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# Role of oxidative stress in the pathogenesis of chronic hepatitis C (CHC)

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**Abstract**

Oxidative stress is an important pathophysiological mechanism in chronic hepatitis with C-virus infection (CHC). Steatosis is frequently observed in CHC and seems to have a significant impact on the natural history of the disease with respect to development of fibrosis. The aim of this study was to investigate the relationship between systemic parameters of oxidative stress, insulin resistance, steatosis degree, and fibrosis in CHC. Fifty patients with chronic hepatitis C (29 men and 21 women with the average age 45 years), with or without steatosis, were tested for: oxidative stress and antioxidant status by measuring serum malondialdehyde (MDA), total blood non-proteic thiols concentration (GSH), gamma glutamyl transpeptidase (GGT) activity, lipid parameters, and liver function tests. Our results show that the prevalence of insulin resistance (IR) in chronic hepatitis with C-virus genotype 1 was 32% and the association with hepatic steatosis was in a proportion of 48%, IR is mediated by both metabolic factors as well as viral factors. Hepatic steatosis was associated with an increase of MDA, correlated with its severity, and secondary with a decrease of GSH. The activity of serum GGT was net superior, in patients with steatosis, proportional with its degree. *Conclusions*: In patients infected by HCV genotype 1, oxidative stress and insulin resistance contribute to steatosis, which in turn exacerbates both insulin resistance and oxidative stress and accelerates the progression of fibrosis. The induction of GGT is an adaptive response against oxidative damage elicited by lipid peroxidation and it may be critical in the progression of the disease.

***Keywords*:** oxidative stress, malondialdehyde, chronic hepatitis C, GGT.

## Introduction

The usual progression of liver disease in patients with hepatitis C (HCV) is a process of inflammation accompanied by periportal necrosis and fibrosis. The inflammation that results from the virus causes stimulation of stellate cells, which ultimately leads to the deposition of collagen, which leads to fibrosis progression within the liver. The hepatitis C virus is not considered to directly injure the liver but it rather triggers an HCV-specific lymphoproliferation. Through profuse inflammatory cytokine production and also a direct cytopathic effect, these T-cells result in hepatocyte apoptosis [1].

Many patients with chronic HCV are also noted to have a degree of steatosis present on their liver biopsies. Hepatic steatosis is defined as excessive lipid accumulation within the hepatocyte cytoplasm and has been more recently recognized as a significant cause for cirrhosis [2]. There are two forms of steatosis present in patients with hepatitis C, HCV-induced steatosis (fatty infiltration is directly elicited by the virus), and metabolic steatosis (process which occurs in the setting of obesity, hyperlipidemia, and insulin resistance) [3].

Insulin resistance has a key role in the development of hepatic steatosis and potentially steatohepatitis [4]. Resistance to the action of insulin results in important changes in lipid metabolism. These include enhanced peripheral lipolysis, increased triglyceride synthesis, and increased hepatic uptake of fatty acids. Each of these may contribute to the accumulation of hepatocellular triglyceride [5].

Excessive fat accumulation in the liver, whatever its cause, is prone to attack by reactive oxygen species (ROS), leading to lipid peroxidation with its cellular consequences. ROS (superoxide anions, hydrogen peroxide, hydroxyl radicals) are relatively short-lived molecules that exert local effects [6]. However, they can attack polyunsaturated fatty acids (PUFAs) and initiate lipid peroxidation within the cell [6], which results in the formation of aldehyde by products such as *trans*-4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA). These molecules have longer half-lives than ROS and have the potential to diffuse from their site of origin to reach distant intracellular and extracellular targets, thereby amplifying the effects of oxidative stress (disturbance in the pro-oxidant–antioxidant balance). The reactive oxygen intermediates, produced in mitochondria, peroxisomes, and the cytosol, are scavenged by cellular defending systems, including enzymatic (ex. superoxide dismutase, glutathione

peroxidase, glutathione reductase, catalase, and gamma glutamyl transpeptidase – GGT), and nonenzymatic antioxidants (ex. G-SH, thioredoxin, lipoic acid, ubiquinol, albumin, uric acid, flavonoids, vitamins A, C and E, etc.). Some are located in cell membranes, others in the cytosol or in the blood plasma [7].

