Pathol. Oncol. Res. (2010) 16:149–157
DOI 10.1007/s12253-009-9195-4

Effect of Liver Steatosis on Therapeutic Response in Chronic Hepatitis C Virus Genotype 1 Infected Patients in Hungary

Klara Werling · Zsuzsa Schaff · Elek Dinya · Zsolt Tulassay

Received: 19 December 2008 / Accepted: 12 August 2009 / Published online: 15 September 2009

Abstract Hepatic steatosis seems a frequent histological alteration seen in chronic hepatitis C virus infected patients. There is still a lot to learn about the exact mechanism of effect of liver steatosis and its influence on the progression of liver diseases. Our study involved 96 chronic hepatitis C genotype 1 infected Hungarian patients who received pegylated interferon and ribavirin treatment for the first time. Degree of steatosis, viral and host factors influencing its development and its effect on the efficiency of antiviral treatment were determined. In 61 (64%) of patients the liver tissue showed varying degree of steatosis, which did not show relationship with level of alcohol consumption (p=0.5792), diabetes mellitus (p=0.5925) or body mass index (p=0.9685) in type 1 chronic hepatitis C patients. Degree of steatosis and virus titer showed strong relationship (OR=2.1). Significant relationship was also found between degree of hepatic steatosis and stage (p=0.0119), as well as between therapeutic response to combined pegylated interferon + ribavirin treatment and steatosis (p=0.0012). Our results demonstrated that steatosis has clinical significance in hepatitis C virus genotype 1 infected patients.

K. Werling (✉) · Z. Tulassay
2nd Department of Internal Medicine, Semmelweis University,
Szentkirályi u. 46,
1088 Budapest, Hungary
e-mail: werling@freemail.hu

Z. Schaff
2nd Department of Pathology, Semmelweis University,
Budapest, Hungary

E. Dinya
Medical Department, EGIS Pharmaceuticals PLC,
Budapest, Hungary

Keywords Hepatitis C virus · Genotype 1 · Steatosis · Therapy

List of abbreviations

CHC	chronic hepatitis C
BMI	body mass index
HCV	hepatitis C virus
SVR	sustained virological response
AST	aspartate aminotransferase
ALT	alanine aminotransferase
GGT	gamma-glutamyl-transferase
AP	alkaline phosphatase
Fe	serum iron
TSH	thyroid stimulating hormone
FT4	free T4
FT3	free T3
MTP	microsomal trygliceride transfer protein
EVR	early virological response

Introduction

Twenty percent of patients suffering from chronic hepatitis C (CHC) develop liver cirrhosis within 20 years [1]. Several factors are responsible for the degree of connective tissue reorganization and the development of hepatic cirrhosis. Gender, age, duration of infection, alcohol consumption may all add to the progression of the disease [2]. Hepatic steatosis seems a frequent histological alteration seen in CHC patients, with an incidence of 40–86% based on the literature [3–16]. There is still a lot to learn about the exact mechanism of effect of liver steatosis and its influence on the progression of liver diseases. Development of steatosis in CHC is influenced by both viral and

host factors, with metabolic factors as obesity, heavy drinking, type 2 diabetes mellitus, hyperlipidaemia all possible contributors [17]. Retrospective studies have pointed towards a relationship between severity of steatosis and degree of connective tissue reorganization [7, 9, 10, 15]. Several studies claim relationship between degree of steatosis and body mass index (BMI) [3–6, 8–16], whereas others do not [7]. The effect of metabolic factors on the development of steatosis is further confirmed by epidemiologic studies demonstrating that type 2 diabetes mellitus is more frequent in CHC patients as compared with the average population [18–22].

The role of viral factors is supported by the fact that genotype 3a infected patients show more severe hepatic steatosis as opposed to non genotype 3a patients [6, 7, 9, 23, 24]. The direct steatogenic effect of HCV is well documented in an experiment in which the HCV core of transgenic mice developed progressive hepatic steatosis [25].

Several studies have indicated that the degree of steatosis has influence on the result of treatment. In genotype 1 infected patients lack of steatosis increases the chances of development of sustained virological response (SVR) [16]. Kumar and coworkers found that despite the therapeutic response in genotype 1 patients, the degree of steatosis did not change [26].

