

Relationship between the production of autoantibodies to oxidized low-density lipoprotein and hepatic steatosis in patients with chronic hepatitis C

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Abstract. Persistent hepatitis C virus (HCV) infection induces oxidative stress and eventually leads to hepatic steatosis. Oxidatively modified autoantigens, including oxidized low-density lipoprotein (ox-LDL), were identified in patients with systemic lupus erythematosus. Chronic HCV infection often evokes autoimmune phenomena, such as autoantibody production and/or concurrent autoimmune diseases. We examined the relationship between the production of antibodies to ox-LDL (anti-ox-LDL) and hepatic steatosis in patients with chronic hepatitis C (CH-C). Anti-ox-LDL levels were determined by the enzyme-linked immunosorbent assay method. The severity of hepatic steatosis was evaluated using the classification proposed by Brunt and colleagues. The effect of antiviral treatment was also investigated. Twenty-two (52%) of the 42 patients with CH-C had no hepatic steatosis (grade 0), while 12 (29%) and 8 (19%) had grade 1 and 2 hepatic steatosis, respectively. The overall serum immunoglobulin G (IgG) level in patients with grade 2 steatosis was significantly higher than that in patients with grade 0 steatosis ($1,999 \pm 340$ vs. $1,465 \pm 196$ mg/dl, $p < 0.0001$). The mean anti-ox-LDL level in grade 2 steatosis patients was also higher than that in grade 0 steatosis patients (754 ± 479 vs. 361 ± 274 mU/ml, $p = 0.0165$). A close correlation was apparent between anti-ox-LDL and serum IgG levels ($r = 0.390$, $p = 0.0107$). There was no significant difference in the level of anti-ox-LDL between CH-C patients who acquired sustained virological response (SVR) and those who exhibited non-SVR. These findings suggest that

anti-ox-LDL in patients with CH-C is induced in the process of hepatic steatosis and that the emergence of anti-ox-LDL does not affect antiviral treatment.

Introduction

Persistent hepatitis C virus (HCV) infection results in reactive oxygen species (ROS) in the liver (1,2), and the oxidative stress induced by HCV infection leads to lipid peroxidation (3), eventually progressing to hepatic steatosis (4,5).

Hepatic steatosis is usually observed, not only in patients with chronic hepatitis C (CH-C), but also in those with alcoholism (6), obesity and diabetes mellitus (DM) (7), and patients who have undergone treatment with tetracycline or corticosteroid (8). Previous studies have found hepatic steatosis in approximately 30-70% of patients with CH-C (9-16). The degree of hepatic steatosis in the majority of these patients was mild to moderate. The prevalence of severe hepatic steatosis, occupying more than 50% of hepatocytes, appears to be relatively low in patients with CH-C.

The clinical significance of hepatic steatosis in patients with CH-C has been well established. Obesity (10-13,15), insulin resistance (14,15), hepatic fibrosis (9,12,13,16) and HCV genotype 3 (10-13,16) are independently associated with hepatic steatosis upon multivariate analysis. Hepatic steatosis also appears to be a predictive hallmark of poor response to antiviral treatments in patients with CH-C (16,17). In addition, hepatic steatosis may be a possible indicator of progression to hepatocellular carcinoma (18,19).

Persistent HCV infection often evokes autoimmune phenomena, such as the production of numerous types of autoantibodies and/or concomitant autoimmune diseases (20,21). Recent studies have revealed the identification of oxidatively modified autoantigens, including oxidized low-density lipoprotein (ox-LDL), from the sera of patients with systemic lupus erythematosus (SLE) (22) and type 1 DM (23). The main purpose of this study was to investigate whether an immune response to ox-LDL may be involved during the process of hepatic steatosis in patients with CH-C.

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Table I. Demographic characteristics of the enrolled patients with CH-C.

	Hepatic steatosis			p-value
	Grade 0 (n=22)	Grade 1 (n=12)	Grade 2 (n=8)	
Age (years)	57.8±13.3	59.2±10.0	63.4±9.1	NS
Gender (male/female)	11/11	6/6	5/3	NS
HCV genotype (1b/2a/2b)	(13/6/3)	(9/2/1)	(4/3/1)	NS
Loads of HCV-RNA (KIU/ml)	996±899	1555.0±1519	1,098.0±504	NS
BMI (kg/m ²)	22±2.8 ^a	23.3±2.6 ^b	27.2±3.7 ^{a,b}	p=0.0004 ^a p=0.0115 ^b
Concurrent type 2 DM	6 (27%)	1 (8%)	4 (50%)	NS

CH-C, chronic hepatitis C; HCV, hepatitis C virus; BMI, body mass index; DM, diabetes mellitus; NS, not significant.

