

Elevation of Serum Apolipoprotein B after Successful Eradication of Hepatitis C Virus in Patients with Chronic Hepatitis C Treated by IFN-Based Therapy

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Abstract

Background: Hepatitis C Virus (HCV) infection is closely tied to the lipid metabolism of liver cells. We recently reported that serum levels of LDL- and VLDL-cholesterol (Cho) increased in patients with Chronic Hepatitis C (CHC) showing Sustained Virologic Response (SVR) after treatment with interferon (IFN)-based therapy. LDL- and VLDL-Cho contained apolipoprotein (apo)-B synthesized in the liver as a major protein component. The goal of the present study was to clarify how serum lipid markers change in CHC patients showing SVR after treatment with IFN-based therapy.

Patients and methods: The study included 121 consecutive patients with CHC infected with HCV genotype 1 (n=66, male/female: 40/26) or HCV genotype 2 (n=55, male/female: 38/17). Ninety-five patients received PEG-IFN alpha and Ribavirin (RBV). Twenty-six patients received PEG-IFN alpha-2a alone. SVR was defined as being negative for serum HCV-RNA on RT-PCR at 24 weeks after the End of Therapy (EOT). Fasting serum triglyceride (T-G), total-Cho, and apo-B were evaluated before starting therapy and at 24 weeks after EOT.

Results: SVR rates were 74% (90/121). Serum levels of total-Cho and apo-B increased significantly ($p < 0.05$ by Wilcoxon test) in patients infected with HCV genotypes 1 and 2 who achieved SVR at 24 weeks after EOT, as compared to before the start of therapy, but no increases were seen in non-SVR patients.

Conclusions: Infection with HCV genotypes 1 and 2 equally lowered serum levels of apo-B and total-Cho, which increased after HCV was successfully eradicated.

Keywords: Chronic hepatitis C; Cholesterol; Apolipoprotein-B; PEG-IFN; Ribavirin

Introduction

As hepatic biosynthesis of lipids has been suggested to play a role in the processes of infection and replication of Hepatitis C Virus (HCV) [1,2], HCV infection and serum lipid markers are thought to be related. Serum cholesterol (Cho) levels have been reported to be a predictor of Sustained Virologic Response (SVR) in patients with Chronic Hepatitis C (CHC) receiving antiviral therapy [3-6], but the precise mechanism for this phenomenon has not yet been confirmed.

We also reported recently that serum levels of total Cho (T-Cho) and its fraction increased in CHC patients showing SVR after treatment with interferon (IFN)-based therapy [7,8], and Corey et al. [9] reported similar observations.

The proteins associated with lipoproteins, known as apolipoproteins (apos), are required for the assembly, structure, and function of lipoproteins, and apos activate enzymes important in lipoprotein metabolism. It is known that apo-B is the major structural protein in chylomicron, Very Low Density Lipoprotein (VLDL)-Cho, Intermediate Density Lipoprotein (IDL)-Cho and Low Density Lipoprotein (LDL)-Cho, which are synthesized in the liver.

The goal of the present study was to clarify how serum levels of lipid markers change in CHC patients showing SVR after treatment with IFN-based therapy.

Patients and Methods

Patients

The study included 121 consecutive patients with CHC who were

infected with genotype 1 (n=66; male/female: 40/26; age: 28 to 72 years) having baseline HCV-RNA ranging from 3.9 to 7.4 log copies/ml, as quantified by real time (RT)-PCR (lower detection limit: 1.2 log copies/ml, upper limit: 9.1 log copies/ml; TaqMan; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), or with genotype 2 (n=55; male/female: 38/17; age: 31 to 76 years) having baseline HCV-RNA ranging from 3.2 to 7.1 log copies/ml, as quantified by RT-PCR. All patients were positive for anti-HCV antibody on testing with third-generation enzyme-linked immunosorbent assay and were positive for serum HCV-RNA on RT-PCR, but were negative for hepatitis B surface antigen. Patients were excluded if they were known to be homosexual or were intravenous drug users, if they were positive for antinuclear antibodies, or if they had metabolic liver dysfunction, history of familial hyperlipidemia, HIV co-infection or renal dysfunction. Furthermore, patients were excluded if they were taking lipid-lowering drugs, if they were taking hypoglycemic agents, or if they had a history of habitual alcohol abuse (daily alcohol consumption > 20 g/day in men and >10 g/day in women).