Chronic over-production of reactive oxygen species (ROS) leads to redox imbalance, favoring depletion of GSH, the major non-enzymatic antioxidant [6]. GSH is known as a substrate in both conjugation and reduction reactions. GGT participates in the transfer of amino acids across the cell membrane, and in glutathione (an anti-oxidant) metabolism. The GSH-antioxidant system has several physiological functions such as maintenance of protein-SH groups in a reduced state, detoxification from oxygen radicals, enzymatic degradation of endogenous peroxides, and formation of bioactive molecules. Furthermore, reduced levels of plasma GSH have been associated with the development of liver fat diseases and cardiovascular disease [8], and with the activation of several types of viruses [6].

The contribution of steatosis and oxidative stress to the pathogenesis of chronic hepatitis C (CHC) is still poorly elucidated. Since GGT affects GSH catabolism, a potential link between raised GGT activity and redox state imbalance can be hypothesized in subjects with CHC. The aim of this study was to investigate the relationship between systemic parameters of oxidative stress, insulin resistance and steatosis degree, and fibrosis in CHC.

## Patients and Methods

### Patients

The study population included 50 consenting subjects with chronic hepatitis C, with or without steatosis, selected among 75 outpatients hospitalized at the University Emergency Hospital in Bucharest for diagnosis and antiviral treatment (29 men and 21 women, aged 21–66 years, average age 45 years). The highest incidence of the C-virus chronic infection was encountered at the age interval ranging from 41–55 years.

Subjects who had advanced cirrhosis, hepatocellular carcinoma associated with the C-virus infection, drugs involved in the occurrence of steatosis, association with hepatitis B-virus, severe obesity – Body Mass Index (BMI) >35 kg/m$^2$ were excluded. Subjects with the following conditions, known to be associated with an altered redox pattern, were also excluded: severe uncontrolled hypertension, autoimmune diseases, acute or chronic inflammation, and alcohol abuse.

Information on common risk factors, general dietary-medical history and lifestyle were obtained from each subject using a questionnaire. Associated conditions of chronic hepatic disorders were: smoking habit, hypertension, diabetes, overweight, hypercholesterolemia. Participants were considered smokers based on the current smoking status (10 cigarettes per day for at least one year without interruption). Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg, or the need for anti-hypertensive drugs; hypercholesterolemia as LDL-cholesterol level ≥160 mg/dL, or the need for lipid-lowering medication; diabetes mellitus as fasting glucose levels ≥126 mg/dL, or the need for insulin or oral hypoglycaemic agents; overweight as a body mass index – BMI [kg/m$^2$] as follows: 25.0–29.9 (weight excess); 30.0–34.9 (moderate obesity); 35.0–39.9 (severe obesity). Patients had not received antioxidant vitamin or selenium supplementation within the two months preceding their inclusion in the study.

### Chemical analysis

Blood samples were obtained under fasting conditions on the same day as the liver biopsy. The blood redox status was evaluated by assaying serum lipid peroxidation products (MDA), total blood non-proteic thiols concentration (GSH), ferittin, bilirubin, and GGT activity. Plasma TBARS (the condensation product between MDA and TBA-thiobarbituric acid at 95$^0$C) was assessed by using high performance liquid chromatography (HPLC) method [9]. Analytical HPLC separations were performed with a liquid chromatograph (Waters) with a spectrophotometric detector monitored at 532 nm, with a C18 column of 10-pm particle size. Flow rate of the mobile phase were 0.8 mL/min., according to column and phosphate buffer molarity. Total blood non-proteic thiols were assayed by the method of Beutler E [10]. The general thiol reagent, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, Ellman's Reagent) reacts with GSH to form the 412 nm chromophore 5-thionitrobenzoic, which is measured spectrophotometrically.

