia < 140 mg/dl, age and gender comparable with the chronic hepatitis C carriers, were assessed to evaluate oxidative stress serum markers and insulin resistance. They all showed hepatic enzymes within normal ranges, as well as no signals of metabolic syndrome.

Statistical analysis

The results are presented as means ± standard deviation. Unpaired *t-test* and χ^2 contingency test were used whenever appropriated. Non-parametric methods were used for non-normally distributed values (Mann-Whitney test). Linear regression analysis was calculated for the parameters associated with insulin resistance and steatosis. A significance level of 0.05 (5%) was considered for all statistical analyses [26].

The study protocol was approved by the Human Ethics Committee of São Paulo Hospital of the Federal University of São Paulo and in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all participant subjects.

Results

The clinical, demographic, virological and histological features of the population under study are listed in Table I.

Initially, all 187 chronic hepatitis C carriers were divided according to their genotype [non-3 genotype (n= 131) and genotype-3 (n = 56)] and presence or absence of steatosis in the biopsy (Tables II and III). Patients with non-3 genotypes with steatosis (n = 71) showed significantly higher values of BMI, HOMA-IR, triglycerides and GGT when compared with those patients with no steatosis (Table II). Patients with genotype 3 and liver steatosis (n = 40) showed significantly higher values of ALT, AST and mean HCV-RNA and lower total cholesterol levels, with no difference regarding the parameters related to insulin resistance or metabolic syndrome (Table III).

In a linear regression analysis, HOMA-IR (p = 0.036) and triglycerides (p = 0.035) were independent predictors for steatosis in non-3 genotype and ALT (p= 0.006), BMI (p = 0.036) and HCV-RNA load (p = 0.041) were independent predictors for steatosis in 3 genotype.

In the last 52 non-3 genotype patients, the values for oxidative stress serum markers were also obtained. In this group with mean age of 49.4 ± 12.6 years old, 27 were women and 25 men; only four (7.7%) showed graded 3 steatosis (> 66% of hepatocytes affected) and 10 (19%) of them had the diagnosis of cirrhosis upon liver biopsy.

Table I. General features of the 187 chronic hepatitis C carriers.

Variables	Mean ± SD (range)
Age (years)	48.9 ± 12.1 (18–78)
Gender (M:W)	110:77
AST (X ULN)	2.12 ± 1.7 (0.36–9.61)
ALT (X ULN)	2.73 ± 1.9 (0.30–10.1)
GGT (X ULN)	2.22 ± 1.14 (0.2–15.1)
Mean HCV-RNA	
<800 000 UI/mL	106
⩾800 000 UI/mL	81
Fibrosis	
0	28 (15%)
1	44 (24%)
2	36 (19%)
3	36 (19%)
4	43 (23%)
Activity (mean ± SD)	1.53 ± 1.0 (0–3)
Steatosis	
0	40%
1	43%
2	18%
3	9%
3 genotype (P:A)	40:16 (71.4%)
non-3 genotype (P:A)	71:60 (54.2%)
BMI (Kg/m^2)	26.6 ± 4.2 (15.4–44.1)
HOMA-IR	3.4 ± 2.8 (0.43–23.42)
Cholesterol (mg/dL)	167.3 ± 32.2 (85–252)
HDL (mg/dL)	50.9 ± 32.2 (18–73.3)
Triglycerides (mg/dL)	123.4 ± 80.5 (21–784)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; BMI, body mass index; HOMA-IR, insulin resistance index; ULN, upper limit normality; P, presence; A, absence.

For comparison with these patients, a control group with 35 healthy individuals, 17 men and 18 women, 48.2 ± 12.8 years of age, was used to assess the HOMA-IR values and oxidative stress serum markers. The insulin resistance value (HOMA-IR) obtained for the control group was 1.27 ± 0.62. The cut-off point value of HOMA-IR for the diagnosis of insulin resistance was calculated as ⩾ 2.5, which corresponds to the upper quartile of the control population.