In Hungary, 90% of CHC patients are infected with genotype 1b, which group shows poorer reaction to treatment. The ratio of genotype 3 infected patients is 0.5% [27]. According to a prospective study, 48% of Hungarian patients developed SVR upon 48 weeks of combined pegylated interferon and ribavirin treatment. The efficiency of the treatment was influenced by the earlier age of the patients and the lack of liver cirrhosis [28].

The present study was undertaken to determine the degree of steatosis, the factors influencing its development and the effect it has on the efficiency of combined pegylated interferon and ribavirin treatment in Hungarian CHC genotype 1 infected patients.

Patients and Methods

Patients

The study involved 96 CHC genotype 1 infected patients who received pegylated interferon and ribavirin treatment for the first time between 2002 and 2006 at the 2nd Department of Internal Medicine of the Semmelweis University. The patients were chosen based on the following criterias: (i) only genotype 1 infected patients were included in the study and (ii) they received pegylated interferon and ribavirin treatment for the first time. Among the patients were 15 who earlier underwent traditional (non-pegylated) interferon and ribavirin treatment, but were relapsers, whereas the rest of the patients had no previous history of antiviral treatment.

Presence of the following diseases was excluded prior to treatment: decompensated liver disease, hepatitis B viral infection, human immunodeficiency virus infection, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, Wilson disease, drug induced liver injury, hypo- and hyperthyreosis. None of the patients took medication causing steatosis prior to and during treatment. The case history of each patient included data related to diabetes, hypertension and average alcohol consumption for the past 6 months and earlier (g/day). Only those patients were included in the study who did not consume alcohol for at least half a year prior to treatment. Liver biopsies were therefore performed before the start of treatment, following 6 months of alcohol abstinence. Before start of therapy, the patients received information pertaining to the course of treatment. Informed consent was obtained from each patient, who all signed a documentation of consent approved by the Ethical Committee of the Semmelweis University.

Before starting treatment patient information on age, gender, body weight and height, any earlier antiviral therapy were registered. Body mass index (BMI) of each patient was calculated by dividing body weight in kilograms by body height squared. The patients were then divided into three groups based on their BMI: normal BMI <25 kg/m^2, slightly obese 25–30 kg/m^2 and obese >30 kg/m^2. Prior to, as well as monthly throughout the therapy, laboratory tests were performed following 12 h fasting, with registration of the following parameters: blood glucose, cholesterol, triglyceride, serum bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (AP), serum albumine, prothrombine, serum iron (Fe) levels.

Every 3 months during the treatment period thyroid stimulating hormone (TSH), free T4 (FT4) and free T3 (FT3) levels were also monitored to exclude the possibility of hypothyroidism. HCV RNA was determined in appropriately collected sera by reverse transcriptase-polymerase chain reaction using commercial kit (Amplicor HCV, Roche Diagnostics, Branchburg, NJ). HCV genotyping was performed using second-generation reverse hybridization line probe assay (Inno-Lipa HCV II; Innogenetics, Zwijndrecht, Belgium). Viral levels were determined in the 12th and 24th weeks as well as at the end of therapy, and also 24 weeks after treatment.

The data of the patients are shown in Table 1.

Liver Histopathology

Blind liver biopsies were performed for 61 patients. The histological specimens were evaluated by a specialized

Table 1 Baseline characteristics and laboratory features of chronic hepatitis C genotype 1 infected patients

Number of patients (n=96)	
Mean age±SD:	48.7±9.8
Sex:	
Male:	52 (54%)
Female:	44 (46%)
Relapse patients:	16 (17%)
Naive patients:	77 (80%)
Body weight±SD:	76.4±16.8
Genotype:	
1b	86 (90%)
1a	10 (10%)
Mean BMI±SD:	26.8±5.2
Significant alcohol intake:	30 (31%)
Hypertension:	38 (40%)
Diabetes:	10 (16%)
Mean Laboratory values±SD	
AST (IU/mL):	92.9±75.7
ALT (IU/mL)::	115.5±76.7
GGT (IU/mL):	104.7±88.1
Blood glucose (mmol/L):	5.8±1.5
Cholesterol (mmol/L):	4.3±0.9
Trygliceride (mmol/L):	1.2±0.7
Steatosis (n=61)	
Codes	
0	17 (28%)
1	25 (41%)
2	10 (16%)
3	9 (15%)
Viral load x 10^6 (IU/ml)	3.1±7.4

hepato-pathologist using the score system introduced by Ishak and coworkers in 1995 [29]. Scoring of the degree of necroinflammation (0–18) was as follows: 0–8 slight, 9–12 moderate, 13–18 severe degree of chronic hepatitis (Fig. 1). Severity of fibrosis (stage) was also calculated by means of the Ishak score system (0–6). Degree of steatosis was measured by the percentage of fatty hepatocytes using a scale of 0–3: grade 0: <5% steatosis, grade 1: 5–33% steatosis, grade 2: 34–66% steatosis, grade 3: hepatocyte involvement of and over 67% [30].