Serum GGT (gamma glutamil transpeptidase) was determined using Dade Behring reactive, and the Dimension RXL analyzer. This method uses γ-glutamil 3-carboxi-4-aniline, using as substrate glycil-glycine. Merck commercial kits were used to determine serum concentrations of ferritin using an automatic analyzer.

Glucose, bilirubin, aminotransferases, total serum cholesterol and triglyceride concentrations were determined using standard laboratory methods. High-density lipoprotein (HDL)-cholesterol was determined by a direct method (Konelab) and low-density lipoprotein (LDL)-cholesterol was estimated by Friedewald's formula [12].

Insulin determinations were performed using the method of indirect chemiluminescence (MEIA). Insulin resistance was calculated according to the HOMA score (homeostasis model assessment): the product between the serum glucose and insulin levels in basal conditions were divided at 22.5. For insulin resistance the cut-off value was considered 2.5.

Steatosis was graded as the percentage of hepatocytes containing the fat droplets. Steatosis was considered absent when 5% or less of hepatocytes contained fat droplets and present when fat droplets were present in 10% or more of hepatocytes. Necro-inflammatory activity (activity grade) was scored according to the METAVIR scoring system in patients with chronic hepatitis [13], and according to the scoring system described by Brunt EM *et al.* [14] in patients with NAFLD (non-alcoholic fatty liver disease).

The diagnosis of C-virus infection was based on the presence in the patients' serum of the antibodies created against the C-virus depicted by the 3rd generation of ELISA (enzyme-linked immunoabsorbent assay). This method has a specificity of 99.5–100% and sensitivity of 100%.

In order to confirm the infection, quantitative determinations of the viral RNA were performed using the COBAS TaqMan HCV test – the test of *in vitro* amplification of the C-virus nucleic acid, which uses the High Pure System Viral nucleic acid kit for manual preparation and the COBAS TaqMan 48 analyzer for automatic amplification and detection. The RNA titter was expressed in international units (IU/mL). The detection limit is >10 IU/mL with a positive rate of 95%. In order to determine the genotype of the C-virus the Linear Array Hepatitis C Genotyping test was used with AMPLICOR R HCV test and COBAS AMPLICOR HCV Test version 2. The test uses the RNA reverse transcriptase to produce complementary RNA (cADN), the Polymerase Chain Reaction (PCR) to amplify the cDNA, and hybridization methods to determine the HCV-genotype. Serum RNA is detected with EDTA as an anticoagulant. The serum detection limit is >500 IU/mL with a positive response rate >95%; the clinic specificity of the test is >99.99%.

Data are expressed as mean ± SD. Results were considered significant at $p<0.05$. Statistical analysis was performed by analysis of variance (ANOVA).

## Results

The study population with chronic hepatitis C included 23 subjects without and 27 with different degree of steatosis. Clinical and biochemical characterristics and the redox state profile of the overall population studied, depending on the degree of steatosis are listed in Tables 1 and 2, respectively.

Fasting glucose and alanin-aminotransferase (ALT) levels fell within the normal range in the overall population, whereas values of GGT activity, ferritin concentration and HOMA score were out of range in patients with steatosis, proportional with its degree.

Also, the total cholesterol was not modified and neither the LDL- or HDL-fractions were different among the groups of steatosis. Liver steatosis was in 81% (22/27) of cases light or moderate. Two patients with high triglyceride levels in the serum have shown moderate steatosis at hepatic biopsies.

Steatosis was associated with an increase of MDA, correlated with its severity, and secondary with a decrease of GSH. There were no significant differences of the biological values of bilirubin between the different forms of steatosis.