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Of the 121 patients, 82 had undergone percutaneous liver biopsy before therapy under ultrasonographic control, and tissue specimens thus obtained were scored according to the Histology Activity Index (HAI) of Knodell et al. [10], and were divided into three grades (Grade 1 for HAI scores of 1-3; Grade 2 for HAI scores of 4-8; and Grade 3 for HAI scores of 9 or more). Specimens were also divided into four groups from stage 1 to stage 4 based on the fibrosis score of Desmet [11].

IFN regimens

After providing informed consent, 58 patients received PEG-IFN alpha-2b (PegIntron®; MSD K.K., Tokyo, Japan) at weekly doses adjusted for body weight according to the manufacturer's instructions (45 kg or less=60 µg/dose; 46 to 60 kg=80 µg/dose; 61 to 75 kg=100 µg/dose; 76 to 90 kg=120 µg/dose; and 91 or more=150 µg/dose) in combination with oral ribavirin (Rebetol®; MSD, K.K.) at daily doses adjusted for body weight according to the manufacturer's instructions (60 kg or less=600 mg/day; 61 to 80 kg=800 mg/day; and 81 kg or more = 1,000 mg/day) for 48 weeks for HCV genotype 1 and high viral load (serum HCV-RNA >5.0 log copies/ml), and for 24 weeks for HCV genotype 2 and high viral load.

Thirty-seven patients received PEG-IFN alpha-2a (Pegasys®; Chugai Pharmaceutical Co., Ltd.) at 180 µg per week in combination with ribavirin (Copegus®; Chugai Pharmaceutical Co., Ltd.) given orally at daily doses adjusted for body weight according to the manufacturer's instructions (60 kg or less=600 mg/day; 61 to 80 kg=800 mg/day; and 81 kg or more=1,000 mg/day) for 48 weeks for HCV genotype 1 and high viral load, and for 24 weeks for HCV genotype 2 and high viral load.

The remaining 26 patients received PEG-IFN alpha-2a (Pegasys®) alone at 180 µg per week for 24 to 48 weeks for HCV genotype 1 or genotype 2 and low viral load (serum HCV-RNA < 4.9 log copies/ml).

Some patients needed slight adjustment of the PEG-IFN and RBV doses, because of decreases in leukocyte count, platelet count or hemoglobin during therapy.

Serum HCV-RNA was measured using RT-PCR before the start of therapy and every 4 weeks thereafter until the End of Therapy (EOT), as well as at 24 weeks after EOT. SVR was defined as being negative for serum HCV-RNA by RT-PCR at 24 weeks after EOT.

Assay

Fasting serum total Cho (T-C), triglyceride (T-G), and apo-A1, -A2 and -B were evaluated [12] before the start of IFN therapy and at 24 weeks after EOT. To assess hepatic synthetic function, serum albumin and peripheral blood prothrombin time (PT: %) were measured by standard methods before starting therapy and at 24 weeks after EOT.

Statistical analysis

Results shown in the table 1 are presented as medians and 10th to 90th percentiles.

Comparisons between two groups were performed by nonparametric Mann-Whitney test or Wilcoxon's signed rank test. Multiple comparisons were performed by parametric Tukey's test. In all analyses, a probability value of less than 0.05 was considered to indicate statistical significance.

			Genotype 1	Genotype 2	
No. of patients			66	55	
Age	(y)	Median	55 (41-66)	54 (40-67)	NS#
Sex	(M/F)		40/26	38/17	NS ^c
Laboratory data					
Serum HCV level	(KIU/ml)		6.3 (4.8-6.9)	5.5 (3.8-6.6)	p < 0.01 ^c
AST	(IU/l)	Median	49 (24-86)	54 (26-86)	NS#
ALT	(IU/l)	Median	54 (25-117)	58 (25-151)	NS#
ChE	(IU/l)	Median	252 (111-380)	295 (205-397)	NS#
Albumin	(g/dl)	Median	4.2 (3.6-4.6)	4.2 (3.4-4.6)	NS#
T-Cho	(mg/dl)	Median	162 (131-212)	166 (137-219)	NS#
T-G	(mg/dl)	Median	87 (63-181)	96 (54-246)	NS#
Peripheral Blood					
WBC	(10 ³ /µl)	Median	4.8 (3.4-7.7)	5.2 (3.5-6.9)	NS#
RBC	(10 ⁶ /µl)	Median	4.5 (3.6-5.0)	4.6 (3.9-5.2)	NS#
PLT	(10 ⁴ /µl)	Median	15.1 (8.2-25.6)	17.1 (10.2-26.6)	NS#
PT	(%)	Median	94 (79-112)	98 (73-114)	NS#
Pathological findings					
Activity (1/2/3/none)			(22 / 23 / 0 / 21)	(16 / 21 / 0 / 18)	NS ^c
Staging (1/2/3/4/none)			(18/13 / 1/13/21)	(21 / 7/3/6 / 18)	NS ^c
IFN regimen					
Ribavirin+PEG-IFN-alpha 2b(n=)			29	29	
Period	(week)		48 (48-72)	24 (24-48)	p < 0.01
Ribavirin+Peg- IFN-alpha 2a(n=)			33	4	
Period	(wk)		48 (32-72)	48 (48-48)	NS#
PEG-IFN-alpha alone (n=)			4	22	
Period	(week)		24 (24-24)	24 (24-24)	NS#
Efficacy of therapy					
(SVR/ Relapser / NVR)			(40 / 13 / 13)	(51 / 3 / 1)	p < 0.01