Materials and methods

Study population. Forty-two patients who had detectable serum HCV-RNA by polymerase chain reaction (PCR) and showed histological findings compatible with CH-C were randomly selected for participation in this study. Administration of pegylated interferon (PEG-IFN) alone or PEG-IFN plus ribavirin was carried out for 24 or 48 weeks in all of the enrolled patients after percutaneous liver biopsy. The evaluation of the antiviral treatment was carried out in 34 of the 42 patients with CH-C.

Clinical assessments. Age at entry, gender and the prevalence of type 2 DM were examined in the enrolled patients. Obesity was evaluated by body mass index (BMI) which was calculated in accordance with the formula: weight (kg) divided by height² (m²).

Laboratory assessments. Anti-ox-LDL levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Biomedica, Vienna, Austria). Biochemical tests, including analysis of alanine aminotransferase (ALT), total cholesterol (T-Chol) and triglyceride (TG) levels, were carried out before the antiviral treatment. Insulin resistance was determined by the Homeostasis Model for Assessment of Insulin Resistance (HOMA-IR) method using the following equation: $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mg/dl)} / 405$. Quantitative detection of serum HCV-RNA was performed by the Amplicor-HCV monitor assay (Roche Molecular Diagnostics, Tokyo, Japan) (24). The HCV genotype was determined by the HCV-RNA genotyping assay system (Home Brew SRL Inc., Tokyo, Japan) (25). Sustained viral response (SVR) was defined as an absence of HCV-RNA in serum 24 weeks after the completion of the treatment. No biochemical or virological response to the treatment was regarded as non-SVR. As immunoserological assessments, antinuclear antibody (ANA) and serum immunoglobulin G (IgG) levels were measured. ANA was tested at a serum dilution of 1:40 by the indirect immunofluorescence method using HEp-2 cells as substrates. Positive reactions were titrated by double dilution to the end point.

Histological assessments. Liver tissue specimens were obtained by liver biopsy under the guidance of ultrasound using 16-gauge needles before treatment. The tissue samples were fixed in 10% formalin and embedded in paraffin. The tissue sections were stained with H&E for morphological evaluation. The severity of hepatic steatosis was graded on the basis of the classification proposed by Brunt and colleagues (26). Briefly, steatosis observed in none, <33, 33-66 or >66% of hepatocytes was defined as grades 0, 1, 2 or 3, respectively. Fibrosis and necroinflammation in the liver were evaluated in accordance with the New Inuyama Classification system (27) and the histological activity index (HAI) scores designed by Knodell *et al* (28). The staging of hepatic fibrosis was classified from F0 to F4. F0 was defined as no fibrosis in the tissue specimen, while F4 was defined as liver cirrhosis.

Statistical analyses. Data values are represented as the mean ± standard deviation (SD). The Mann-Whitney U test was applied for comparison of continuous variables. Linear regression analysis was used to analyze the relationships between titers of anti-ox-LDL and serum IgG, ALT levels or values of HOMA-IR. We used the Fisher's exact test to compare the differences in frequencies. p-values of <0.05 were considered to indicate a significant difference between groups.

Results

Demographic features of the enrolled patients with CH-C. Among the enrolled patients, 22 cases were male and 20 were female. HCV genotypes of the enrolled patients were 1b in 26 (62%) patients, 2a in 11 (26%) and 2b in 5 (12%) patients. The ages at entry in the enrolled patients ranged from 23 to 76 years.

As shown in Table I, 22 (52%) of the 42 patients with CH-C had no hepatic steatosis (grade 0), while 12 (29%) patients had hepatic steatosis of grade 1 and 8 (19%) of grade 2. None of the patients showed grade 3 hepatic steatosis. There were no significant differences in age or gender between CH-C patients with grade 0, 1 or 2 hepatic steatosis. The prevalence of concurrent type 2 DM in CH-C patients with grade 2 hepatic steatosis was higher than that in the other groups, although no significant differences were apparent. The mean BMI in the

Table II. Laboratory data of the patients according to hepatic steatosis group.