Treatment

The patients chosen for the study had no previous treatment with peginterferon and ribavirin. They received either peginterferon alpha-2a 180 μg/week, or peginterferon alpha-2b 1.5 μg/body weight kg/week in the form of subcutaneous injection and ribavirin 10.6 mg/body weight kg/die treatment. Those patients continued their therapy till the 52nd week whose PCR results proved negative in the 24th week of treatment. Therapy was discontinued in case of four patients owing to side effects (angina pectoris, lung fibrosis, atrium fibrillation, depression) presenting before the 24th week. These patients were excluded from the study.

Those patients were regarded responders who became virus free within 6 months after therapy, i.e. who reached the stage of sustained virological response (SVR). Relapsers were those patients who became virus free by the end of therapy, but showed PCR positivity again within 6 months following their treatment. Non-responders were the patients who remained PCR positive by the 24th week of treatment or by the end of therapy.

Statistical Method

The results of continuous variables were given by descriptive method, as sample size, means ± SD, and their normality was checked by Leven's test. Statistical analysis was performed using one-way analysis of variances (ANOVA or non parametric Kruskal-Wallis ANOVA) followed by Tukey's test, or multiple comparisons of mean ranks were applied if significant differences between means were detected.

Contingency tables were adapted for categorical variables and Fisher's exact test (with Monte-Carlo estimation) was used to draw the inferences.

Logistic regression model was used for virus titer (values were grouped into two classes, cutpoint: < and ≥800.000 IU/ml) and steatosis variables to estimate the odds ratio.

Differences were considered to be statistically significant at $p<0.05$. Each analysis was performed using the SAS statistical software package (SAS/STAT, Software Release 9.1.3., SAS Institute Inc., Cary, North Carolina 27513, USA).

Results

Patient Characteristics

From the 96 genotype 1 infected patients 86 proved to be of genotype 1b (90%), 10 of genotype 1a (10%). Data of these patients are summarized in Table 1. Degree of steatosis was determined in 65 patients (biopsy was not performed in the rest of the patients). Treatment had to be stopped due to side effects in case of four patients, their results were therefore not processed, so we analyzed 61 patients. In 64% of the patients the liver tissue showed steatosis of varying degree: stage 0 in 28% (17 patients), stage 1 in 41% (25