In parentheses, range represents 10th and 90th percentiles.

Table 1: Clinical background of patients with chronic hepatitis C.

Results

Response of CHC to treatment

Of the 66 CHC patients (61%) infected with HCV genotype 1, 40 achieved SVR, while 50 of 55 CHC patients (91%) infected with genotype 2 achieved SVR.

Changes in serum lipids in CHC patients

There were no significant differences in baseline serum levels of T-Cho, T-G, apo-A1, apo-A2 and apo-B between CHC patients infected with HCV genotypes 1 and 2. However, the serum levels of T-Cho and apo-B had increased significantly ($p < 0.05$, Wilcoxon's test) at 24 weeks after EOT, as compared to before the start of therapy, in CHC patients with both HCV genotypes 1 and 2 (Figure 1a and Figure 1e). Serum levels of apo-A2 had decreased significantly ($p < 0.05$ by Wilcoxon's tests) at 24 weeks after EOT, as compared to before the start of therapy, in CHC patients with only HCV genotype 1, while there were no differences between pre-therapy values and those at 24 weeks after EOT in CHC patients infected with genotype 2 (Figure 1d).

There were no significant differences in serum levels of T-G or apo-A1 before the start of therapy and at 24 weeks after EOT in CHC patients infected with either HCV genotype 1 or 2 (Figure 1b and Figure 1c).

Changes in other serum liver marker in CHC patients

Serum levels of albumin showed a median value of 4.2 g/dl (10th-90th range: 3.7 - 4.6 g/dl) before the start of therapy, and a median 4.4 g/dl (10th-90th range: 3.9 - 4.7 g/dl) at 24 weeks after EOT in SVR patients, while albumin levels were a median 4.0 g/dl (10th-90th range: 3.2 - 4.5 g/dl) before the start of therapy and a median 4.0 g/dl (10th - 90th range: 3.2 - 4.3 g/dl) at 24 weeks after EOT in non-SVR patients.

Peripheral blood PT was a median 96% (10th-90th range: 75% - 114%) before the start of therapy and a median 97% (10th - 90th range: 82% - 111%) at 24 weeks after EOT in SVR patients, while PT was a median 93% (10th - 90th range: 79% - 111%) before the start of therapy and a median 90% (10th-90th range: 79% - 117%) at 24 weeks after EOT in non-SVR patients. There were no significant differences in serum

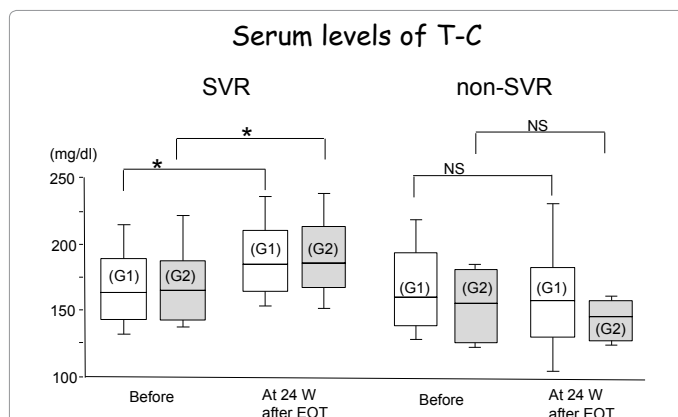


Figure 1a: Serum levels of Total Cholesterol (T-Cho) before the start of therapy and at 24 weeks after the End of Therapy (EOT) in Chronic Hepatitis C (CHC) patients infected with genotypes 1 and 2 who showed SVR and non-SVR. Each box represents the range from the 2nd to 3rd quartiles (25th to 75th percentile) and each horizontal line represents the median. Upper and lower bars represent the 10th and 90th percentiles. NS: No significant differences by Wilcoxon's signed rank test.