	Hepatic steatosis			p-value
	Grade 0 (n=22)	Grade 1 (n=12)	Grade 2 (n=8)	
ALT (IU/l)	81±64	69±37	112±66	NS
TG (mg/dl)	89±40	89±35	118±35	NS
T-Cho (mg/dl)	157±39	162±29	162±39	NS
IgG (mg/dl)	1,465±196 ^{a,b}	1,868±382 ^a	1,999±340 ^b	p=0.0003 ^a p<0.0001 ^b
Prevalence of ANA	5 (23%)	4 (33%)	4 (50%)	NS

ALT, alanine aminotransferase; TG, triglyceride; T-Cho, total cholesterol; IgG, immunoglobulin G; ANA, antinuclear antibodies; NS, not significant.

grade 2 group was significantly higher than that in the grade 0 group (27.2 ± 3.7 vs. 22.0 ± 2.8 , $p=0.0004$) and that in the grade 1 group (27.2 ± 3.7 vs. 23.3 ± 2.6 , $p=0.0115$). The severity of hepatic steatosis was independent of HCV genotypes and loads of HCV-RNA (data not shown).

Correlation of hepatic steatosis with laboratory findings in patients with CH-C. Table II summarizes the values of the biochemical and immunological parameters measured in each group. There were no significant differences in serum ALT, T-Cho or TG levels among the three groups. CH-C patients with grade 1 or 2 hepatic steatosis had significantly higher values of serum IgG levels than those with grade 0 hepatic steatosis ($1,868\pm382$ vs. $1,465\pm196$ mg/dl, $p=0.0003$; $1,999\pm340$ vs. $1,465\pm196$ mg/dl, $p<0.0001$). The prevalence of ANA in CH-C patients with grade 2 hepatic steatosis was higher than ANA in the other groups, although no significant differences were found among the three groups.

Correlation of hepatic steatosis with anti-ox-LDL. The levels of anti-ox-LDL in each group are shown in Fig. 1. The mean titer of anti-ox-LDL in patients with grade 2 hepatic steatosis was significantly higher than that in patients with grade 0 hepatic steatosis (754 ± 479 vs. 361 ± 274 mU/ml, $p=0.0165$). Fig. 2A demonstrates the close correlation between titers of anti-ox-LDL and serum IgG levels ($r=0.390$, $p=0.0107$). However, the titers of this autoantibody were not associated with serum ALT levels ($r=0.208$, $p=0.1865$) or values of HOMA-IR ($r=0.192$, $p=0.2627$), as shown in Fig. 2B and C, respectively.

Relationship between titers of anti-ox-LDL and concurrent type 2 DM. The levels of anti-ox-LDL in CH-C patients with type 2 DM were compared to those in CH-C patients without type 2 DM. As shown in Fig. 3, there was no significant difference in the levels of the autoantibodies between CH-C patients with type 2 DM and those without type 2 DM (337 ± 298 vs. 497 ± 376 mU/ml, $p=0.2518$).

Relationship between the titers of anti-ox-LDL and the effect of antiviral treatment. The effect of antiviral treatment was evaluated in 20 patients with CH-C of genotype 1b and 14 patients with CH-C of genotype 2a/2b. Among patients with

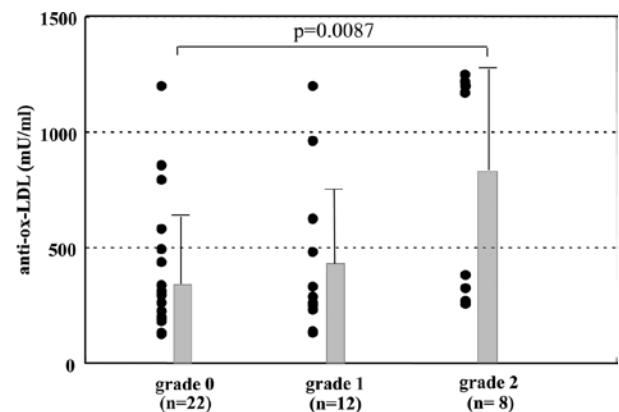


Figure 1. Relationship between the severity of hepatic steatosis and the levels of anti-ox-LDL.