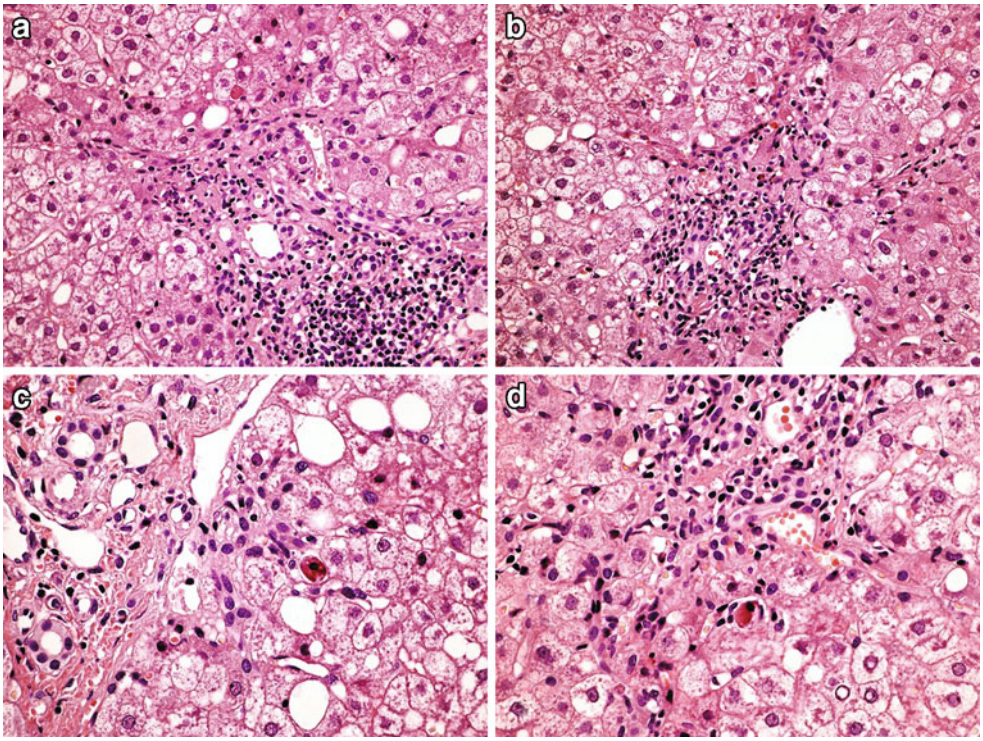

Fig. 1 Histology of liver biopsy specimens with CHC. Portal inflammation and steatosis (**a**, **b**). Apoptotic bodies and inflammatory cells in the periportal areas (**c**, **d**). (Haematoxylin and eosin; a, b: 250x; c, d: 400x)

patients), stage 2 in 16% (ten patients) and stage 3 in 15% (nine patients).

Relationship Between Steatosis and Host or Viral Factors

In 38% of cases (23 patients) case history revealed alcohol consumption 6 months prior to treatment, with grade 0–1 steatosis present in 65% of these cases (Table 2). Based on the results, the degree of hepatic steatosis did not show any relationship with the level of alcohol consumption in the type 1 CHC patients (p=0.5792).

Case history denoted type 2 diabetes in 16% of the cases (ten patients), 60% of which showed grade 0–1 steatosis further, 70% of the patients not revealing diabetes also presented steatosis to a similar degree (Table 2). No significant relationship could be found between degree of steatosis and diabetes mellitus (p=0.5925).

Fifty-nine percent of the patients in whom liver steatosis was not manifest had BMI values >25 kg/m^2. From 19 patients with grade 2–3 steatosis, BMI values greater than 30 kg/m^2 were found in 31% (Table 3). The studied genotype 1 infected patients showed no relationship in regard to steatosis and BMI (p=0.9685).

In the study, values below 890,000 IU/ml were considered low virus levels. Logistic regression analyses were carried out to find out how virus levels influenced severity of steatosis. Considering the various degrees of steatosis, the odds ratio equalled 2.1 (Fig. 2). The degree of steatosis and the virus titer showed significant relationship.

Relationship Between Steatosis and Laboratory Parameters Prior to Therapy

The relationships found between pre-treatment laboratory values and severity of steatosis are summarized in Table 4. Degree of hepatic steatosis and blood glucose, cholesterol, trygliceride, ALT, AP, albumin, prothrombin and Fe values prior to treatment showed no relationship. The AST value increased with the degree of steatosis. The patients presenting no steatosis had pre-treatment AST levels of 52.6±20.7, whereas this value was found to be 115.7±69.5 for patients with grade 3 steatosis. Significant differences were noted

Table 2 Effect of alcohol consumption, diabetes mellitus on steatosis

	Alcohol		Diabetes Mellitus	
Steatosis	No	Yes	No	Yes
0	12 (32%)	5 (22%)	15 (29%)	2 (20%)
1	15 (40%)	10 (43%)	21 (41%)	4 (40%)
2	7 (18%)	3 (13%)	9 (18%)	1 (10%)
3	4 (10%)	5 (22%)	6 (12%)	3 (30%)
Total	38 (100%)	23 (100%)	51 (100%)	10 (100%)

Steatosis-Alcohol:

Fisher's exact test (Monte-Carlo estimate) and 95% CI limits: p=0.5792 (0.5695, 0.5889)

Steatosis-Diabetes Mellitus:

Fisher's exact test (Monte-Carlo estimate) and 95% CI limits: p=0.5925 (0.5829, 0.6021)

Table 3 Effect of BMI (Body Mass Index, kg/m^2) on steatosis

	BMI			
Steatosis	<25	25–30	>30	Total
0	7 (41%)	6 (35%)	4 (24%)	17 (100%)
1	8 (32%)	11 (44%)	6 (24%)	25 (100%)
2	3 (30%)	5 (50%)	2 (20%)	10 (100%)
3	3 (33%)	5 (56%)	1 (11%)	9 (100%)
Total	21 (34%)	27 (44%)	13 (22%)	61 (100%)

Fisher's exact test (Monte-Carlo estimate) and 95% CI limits: p=0.9685 (0.9651, 0.9719)

regarding the enzyme levels of the grade 0,2 (p=0.0325) and grade 0,3 steatosis groups (p=0.0169). The GGT enzyme level also showed elevation in parallel with the degree of steatosis: grade 0: 54.9±54.1, grade 1: 105.4±95.4, grade 2: 108.4±74.8, grade 3: 180.4±92.6. Significant differences were observed between the grade 0,1 (p=0.0197), 0,2 (p=0.0271) and 0,3 (p=0.0009) steatosis groups.