Insulin resistance was present in 16 patients (32%) and out of the 27 cases of steatosis 13 (48%) disclosed hyperinsulinemia through peripheral resistance. The cut-off value of the HOMA test was considered 2.5. Steatosis was more frequent in women (66%) than in men (48%). Also in women, moderate forms were prevalent (28%) and severe (28%), while in men the predominant form was the light one (27%). Also, steatosis was not associated with viral replication, viral RNA having comparable values regardless of the presence or absence of hepatic steatosis and its severity. All the 50 patients had genotype 1 (Tables 1 and 2).

**Table 1** – ***Clinical and biological data of the CHC patients studied (n=50) depending on the degree of steatosis (number or mean ± SD)***

| Variable | Absence | Light | Moderate | Severe |
|---|---|---|---|---|
| | *n=23* | *n=11* | *n=10* | *n=6* |
| *Average age [years]* | 45.3±10.6 | 43.2±11.2 | 52.3±9.9 | 54.1±11.9 |
| Age [years] | | | | |
| *<45* | 12 (52%) | 4 (36%) | 2 (20%) | 0 |
| *>45* | 11 (48%) | 7 (44%) | 8 (80%) | 6 (100%) |
| Sex | | | | |
| *Men* | 15 (52%) | 8 (27%) | 4 (14%) | 2 (7%) |
| *Women* | 7 (33%) | 2 (10%) | 6 (28%) | 6 (28%) |
| BMI [kg/m$^2$] | | | | |
| *<25* | 15 (66%) | 7 (44%) | 5 (50%) | 0 |
| *25–29* | 8 (34%) | 3 (27%) | 3 (30%) | 2 (23%) |
| *>30* | 2 (9%) | 0 | 1 (10%) | 4 (67%) |
| Waist [cm] | | | | |
| *<88* | 8 | 1 | 0 | 0 |
| *>88* | 0 | 2 | 6 | 4 |
| *<102* | 15 | 1 | 0 | 0 |
| *>102* | 0 | 7 | 4 | 2 |
| ALT | 3×N | 3.1×N | 2.9×N | 3.2×N |
| Glucose [mmol/L] | 4.63±0.82 | 4.52±0.91 | 4.62±0.85 | 4.86±0.76 |
| HOMA | 2.3±2.0 | 2.4±2.1 | 2.5±2.2 | 3.4±3.1 |
| Cholesterol [mg/dL] | 195±32.7 | 192±31.4 | 195±36.9 | 184±34.2 |
| Triglycerides [mg/dL] | 103±43.4 | 105±46.1 | 108±44.2 | 117±.52.3 |
| $Log_{10}$ HCV–RNA [IU/mL] | 12.5±2.1 | 12.7±2.4 | 12.8±1.8 | 12.5±2.3 |

**Table 2** – ***Biological parameters of oxidative stress in the CHC-patients studied (n=50) depending on the degree of steatosis (number or mean ± SD)***

| Variables | Absence | Light | Moderate | Severe |
|---|---|---|---|---|
| MDA [nmol/dL] | | | | |
| *<250* | 20 (87%) | 7 (64%) | 1 (10%) | 0 |
| *>250* | 3 (13%) | 4 (36%) | 9 (90%) | 6 (100%) |
| GSH [µmol/dL] | | | | |
| *>40* | 22 (96%) | 8 (73%) | 1 (10%) | 0 |
| *<40* | 1 (4%) | 3 (7%) | 9 (90%) | 6 (100%) |
| GGT | 1.2×N | 1.5×N | 1.9×N | 2.2×N |
| Ferritin [ng/mL] | 250±165 | 307±182 | 326±176 | 332±297 |
| Bilirubin [mg/dL] | 0.8±0.3 | 0.8±0.4 | 0.9±0.2 | 0.9±0.3 |

In order to establish the predictive factors for steatosis in patients with hepatitis C-virus, a univariate analysis was performed. Univariate analysis compared the basal values between the patients with steatosis and the ones without steatosis. After the univariate analysis the predictive factors of the steatosis were consider to be the following (Table 3): age >45 years, female sex, raised body mass index (BMI >30 kg/m$^2$), visceral obesity (waist >88 cm for women and >102 cm for men), HOMA score, and raised values of GGT.