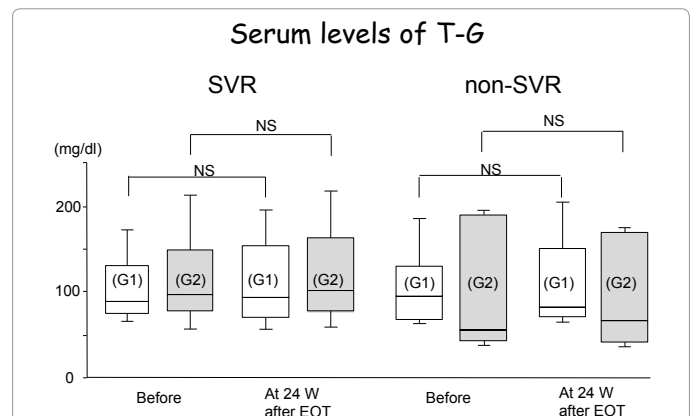


Figure 1b: Serum levels of triglyceride (T-G) before the start of therapy and at 24 weeks after EOT in CHC patients infected with genotypes 1 and 2 who showed SVR and non-SVR. NS: No significant differences by Wilcoxon's signed rank.

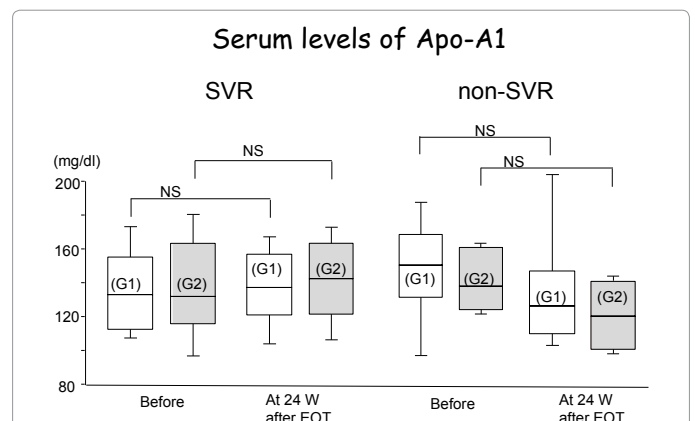
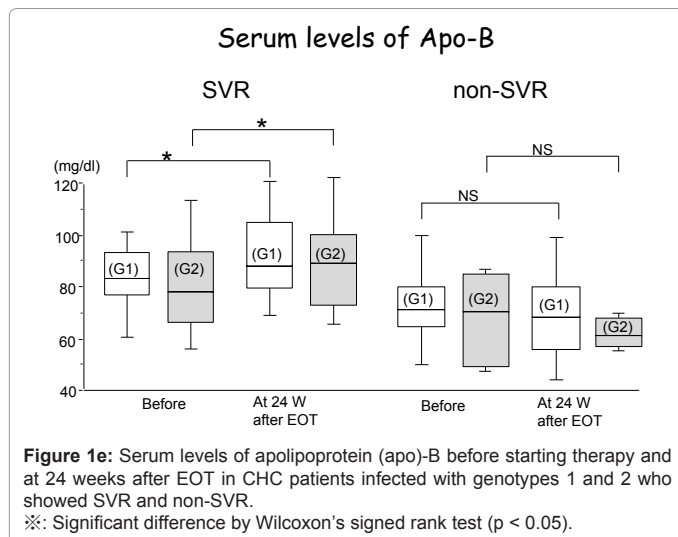
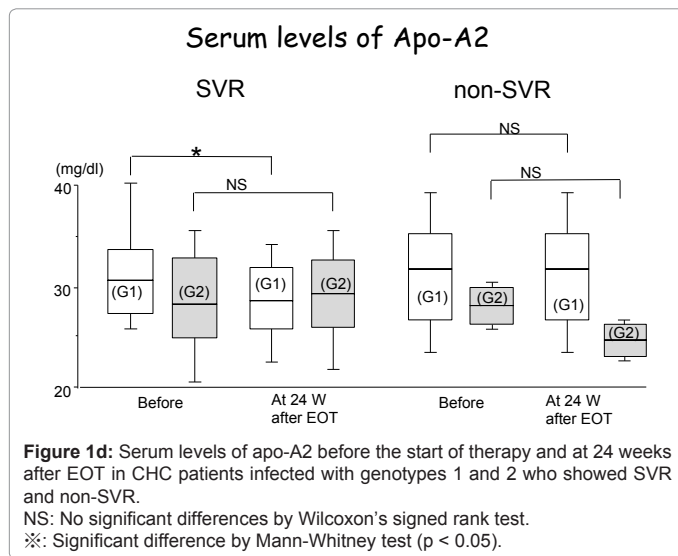


Figure 1c: Serum levels of apo-A1 before the start of therapy and at 24 weeks after EOT in CHC patients infected with genotypes 1 and 2 who showed SVR and non-SVR. NS: No significant differences by Wilcoxon's signed rank test.

levels of albumin or peripheral blood PT between pre-treatment values and those at 24 weeks after EOT in either group.