When compared with the control group, the non-3 genotype hepatitis C group presented significant differences regarding TBARS levels (4.6±1.5 × 3.7± 1.4; p = 0.020), glutathione peroxidase activity (11.8 ± 3.0 × 10.3 ± *2.5;* p = 0.045), glutathione plasmatic levels (6.0 ± 1.2 × 6.5 ± 0.9; p = 0.050), and HOMA-IR (4.47 ± 4.40 × 1.27 ± 0.62; p = 0.001).

Patients with non-3 genotype hepatitis C when divided according to the presence or absence of steatosis on liver biopsy presented significant differences in the HDL cholesterol, triglycerides concentration and HOMA-IR values (Table IV), but no differences could be found for hepatic enzymes or the oxidative stress serum markers concentration. On the other hand, when these patients were separated according to the 2.5 cut-off point for HOMA-IR, serum levels of AST, ALT, GGT, ferritin, C-peptide, triglycerides, HDL cholesterol, total GSH, beta-carotene, lycopene, GPx, catalase, BMI and degree of fibrosis in patients with insulin resistance were significantly different from the serum levels found in patients with hepatitis C and HOMA-IR < 2.5 (Table V).

In a linear regression analysis, BMI (p= 0.031) and serum total glutathione (p = 0.037) were independent predictors for insulin resistance in these patients with hepatitis C non-3 genotype (Table VI).

Discussion

Steatosis is found with a variable frequency in chronic hepatitis C, with a prevalence that can range from 30–70%, being more frequent in patients with genotype 3 [10,27]. In those carrying that genotype, the presence of steatosis is associated to higher HCV-RNA and reduction of serum cholesterol values, as we found among our patients, and is accompanied by a tendency to recede with viral eradication. These facts suggest that, in these cases, steatosis derives from direct cytopathologic viral action [10,28]. Conversely, in non-3 genotype patients, steatosis tends to persist after treatment and is associated with other factors more connected with the host, such as alcoholism, hypothyroidism and metabolic syndrome features [11–13]. As confirmed in the first part of this

Table II. Demographic, biochemical, histological and virological features in chronic hepatitis C carriers, non-3 genotypes, with and without liver steatosis *(n* = 131).

Variables	Steatosis *(n* =71)	No steatosis *(n* = 60)	*p*
Age (years)	52.7 ± 11.9	47.4 ± 13.6	0.019*
Gender (M:W)§	38:33	31:29	0.971
AST (X ULN)	2.24 ± 1.9	1.73 ± 1.19	0.260
ALT (X ULN)	2.78 ± 2.0	2.22 ± 1.7	0.082
GGT(X ULN)	2.77 ± 2.5	1.97 ± 2.2	0.006*
Platelets ($\times 10^3/mm^3$)	192.8 ± 61.9	199.4 ± 69.4	0.557
Mean HCV-RNA ($\times 10^3$ UI/mL)	1210 ± 1566	872 ± 852	0.965
Fibrosis (mean±SD)	2.1 ± 1.4	1.9 ± 1.3	0.301
BMI (Kg/m^2)	28.1 ± 3.5	25.4 ± 4.8	<0.001*
Insulin (μU/L)	24.9 ± 2.1	11.5 ± 2.0	0.027*
Glucose (mg/dL)	104.7 ± 30.4	93.1 ± 20.3	<0.001*
HOMA-IR	4.35 ± 2.98	2.45 ± 1.56	<0.001*
Cholesterol (mg/dL)	172.7 ± 33.4	164.6 ± 27.8	0.144
HDL (mg/dL)	47.2 ± 12.7	48.0 ± 10.7	0.846
Triglycerides (mg/dL)	149.6 ± 104.9	101.4 ± 38.7	<0.001*

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; BMI, body mass index; HOMA-IR, insulin resistance index; HDL, high density lipoprotein.
§ χ^2.
*p-significance level.

research work, patients with non-3 genotype and hepatic steatosis presented higher values of serum triglycerides and glucose, BMI and HOMA-IR values than those patients with the same genotype but no fat tissue infiltration, characterizing the relation between IR, metabolic syndrome and the presence of steatosis. Despite the fact that other mechanisms can be involved in the genesis of steatosis in hepatitis C, such as hyperhomocysteinemia and obesity itself [29,30], IR is considered the main cause of steatosis in these non-3 genotype patients.