CH-C of genotype 1b, 2 (25%) of 8 patients with grade 0 hepatic steatosis and 3 (38%) of 8 patients with grade 1 hepatic steatosis acquired SVR, while none (0%) of those with grade 2 hepatic steatosis did. The frequency of F3 in patients with CH-C of genotype 1b who acquired SVR was significantly lower than those who exhibited non-SVR (20 vs. 80%, $p=0.0176$), which suggests that hepatic fibrosis affected the outcome of antiviral treatment in patients with CH-C of genotype 1b (Table III). However, there was no significant difference in the levels of anti-ox-LDL between patients who acquired SVR and those who showed non-SVR (273 ± 118 vs. 521 ± 424 mU/ml, $p=0.2204$) (Table III). On the other hand, the levels of anti-ox-LDL were also independent of the effect of antiviral treatment in CH-C patients of genotype 2a/2b (477 ± 376 mU/ml in the SVR group vs. 554 ± 232 mU/ml in the non-SVR group, $p=0.2424$).

Discussion

In this study, we demonstrated that the degree of hepatic steatosis was significantly associated with the elevation of serum IgG levels (Table II) and anti-ox-LDL levels (Fig. 1) in patients with CH-C, which indicated that these autoantibodies were produced during the process of hepatic steatosis. These autoimmune responses were independent of HCV genotypes and loads of HCV-RNA, which suggests that host factors may

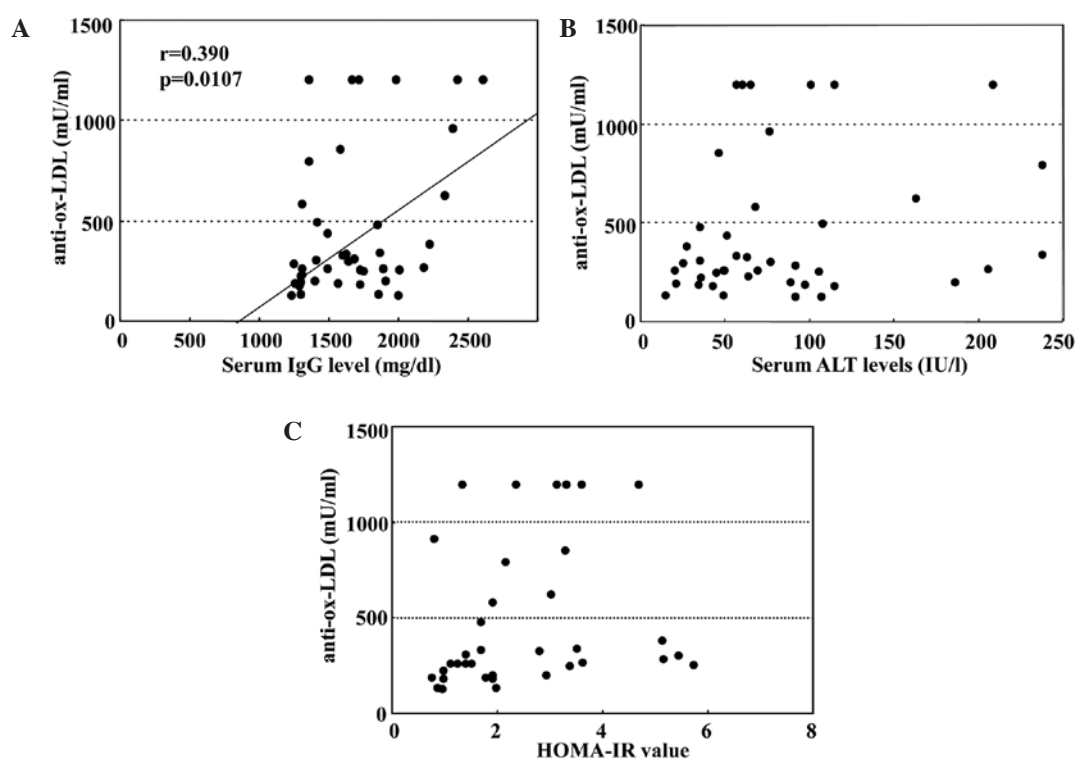


Figure 2. Relationship between the levels of anti-ox-LDL and (A) serum IgG, (B) serum ALT or (C) HOMA-IR values.

Table III. Efficacy of the antiviral treatment in patients with CH-C of genotype 1b.