Relationship Between Steatosis, Stage and Grade

Relationship between steatosis, stage and grade are observable in Tables 5 and 6. No relationship was found between severity of steatosis and degree of necroinflammation (p=0.3247). There was however relationship between the degree of hepatic steatosis and stage of CHC (p=0.0119). From the 44 patients showing some degree of liver steatosis, 68% (30 patients) had a stage lower than grade 3, from which 43% (19 patients) presented the lowest degree of steatosis. From patients with fibrosis greater than stage 3, 79% (11 patients) revealed steatosis also of significant degree, grade 2–3.

Effect of Steatosis, Grade and Stage on Therapeutic Response

By the end of the combined pegylated interferon and ribavirin treatment 57% of the genotype 1 infected patients became HCV PCR negative, 43% were non-responders. After the offset of therapy 21% of the patients became relapsers, SVR was manifest in 36% of cases. A total of 65% of responder and 35% of non-responder patients were naive cases. Twenty-nine percent of the non-responders received interferon and ribavirin treatment earlier. No liver steatosis was detected in 55% of the responders (12 out of 22 patients). This ratio was 19% in case of non-responders (5 out of 26 patients), whereas all relapse cases exhibited steatosis to a certain degree (grade 1 in 62%, grade 2 in 31% and grade 3 in 7% of the patients). Severe steatosis of grade 2–3 was denoted in 26% of responding patients, 48% of non-responding patients and 26% of relapse patients (Table 7).

Significant relationship was found between therapeutic response to combined pegylated interferon + ribavirin treatment and steatosis (p=0.0050). Slight necroinflammation was evidenced in 83% of the patients (51 out of 63), independent of therapeutic response.

Severe grade was observed in case of one non-responding patient. Effectiveness of treatment was not influenced by grade (p=0.9413).

Fibrosis was slighter than stage 3 in 91% of responding (21 out of 23) and 57% of non-responding (16 out of 28) patients. More severe fibrosis was detected in 43% of non-responding and 31% of relapse (4 out of 13) patients. No correlation was found between stage severity and effectiveness of treatment (p=0.3164).

Discussion

The results of this study demonstrate that steatosis was present in the liver in 64% of the CHC genotype 1 infected patients, though its degree was only slight in the majority of cases.

Antiviral therapy proved useful for those patients who had no liver steatosis. The non-responding and relapse patients presented higher degree of hepatic steatosis. Earlier alcohol consumption, type 2 diabetes mellitus or high BMI did not influence the severity of hepatic steatosis in the studied patients. Further, the degree of steatosis was not influenced by glucose level, cholesterol or trygliceride values, or Fe level. Patients with high viral load demonstrated a more severe grade of steatosis. Relationship

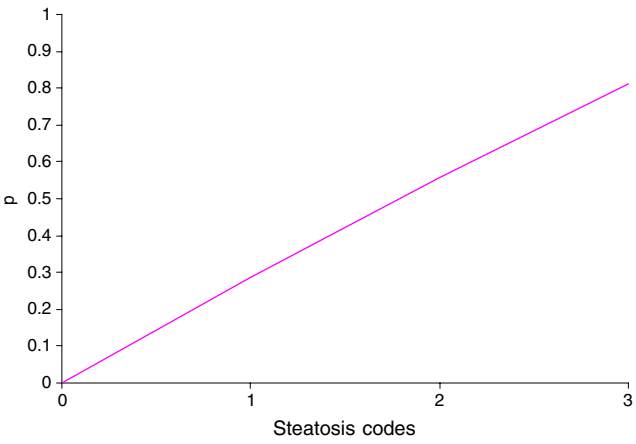

Fig. 2 Relationship between viral level and steatosis codes estimated p values of viral levels by logistic regression. At logistic regression the cut-off point of viral level was the medium value, that is 890000 IU/ml. So, the codes of the viral groups in logistic regression were 0 (<890,000 IU/ml) and 1 (>=890,000 IU/ml)