Since IR is associated to steatosis and both steatosis and IR [11–13,31–33] are associated to fibrosis progression, the way in which these factors may interact is yet to be determined. By analogy with non-alcoholic fatty liver disease (NAFLD) where oxidative stress is related to fatty liver progression to NASH [14,34], in non-3 genotype HCV carriers with steatosis, oxidative stress could be related to the disease progression.

Several studies have shown modifications in serum concentration of oxidative stress markers in HCV carriers that could be associated with liver fibrosis,

Table III. Demographic, biochemical, histological and virological features in chronic hepatitis C carriers, genotype 3, with and without liver steatosis *(n* =56).

	Genotype 3		
Variables	Steatosis *(n* =40)	No steatosis *(n* = 16)	*p*
Age (years)	45.8 ± 8.8	45.4 ± 11.1	0.891
Gender (M:F)§	25:15	10:6	0.760
AST (X ULN)	2.71 ± 1.9	1.63 ± 8.3	0.030*
ALT (X ULN)	3.79 ± 2.2	1.81 ± 0.6	0.001*
GGT (X ULN)	1.91 ± 1.35	1.56 ± 0.9	0.474
Platelets ($\times 10^3/mm^3$)	164.3 ± 63.0	184.7 ± 69.5	0.265
mean HCV-RNA (10^3 UI/mL)	1813 ± 245	1427 ± 454	0.007*
Fibrosis (mean±SD)	2.3 ± 1.5	2.4 ± 1.6	0.823
BMI (kg/m^2)	26.6 ± 4.2	24.6 ± 3.8	0.030*
Insulin (μU/L)	13.7 ± 2.1	17.5 ± 2.4	0.993
Glucose (mg/dL)	95.9 ± 22.9	97.4 ± 24.3	0.723
HOMA-IR	3.57 ± 3.79	2.31 ± 1.6	0.182
Cholesterol (mg/dL)	156.7 ± 35.1	175.2 ± 29.9	0.049*
HDL (mg/dL)	61.6 ± 6.9	58.2 ± 2.2	0.107
Triglycerides (mg/dL)	115.8 ± 58.6	108.9 ± 93.2	0.253

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; BMI, body mass index; HOMA-IR, insulin resistance index; HDL, high density lipoprotein.
§ χ^2.
*p-significance level.

Table IV. Demographic, biochemical, histological features and oxidative stress serum markers in chronic hepatitis C carriers, non-3 genotypes, with and without liver steatosis *(n* =52).

Varables	No steatosis *(n* = 28)	Steatosis *(n* = 24)	*p*
BMI (kg/m^2)	25.5 ± 4.2	28.6 ± 3.7	0.045*
HOMA-IR	3.1 ± 2.2	5.6 ± 5.4	0.040*
HDL (mg/dL)	53.7 ± 14.4	46.8 ± 14.4	0.053
Triglycerides (mg/dL)	106.6 ± 74.9	148.5 ± 105.5	0.039*
C-peptide (μU/mL)	3.4 ± 2.8	3.9 ± 1.9	0.083
TBARS (μM)	4.29 ± 1.49	4.75 ± 1.48	0.430
Total GSH (nmol/mg haemoglobin)	6.16 ± 1.32	5.94 ± 1.06	0.645
GPX (U/g haemoglobin)	12.00 ± 2.91	11.70 ± 3.21	0.595
Lycopene (μM)	0.68 ± 0.36	0.71 ±0.51	0.627
β-carotene (μM)	0.44 ± 0.37	0.42 ± 0.49	0.520
Catalase (U/mg haemoglobin)	156.9 ± 28.3	158.6 ± 31.7	0.811
Cu/ZnSOD (U/mg haemoglobin)	4.97 ± 0.99	5.42 ± 1.32	0.247
Oxidized protein (nmol/ml plasma)	0.23 ± 0.21	0.21 ± 0.19	0.857
α-tocopherol (μM)	12.07 ± 6.60	11.14 ± 4.46	0.854
Vitamin C (μM)	50.25 ± 19.03	45.38 ± 18.68	0.404
Uric acid (mg/dL)	4.97 ± 1.38	5.33 ± 1.22	0.171
Fibrosis (0.1 × ⩾2)§	12:16	16:8	0.150
Activity (0.1 × ⩾2)§	5:23	8:16	0.335