According to the univariate analysis, the factors associated with fibrosis were (Table 4): age >45 years,

HOMA >2.5, moderate or severe steatosis, $Log_{10}$ HCV–RNA >12.7 IU/mL, GSH <40 μmol/dL, MDA >250 nmol/dL (Table 4).

**Table 3 –** ***Predictive factors for steatosis in C-virus chronic infection (univariable linear regression analysis)***

| Variables | No. of patients | Steatosis Moderate/Severe | *p* |
|---|---|---|---|
| Age [years] | | | |
| *<45* | 18 | 2 (11%) | <0.05 |
| *>45* | 32 | 14 (43%) | |
| Sex | | | |
| *Men* | 29 | 6 (21%) | <0.05 |
| *Women* | 21 | 12 (67%) | |
| BMI [kg/m$^2$] | | | |
| *<30* | 43 | 10 (23%) | <0.05 |
| *>30* | 7 | 5 (71%) | |
| Visceral obesity | | | |
| *<88/102* | 9/16 | 0 (0) / 0 (0) | <0.05 |
| *>88/102* | 12/13 | 10 (83%) / 6 (46%) | |
| HOMA | | | |
| *<2.5* | 23 | 3 (13%) | <0.05 |
| *>2.5* | 27 | 13 (49%) | |
| ALT | | | |
| *<3×N* | 33 | 10 (33%) | ns |
| *>3×N* | 17 | 6 (35%) | |
| $Log_{10}$ HCV–RNA [IU/mL] | | | |
| *<12.7* | 24 | 7 (29%) | ns |
| *>12.7* | 26 | 9 (34%) | |
| Cholesterol [mg/dL] | | | |
| *>260* | 15 | 6 (40%) | ns |
| *<260* | 35 | 10 (35%) | |
| GGT | | | |
| *<1.8×N* | 34 | 0 (0) | <0.05 |
| *>1.8×N* | 16 | 16 (100%) | |

**Table 4 –** ***Predictive factors for fibrosis in C-virus chronic infection (univariable linear regression analysis)***

| Variables | No. of patients | Fibrosis METAVIR F3/F4 | *p* |
|---|---|---|---|
| Age [years] | | | |
| *<45* | 18 | 1 (5%) | <0.05 |
| *>45* | 32 | 10 (30%) | |
| Sex | | | |
| *Men* | 29 | 7 (24%) | ns |
| *Women* | 21 | 4 (19%) | |
| HOMA | | | |
| *<2.5* | 23 | 2 (9%) | <0.05 |
| *>2.5* | 27 | 9 (33%) | |
| ALT | | | |
| *<3×N* | 33 | 9 (27%) | ns |
| *>3×N* | 17 | 2 (12%) | |
| $Log_{10}$ HCV–RNA [IU/mL] | | | |
| *<12.7* | 24 | 1 (4%) | <0.05 |
| *>12.7* | 26 | 10 (38%) | |
| Steatosis | | | |
| *0.1* | 34 | 3 (9%) | <0.05 |
| *2.3* | 16 | 8 (50%) | |
| MDA [nmol/dL] | | | |
| *<250* | 28 | 2 (7%) | <0.05 |
| *>250* | 22 | 9 (40%) | |
| GSH [μmol/dL] | | | |
| *>40* | 31 | 3 (9%) | <0.05 |
| *<40* | 19 | 8 (42%) | |