Table 4 ANOVA results between severity of steatosis and laboratory parameters

Parameters	Steatosis 0[a] (*N*=17)	1[b] (*N*=25)	2[c] (*N*=10)	3[d] (*N*=9)	p
Blood glucose /mmol/L/	5.6±1.4	5.7±0.9	5.3±0.5	6.6±2.2	0.1099
Cholesterol /mmol/L/	4.6±0.9	4.5±0.8	4.2±0.6	4.0±1.1	0.3304
Trygliceride /mmol/L/	1.5±1.4	1.1±0.4	0.9±0.4	1.2±0.4	0.3889
AST /IU/ml/	52.6±20.7	89.1±70.6	99.3±48.5[b]	115.7±69.5[c]	*a,c: 0.0325** *a,d: 0.0169**
ALT /IU/ml/	87.5±48.3	112.5±84.9	126.0±79.3	141.3±95.9	0.2529
GGT /IU/ml/	54.9±54.1	105.4±95.4[a]	108.4±74.8[b]	180.4±92.6[c]	*a,b: 0.0197** *a,c: 0.0271** *a,d: 0.0009**
AP /IU/ml/	176.1±48.8	219.1±84.5	245.5±96.5	221.1±63.7	0.1174
Albumine /g/l/	45.4±2.2	44.5±2.7	43.7±1.8	44.7±2.7	0.5954
Prothrombine INR	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	0.6917
Fe (μmol/l)	24.3±11.7	28.3±13.9	28.9±9.5	25.1±12.2	0.6905

*: significant difference at $p<0.05$

between steatosis and stage suggested steatosis to have influence on the development of fibrosis.

An earlier Hungarian study related to CHC patients proved relationship between lipid metabolism and degree of steatosis [31]. The study revealed hepatic steatosis in 64% of CHC patients. Other literary data refer to the presence of liver steatosis in 36% to 66% of genotype 1 infected cases [7, 16, 26, 31]. Our study verified steatosis in a fairly higher ratio of CHC genotype 1 infected patients. Similar to earlier reports, the majority of our patients also showed slight degree of steatosis (<33%).

A further feature of our study was to found out which factors play role in the occurrence of steatosis. Previous studies have pointed to the role of BMI in the development of steatosis in HCV infected patients [3–7, 9–16]. We did not find such correlation between BMI and severity of steatosis. A possible explanation to our differing results could be that most of our patients had low BMI values. Further, alcohol had no role in the development of steatosis in our study, since liver biopsies were performed after 6 months of alcohol abstinence. Our results are in accord with previous study results [9, 16] also demonstrating that earlier alcohol consumption has no role in the development of liver steatosis in CHC patients. In this context, literary data have verified the role played by metabolic factors in CHC genotype 1 infected patients [4, 5]. CHC patients with type 2 diabetes mellitus did not show significant differences in the degree of steatosis as compared with non-diabetic patients. Patton and co-workers [16] found no differences in the degree of steatosis regarding diabetic and non-diabetic CHC patients.

Our results suggest that the presence of type 2 diabetes mellitus does not play significant role in the steatosis of the liver in CHC genotype 1 infected patients. Relationship between virus titer and severity of steatosis in genotype 1 infected patients raises the possiblity that hepatic steatosis is caused by the direct effect of HCV. So far, this effect of HCV has only emerged in relation to genotype 3 infected patients [14, 24]. The degree and frequency of occurrence of steatosis in case of this genotype is higher than in other genotypes, at the same time the decrease or even disappearance of steatosis has been observed in patients responding to therapy [16, 24]. The role of metabolic factors has mostly been raised in regard to the steatosis-inducing effect of HCV genotype 1 infection [3–16]. It has gained verification, however, that the core protein of genotypes 1b and 3a is also capable of trygliceride accumulation on Huh-7 hepatoma cells, though this is more significant in case of genotype 3a infection [32].