BMI, body mass index; HOMA-IR, insulin resistance index; HDL, high density lipoprotein; TBARS, thiobarbituric acid reactants; SOD, superoxide dismutase; GPx, glutathione peroxidase; Total GSH, total glutathione.
§χ^2.
**p*-significance level.

through the release of cytokines or through lipid peroxidation byproducts, which are capable of activating stellate cells [35,36]. In addition, an immunohistochemistry study associated positive oxidative stress tissue markers with the presence of steatosis [37]. Nevertheless, very few studies evaluated the relationship between the presence of steatosis and oxidative stress in these patients.

In order to study the relation between steatosis, IR and oxidative stress, we evaluated 52 sequential patients with non-3 genotype. This sub-group was comparable to the whole group in terms of the number of patients with 1-genotype, with cirrhosis and degree of steatosis on hepatic biopsy. They also showed a relation between steatosis and parameters related with metabolic syndrome (Table V). In comparison with healthy individuals of similar age and gender, these patients presented higher levels of TBARS, reduction of total glutathione levels and increased glutathione peroxidase activity, as shown by others [13–16,38]. Serum oxidative stress markers, such as antioxidant compounds and enzymes and lipid peroxidation markers are frequently altered in the peripheral blood of patients who are chronic hepatitis C

Table V. Demographic, biochemical, histological features and oxidative stress serum markers in chronic hepatitis C carriers, non-3 genotypes, with and without insulin resistance *(n* =52).

Variables	HC HOMA<2.5 (*n*=24)	HC HOMA ⩾2.5 (*n*=28)	*p*
BMI (Kg/m^2)	25.4 ± 3.8	29.0 ± 3.9	0.002
ALT (xULN)	2.0 ± 1.8	3.1 ± 1.9	0.015
GGT (xULN)	1.6 ± 1.9	3.8 ± 2.5	0.001
Triglycerides (mg/dL)	102.9 ± 79.2	145.1 ± 104.0	0.006
Ferritin (mg/dL)	219.5 ± 249.6	410.8 ± 346.9	0.006
C-peptide (μU/mL)	2.9±2.7	4.4 ± 1.7	<0.001
TBARS (μM)	4.3 ± 1.3	4.7 ± 1.6	0.368
Total GSH (nmol/mg haemoglobin)	6.6 ± 1.3	5.6 ± 0.9	0.019
GPX (U/g haemoglobin)	10.7±2.7	13.1 ±2.9	0.009
β-carotene (μM)	0.6±0.5	0.3 ± 0.3	0.006
Lycopene (μM)	0.9±0.5	0.5 ± 0.3	0.005
Uric acid (mg/dL)	4.7 ± 1.2	5.6 ± 1.2	0.013
Fibrosis (0.1 × ⩾2)§	17:7	11:17	0.046
NA (0.1 × ⩾2) §	11:13	6:22	0.116
Steatosis (P:A)§	8:16	16:12	0.150

BMI, body mass index; GGT, gamma glutamyltransferase; ALT, alanine aminotransferase; Total GSH, total glutathione; NA, necroinflamatory activity; GPx, glutathione peroxidase; ULN, upper limit normality; P, presence; A, absence.
§χ^2.
**p*-significance level.

Table VI. Linear regression analysis final model to define factors independently related to insulin resistance in hepatitis C.