## Discussion

Hepatic steatosis, a common feature that was encountered in our study with a prevalence of 57%, supports the cytopathic effect of the C-virus. All the randomized patients had genotype 1, a well-known condition for our country. The factors associated with hepatic steatosis described up to present include: chronic alcohol consumption, obesity, dyslipidemia, diabetes mellitus, drugs, and genotype 3 of the C-virus. Metabolic factors were encountered in 16 patients (32%) and 12/27 (44%) of them had hepatic steatosis. Metabolic steatosis is the consequence of the mitochondrial dysfunction involved in the process of beta-oxidation of the free fatty acids. Metabolic steatosis is not induced by the C-virus itself, but the combination between this form of steatosis and the presence of the virus is associated with an accelerated progression towards fibrosis.

The link we have discovered between steatosis and insulin resistance through univariate analysis is suggestive for the hypothesis that in C-virus induced chronic hepatitis, IR represents a risk factor for steatosis. The prevalence of the IR in chronic hepatitis shows geographical variations between 20–70%. In our study, IR was present in 16 patients (32%). Among the patients with steatosis, 13 (48%) disclosed insulin resistance. A tight association between IR and C-virus infection was observed on one hand, and on the other hand, an association between insulin resistance and steatosis was noticed. It is worth mentioning that IR was encountered in the presence as well as in the absence of metabolic factors [15]. This last argument suggests a direct viral implication as well, not only through the agency of metabolic factors [16].

Recent data reveal the fact that hepatic insulin resistance has two main mechanisms: lipolysis and hyperinsulinemia. Lipolysis increases circulating fatty acids. Increased uptake of fatty acids by hepatocytes leads to mitochondrial $\beta$-oxidation overload, with the consequent accumulation of fatty acids within hepatocytes. FFAs are inducers of several cytochrome P-450 microsomal lipoxygenases, capable of producing hepatotoxic free oxygen radical species. Free radicals and lipid peroxidation can deplete antioxidants system, thus rendering the liver susceptible to oxidative injury [17].

Hyperinsulinemia resulting from insulin resistance increases the synthesis of fatty acids in hepatocytes by increasing glycolysis and favors the accumulation of triglycerides within hepatocytes by decreasing hepatic production of apolipoprotein B-100 [5]. Hepatic fatty acids are normally esterifies into triglycerides, some of which are exported out of hepatocytes as very-low-density lipoproteins (VLDL). The increased level of lipids, mostly in the form of triglycerides, within hepatocytes in patients with fatty liver disease results from an imbalance between the enzyme systems that promote the uptake and synthesis of fatty acids and those promote the oxidation and export of fatty acids [5].

Resistance to the action of insulin results in important changes in lipid metabolism. These include enhanced peripheral lipolysis, increased triglyceride synthesis, and increased hepatic uptake of fatty acids. Each of these may contribute to the accumulation of hepatocellular triglyceride [5].The association between insulin resistance and steatosis is important because of the cumulated risk for fibrosis and because of the

reduction in the sustained virologic answer to antiviral treatment.

Some studies have suggested that, apart from hyperinsulinemia, oxidative stress could play a role in the transition between simple fatty liver and steatohepatitis (i.e. fatty liver coexistent with hepatocyte necrosis and inflammation) [18]. In chronic liver disease, the production of ROS is a multifactorial process. Longstanding necroinflammatory conditions can result in ROS formation irrespective of the presence of steatosis. For instance, in chronic hepatitis C, ongoing hepatocytic necrosis and inflammation are associated with an increased production of ROS [19]. This oxidative stress induces hepatic damage and membrane lipid peroxidation, leading to a malondialdehyde (MDA) release and depletion of reduced glutathione (GSH) [20]. In our studies, hepatic steatosis was associated with an increase of MDA, correlated with its severity, and secondary with a decrease of GSH.