Discussion

The present study demonstrated that serum levels of T-Cho and apo-B increased significantly in patients infected with genotypes 1 and 2 who achieved SVR at 24 weeks after EOT, as compared to pre-therapy levels, but such increases were not seen in non-SVR patients. In addition, serum levels of apo-A2 decreased significantly in patients infected with only genotype 1 who achieved SVR at 24 weeks after EOT, as compared to pre-therapy levels. These changes suggest that infection with HCV genotypes 1 and 2 equally lowered host serum levels of T-Cho and apo-B, which increased after HCV was successfully eradicated. It was also suggested that HCV genotype 1 infection increased serum levels of apo-A2, which decreased at 24 weeks after EOT following successful HCV eradication, but such increases were not seen in CHC patients with HCV genotype 2 infection. We previously reported that serum T-Cho, including LDL- and VLDL-Cho, increased at 24 weeks after EOT when SVR was obtained in patients with CHC treated with IFN-based therapy [7,8], irrespective of their HCV genotypes. Corey et al. [9] also reported that serum T-Cho in CHC patients infected with genotype 1 increased significantly more at 24 weeks after EOT.



It is known that apo-B is present as a lipoprotein in LDL-Cho and VLDL-Cho. VLDL-Cho is secreted into the bloodstream after assembly with apo-B. HCV is reported to cause acquired Hypo Beta Lipoproteinemia (HBL), in which fat accumulates in hepatocytes, steatosis develops and serum levels of apo-B decrease in those infected with HCV genotype 3 [13,14] or HCV genotype 1 [12]. Serum levels of these lipid markers increased when SVR is obtained among treated CHC patients [13]. In the present study, serum levels of T-Cho and apo B increased equally in CHC patients infected with HCV genotypes 1 and 2 who achieved SVR. Therefore, it has been suggested that both HCV genotype 1 and HCV genotype 2 equally inhibit assembly and/or secretion of VLDL-Cho, and that serum levels of T-Cho and apo-B decrease before the start of IFN-based therapy. These levels then increase after HCV is successfully eradicated from CHC patients. The precise mechanism through which HCV induces acquired HBL remains unknown, but this mechanism should soon be elucidated, as interactions between HCV, apo-B and microsomal triglyceride transfer protein are now being clarified [15,16].

It was recently reported that apo-B acts as a molecular link between lipid-induced endoplasmic reticulum stress and hepatic insulin resistance [17], both of which are known to be caused by HCV

infection [18,19]. More recently, it was reported that host cellular factor apo-B messenger RNA-editing enzyme catalytic polypeptide-like 3G (hA3G), a cytidine deaminase, increases in patients with HCV infection. hA3G appears to be a cellular restricting factor against HCV, however, this enzyme is known to be degraded by viruses [20]. If HCV has been eliminated from hepatocytes such as CHC patients having SVR, apo-B may be released into the blood as a result of improvement of metabolism in hepatocytes.

In the present study, serum levels of apo-A2 had decreased significantly at 24 weeks after EOT, as compared to before the start of therapy, in CHC patients with only HCV genotype 1, but not in CHC patients infected with genotype 2. It is reported to be inverse relationship between LDL cholesterol and HDL cholesterol [21]. Therefore, serum HDL-C which is including Apo-A2 might be compensatory increased with decreased serum LDL cholesterol which is including apo-B before the start of treatment, and serum Apo-A2 included in HDL-C decreased with increased Apo-B included in LDL-C after successful elimination of HCV. However, it must be left to the future study of whether this phenomenon occurs only in CHC patients infected with HCV genotype 1.

When cholesterol levels are discussed, the relationship with liver function becomes a problem, as serum cholesterol levels tend to decrease in patients who show histological progression of chronic liver disease [22]. In the present study, serum albumin and peripheral blood PT were measured in order to assess hepatic synthetic function, but there were no significant differences in these parameters between baseline and assessment. Therefore, the changes in serum lipid markers do not necessarily depend on amelioration of liver function brought by eradication of HCV.

In conclusion, our results suggest that HCV genotypes 1 and 2 equally reduce serum levels of T-Cho and apo-B, which increase after HCV is successfully eradicated.

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