Variables	T	p
BMI (Kg/m^2)	2.254	0.031
Total GSH (nmol/mg)	–2.161	0.037

BMI, body mass index; Total GSH, total glutathione.

carriers and, although serum concentrations of oxidative stress markers are indicators of total systemic response, their serum concentrations have a significant correlation with the hepatic expression of such compounds [15,16].

In spite of what would be expected, we found no alterations in oxidative stress markers when comparing patients with hepatitis C in accordance to the presence or absence of steatosis (Table IV). This result was similar to the findings of Bonnefont-Rousselot et al. [39] when they studied patients with hepatitis B and C, with and without steatosis.

With the cut-off point for HOMA-IR defined for the comparative study with the control group, we observed that patients with IR (HOMA-IR $\geq$ 2.5) presented significant differences regarding hepatic enzymes, BMI, triglycerides, ferritin and several serum oxidative stress markers such as total glutathione, glutathione peroxidase activity, lycopene, beta-carotene and uric acid, in addition to a higher degree of structural alteration as compared with patients with no IR. A regression analysis showed that the main factors associated with IR were serum glutathione values and BMI (Table VI). These results are in agreement with the findings of Mitsuyoshi et al. [40] that showed a significant correlation between HOMA-IR and serum and hepatic levels of thioredoxin, which are markers of oxidative stress. Furthermore, the increased activity of GPx and reduced levels of glutathione and others antioxidants suggest an increased in GSH turnover, as demonstrated by others [18,41].

It has been shown that oxidative stress serum markers [42,43] and insulin resistance levels measured by HOMA-IR [44] tend to normalize when the patient reached a sustained virological response after antiviral treatment. Experimental studies with transgenic animal models and cell cultures with C virus core and also with human liver biopsy specimens have managed to prove that the C virus itself could be capable of inducing the development of insulin resistance [45–7] and oxidative stress [48,49].

Thus, our findings support the hypothesis that oxidative stress directly contributes to IR in chronic hepatitis C. The over-production of reactive oxidative stress (ROS) could result from inflammatory cells or more probably by the direct association of HCV core protein with mytochondria in hepatocytes. This abundance of ROS and the glutathione depletion can inhibit tyrosine phosphorylation of insulin receptor substrate (IRS), via the activation of stress-sensitive pathways, such as the c-Jun N-terminal kinase (JNK) and Nuclear factor (NF)-kB pathways [40,50].

Since the oxidative stress has been related to fibrosis in hepatitis C, our results showing a relationship between oxidative stress and insulin resistance in non-3 genotype chronic hepatitis C carriers, regardless the presence of hepatic steatosis. It could suggest that IR and not steatosis is the main determinant of disease progression among these patients. These results would be in accordance with the findings of Moucari et al. [51] that in non-3 genotypes there is a significant association between fibrosis and IR regardless of the presence of steatosis, and also with the findings of Bugianesi et al. [52] that even in genotype 3 patients the progression of the disease is associated with the presence of IR and not with steatosis.

On the other hand, they apparently seem to be contrary to the observations of Vidali et al. [53], who recently published a paper showing that in chronic hepatitis C non-3 genotype, oxidative stress, measured as malondialdehyde-albumin adducts antibodies, is primarily correlated with hepatic steatosis and not with IR. It is of relevance that in that work the most significant effect regarding lipoperoxidation occurred when steatosis affected more than 66% of the hepatocytes. As in our case series less than 10% of the cases showed steatosis exceeding 66% of the hepatocytes and there was no differences in serum levels of lipid peroxidation and protein oxidation markers among our patients with or without IR, we can interpret our findings as the initial phase of the disease, where HCV non-3 genotype induces oxidative stress that leads to insulin resistance. At this stage, the antioxidant system could prevent the formation of large quantities of protein and lipid peroxides. It is possible that with increased accumulation of triglycerides, lipid peroxidation will occur in a more meaningful way and can have greater impact on the development of liver injury and in disease progression.

Thus, at this stage of the disease, insulin resistance and not steatosis is associated with significant alterations of oxidative stress serum markers. More extensive and longitudinal studies can help us gaining better understanding on the correlations observed between IR, steatosis and chronic hepatitis C progression.

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