GSH is known as a substrate in both conjugation and reduction reactions. The GSH-antioxidant system has several physiological functions such as maintenance of protein-SH groups in a reduced state, detoxification from oxygen radicals, enzymatic degradation of endogenous peroxides, and formation of bioactive molecules. Furthermore, reduced levels of plasma GSH have been associated with the development of liver fat diseases and cardiovascular disease [8] and with the activation of several types of viruses [21]. HCV core protein has been observed to induce mitochondrial injury resulting in oxidative stress. Oxidative stress perturbs lipid peroxidation, thereby contributing to the development of steatosis [21].

The studies from our laboratory and others have shown increased of serum gamma-glutamyl transpeptidase (GGT) levels in chronic hepatitis C-virus (HCV) infection [22]. GGT is a cell-surface protein contributing to the extracellular catabolism of GSH [23]. The enzyme is produced in many tissues, but most GGT in serum is derived from the liver [24]. Serum levels of GGT are determined by several factors: alcohol intake, body fat content, plasma lipid/lipoproteins and glucose levels, and various medications. The mechanisms whereby elevated GGT is related to hepatic steatosis have not been determined, but Ortega E *et al.* [25] have proposed several possibilities. For example, fatty liver could cause hepatocellular damage that would simulate the synthesis of GGT. Alternatively, excess fat in the liver could enhance oxidative stress, leading to overconsumption of GSH with a compensatory increase in GGT synthesis. Finally, a higher GGT production could be secondary to a low-grade hepatic inflammation induced by hepatic steatosis.

The concept of serum GGT as primarily either an antioxidant or a pro-oxidant marker presents a challenge in understanding the GGT and disease relationships. Accumulating experimental evidence suggests an important role for GGT in extracellular catabolism of glutathione, the principal thiol antioxidant in humans. GGT enhances the availability of cysteine to promote intracellular glutathione (GSH) resynthesis, thereby counteracting oxidant stress [26]. GGT may also be proinflammatory, because it mediates interconversion of the glutathione-containing inflammatory mediator leukotriene C4 into leukotriene D4 [27]. Additionally, GGT-activity can give rise to redox reactions, due to the interplay of reactive thiol metabolites of GSH (cysteinyl-glycine in the first place) with transition metal ions. Some studies have suggested that iron may catalyze the formation of hydroxyl radicals, which contribute to the development of insulin resistance [28]. In our study, serum-ferittin concentration was correlated with GGT activity and with steatosis degree.

In this study, moderate and severe steatosis were independent risk factors for advanced fibrosis. Although recent studies have suggested that insulin resistance may contribute to the progression of fibrosis in HCV chronic hepatitis, in the study we have performed we have not found any direct association between the HOMA score and the severity of the hepatic fibrosis.

The studies performed in the last years tried to explain the mechanism by which steatosis associated with hepatitis C-virus contributes to the emergence of hepatic lesions. The hypothesis by which progression towards fibrosis may occur through non-inflammatory mechanisms was postulated. Such a mechanism might be represented by the C-virus induced lipid peroxidation, followed by free radical arousal, secondary activation of the stellate cells and collagen production.

In order to support this hypothesis we have considered in our study that the increase of malondialdehyde (MDA), which is a marker of the oxidative stress, along with the decrease in glutathione (GSH), which is an antioxidant protector, to be suggestive for the process of lipid peroxidation. This process is situated at the origin of liver lesions induced by steatosis and is accompanied by the stimulation of fibrogenesis. In patients infected by HCV genotype 1, oxidative stress and insulin resistance contribute to steatosis, which in turn exacerbates both insulin resistance and oxidative stress and accelerates the progression of fibrosis. The induction of GGT is an adaptive response against oxidative damage elicited by lipid peroxidation and it may be critical in the progression of the disease.

## Conclusions

In patients infected by HCV genotype 1, oxidative stress and insulin resistance contribute to steatosis, which in turn exacerbates both insulin resistance and oxidative stress and accelerates the progression of fibrosis. The induction of GGT is an adaptive response against oxidative damage elicited by lipid peroxidation and it may be critical in the progression of the disease.

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