The role played by HCV core and NS5A proteins in trygliceride accumulation has been justified by a number of in vitro studies [33, 34]. The NS5A apolipoprotein steps into reaction with A1 and A2, contributing to the fatty degeneration of hepatocytes. Animal experiments have

Table 5 Steatosis and histological grade/stage (Steatosis – Grade)

Steatosis	Grade 1	2	3	Total
1	22 (88%)	2 (8%)	1 (4%)	25 (100%)
2	8 (80%)	2 (20%)	0	10 (100%)
3	6 (67%)	3 (33%)	0	9 (100%)
Total	36 (82%)	7 (16%)	1 (2%)	44 (100%)

Fisher's exact test (Monte-Carlo estimate) and 95% CI limits: $p=0.3247$ (0.3155, 0.3339)

Table 6 Steatosis and histological grade/stage (Steatosis – Stage)

	Stage						
Steatosis	1	2	3	4	5	6	Total
1	6 (24%)	10 (40%)	6 (24%)	0	2 (8%)	1 (4%)	25 (100%)
2	1 (10%)	1 (10%)	4 (40%)	0	2 (21%)	2 (20%)	10 (100%)
3	0	1 (11%)	1 (11%)	2 (22%)	4 (44%)	1 (11%)	9 (100%)
Total	7 (16%)	12 (27%)	11 (25%)	2 (5%)	8 (18%)	4 (9%)	44 (100%)

Fisher's exact test (Monte-Carlo estimate) and 95% CI limits: p=0.0119 (0.0098, 0.0140)

proved that the HCV core protein inhibits the activity of the microsomal trygliceride transfer protein (MTP), an enzyme playing important role in VLDL assembly and trygliceride selection [35]. Based on our results the direct steatosis causing effect of the HCV core protein in genotype 1 infected patients cannot be excluded.

There are previous studies showing that steatosis plays role in the progression of fibrosis [3, 5–8, 10, 12, 13, 15–17], whereas others have not confirmed this [4, 9, 11, 14]. An European study demonstrated relationship between steatosis and degree of fibrosis in HCV genotype 3 infected cases [15]. Steatosis may enhance the progression of fibrosis, as found in a study on genotype 1 infected American patients [16]. In their study related to genotype 3- and other genotype infected cases, Fartoux and coworkers [36] found that steatosis of the same grade showed no differences in relation to fibrotic stage. Our results also demonstrated significant relationship between the degrees of steatosis and fibrosis, referring to the fact that steatosis might have role in the progression of fibrosis.

The mechanism through which steatosis contributes to the progression of fibrosis is unknown as yet. Steatosis causes via inflammation mitochondrial damage, oxidative stress and enhanced lipid peroxidation [37]. The latter leads to activation of stellate cells, which synthesize type 1 collagen. This is the main collagen in fibrotic liver tissue [5, 38–40]. Relationship was found between steatosis and lipid peroxidation [41] further, enhanced lipid peroxidation was demonstrated at fibrotic sites [42] in chronic HCV cases. All these findings confirm the notion that steatosis leads to increase in fibrosis through lipid peroxidation.

In recent years studies have focused on the influence of steatosis on the results of antiviral therapy. The study of Patton and coworkers [16] showed that the ratio of patients with no steatosis was higher among those who proved to be genotype 1 infected and gave early virological response (EVR). These authors also demonstrated that the degree of steatosis was slighter in SVR patients as compared with non-responders. No such relationship was found in relation to genotype 3 infected patients. In their study, these authors subjected only 17% of their patients to pegylated interferon and ribavirin treatment, the rest received either interferon or pegylated interferon monotherapy, or combined interferon and ribavirin treatment.

Our study involved combined pegylated interferon and ribavirin treatment, related to the effect of liver steatosis on effectiveness of treatment. We found that the severity of steatosis has negative predictive value as regards the outcome of combined pegylated interferon and ribavirin treatment. Steatosis may influence the effectiveness of therapy through a number of factors. Steatosis, via enhancement of fibrosis, has unfavourable effect on the outcome of therapy, advanced connective tissue reorganization results in less effective treatment. Another means by which steatosis influences the success of therapy is that its presence alters the hepatic metabolism of antiviral drugs, thus also worsening the chances of recovery.

Taken together, our results demonstrate that the course of CHC and the effectiveness of therapy are both influenced by the presence of steatosis in case of genotype 1 infected patients. Despite the finding that in CHC genotype 1 infected patients hepatic steatosis was of slighter degree and less frequent than reported in other studies related to genotype 3 infected patients, our findings suggest that steatosis exerts influence on the effectiveness of combined pegylated interferon and ribavirin treatment. Our results demonstrated that as in genotype 3 infected cases, steatosis has clinical significance also in genotype 1 infected patients.