	SVR (n=5)	Non-SVR (n=15)	p-value
Age (years)	58.4±12.5	64.7±7.7	NS
ALT (IU/l)	59.0±33.0	81.0±39	NS
Hepatic steatosis (grade 0/1/2)	2/3/0	6/5/4	NS
Loads of HCV-RNA (KIU/ml)	878±971	1,249±1,140	NS
Hepatic fibrosis (F0/F1/F2/F3)	0/4/0/1	0/3/0/12	p=0.0176
HAI score	10±4.6	11.8±3.9	NS
Anti-ox-LDL (mU/ml)	273±118	521.0±424	NS

CH-C, chronic hepatitis C; SRV, sustained virological response; ALT, alanine aminotransferase; HCV, hepatitis C virus; HAI, histological activity index; anti-ox-LDL, antibodies to oxidized low-density lipoprotein; NS, not significant.

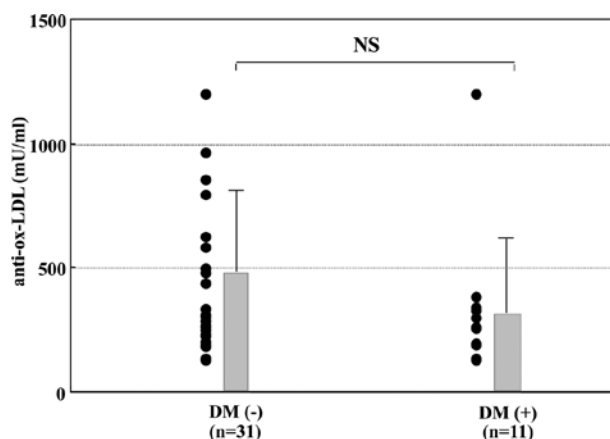


Figure 3. Relationship between the levels of anti-ox-LDL and concurrent type 2 DM. NS, not significant.

contribute to the autoimmune phenomena. Accumulation of ox-LDL in the liver tissues during the process of hepatic steatosis has been postulated as a possible mechanism of autoantibody production in patients with CH-C. Persistent HCV infection exerts the formation of ROS in the liver (1,2). Oxidative stress caused by HCV infection facilitates lipid peroxidation (3) and consequently promotes hepatic steatosis (4,5). We speculated that oxidative modification of LDL induced immunogenic epitopes in the LDL molecule and that ox-LDL accumulated in the liver during the process of hepatic steatosis. The accumulation of ox-LDL may trigger the production of anti-ox-LDL. However, there were previous reports in which no correlation (29) or inverse correlation (30) was found between the levels of ox-LDL and anti-ox-LDL. Therefore, further studies are required to confirm this speculation on the production of anti-ox-LDL in patients with CH-C.

Recently, Vidali and colleagues demonstrated that the levels of circulating IgG against malondialdehyde (MDA)-albumin adducts were significantly higher in CH-C patients with hepatic steatosis than in those without hepatic steatosis (31). This result implies that the autoimmune response to MDA, which is a product of polyunsaturated fatty acid peroxidation, is involved in the process of hepatic steatosis in patients with CH-C.

On the other hand, recent studies have found that 10-30% of patients with non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) have autoantibodies, including ANA and/or smooth muscle antibodies (SMA), with elevated serum IgG levels (32,33). These findings suggest that hepatic steatosis or steatohepatitis are strictly associated with autoimmune reactions.

By contrast, Czaja and colleagues previously reported that CH-C patients with hepatic steatosis had lower serum concentrations of IgG and a lower frequency of ANA than those of CH-C patients without steatosis (34). However, our data in the present study are in direct opposition to their results (Table II). This difference may have been derived from the genotypes of HCV or the genetic backgrounds of patients with CH-C.

The association of insulin resistance with autoimmune disorder has been widely discussed. Patients with SLE frequently exhibit insulin resistance (35). Loria and colleagues showed that high titers of ANA are favorable indicators of insulin resistance in patients with NAFLD (32). We investigated the correlation between the levels of anti-ox-LDL and insulin resistance in patients with CH-C, but observed no such correlation (Fig. 2).

Previous reports have found that the emergence of non-organ-specific autoantibodies, including ANA, SMA or parietal cell autoantibodies at baseline or an increase in their titers during antiviral treatments, appear to be a predictive indicator of a poor response in patients with CH-C (36,37). Therefore, we examined the relationship between the levels of anti-ox-LDL and the outcomes of antiviral treatments in the CH-C patients (Table III). However, the levels of anti-ox-LDL did not affect the outcomes of antiviral treatments in the patients with CH-C.

In conclusion, we demonstrated that hepatic steatosis is strongly associated with the emergence of anti-ox-LDL in patients with CH-C, which indicates that autoimmune responses to ox-LDL are involved in the pathogenesis of hepatic steatosis in patients with CH-C.

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