Table 7 Relationship between therapeutic response and steatosis

	Therapeutic Response			
Steatosis	RL	NR	R	Total
0	0	5 (29%)	12 (71%)	17 (100%)
1	8 (32%)	12 (48%)	5 (20%)	25 (100%)
2	4 (40%)	2 (20%)	4 (40%)	10 (100%)
3	1 (11%)	7 (78%)	1 (11%)	9 (100%)
Total	13 (21%)	26 (43%)	22 (36%)	61 (100%)

Abbreviations: RL relapser, *NR* non-responder, *R* responder

Fisher's exact test (Monte-Carlo estimate) and 95% CI limits: p=0.0012 (0.0005, 0.0019)

Conflict of interest statement The authors of the present study confirm that there is no conflict of interest to be declared.

References

1. Seeff LB, Buskell-Bales Z, Wright EC, National Heart, Lung and Blood Institute Study Group et al (1991) Long-term mortality after transfusion-associated non-A,non-B hepatitis. N Engl J Med 327:1906–1911
2. Poynard T, Bedossa P, Opolon P (1997) Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 349(9055):825–832
3. Mihm S, Fayyazi A, Hartmann H et al (1997) Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. Hepatology 25:735–739
4. Czaja AJ, Carpenter HA, Santrach PJ et al (1998) Host- and disease-specific factors affecting steatosis in chronic hepatitis C. J Hepatol 29:198–206
5. Hourigan LF, Macdonald GA, Purdie D et al (1999) Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 29:1215–1219
6. Rubbia-Brandt L, Quadri R, Abid K et al (2000) Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotypes 3. J Hepatol 33:106–115
7. Adinolfi LE, Gambardella M, Andreana A et al (2001) Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 33:1358–1364
8. Serfaty L, Andreani T, Giral P et al (2001) Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. J Hepatol 34:428–434
9. Monto A, Alonzo J, Watson JJ et al (2002) Steatosis in chronic hepatitis C: relative contribution of obesity, diabetes mellitus, and alcohol. Hepatology 36:729–736
10. Westin J, Nordlinder H, Lagging M et al (2002) Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients. J Hepatol 37:837–842
11. Hui JM, Kench J, Farrell GC et al (2002) Genotype-specific mechanisms for hepatic steatosis in chronic hepatitis C infection. J Gastroenterol Hepatol 17:873–881
12. Castéra L, Hézode C, Roudot-Thoraval F et al (2003) Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut 52:288–292
13. Poynard T, Ratziu V, McHutchinson J et al (2003) Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 38:75–85
14. Asselah T, Boyer N, Guimont MC et al (2003) Liver fibrosis is not associated with steatosis but with necroinflammation in French patients with chronic hepatitis C. Gut 52:1638–1643
15. Rubbia-Brandt L, Fabris P, Paganin S et al (2004) Steatosis affects chronic hepatitis C progression in a genotype-specific way. Gut 53:406–412
16. Patton HM, Patel K, Behling C et al (2004) The impact of steatosis on disease progression and early sustained treatment response in chronic hepatitis C patients. J Hepatol 40:484–490
17. Asselah T, Rubbia-Brandt L, Marcellin P et al (2006) Steatosis in chronic hepatitis C: why does it really matter? Gut 55:123–130
18. Allison ME, Wreghitt T, Palmer CR et al (1994) Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. J Hepatol 21:1135–1139
19. Caronia S, Taylor K, Pagliaro L et al (1999) Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. Hepatology 30:1059–1063
20. Mason AL, Lau JY, Hoang N et al (1999) Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology 29:328–333
21. Petit JM, Bour JB, Galland-Jos C et al (2001) Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol 35:279–283
22. Mehta SH, Brancati FL, Strathdee SA et al (2003) Hepatitis C infection and incident type 2 diabetes. Hepatology 38:50–56
23. Hui KQ, Esrailian E, Runyon B et al (2002) Clinical implication of hepatic steatosis in patients with chronic hepatitis C. Hepatology 34:187A
24. Kumar D, Farell GC, Fung C et al (2002) Hepatitis C virus genotype 3 is cytopathic to hepatocytes: reversal of hepatic steatosis after sustained therapeutic response. Hepatology 36:1266–1272
25. Moriya K, Yotsuyanagi H, Shintani Y et al (1997) Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 78:1527–1531
26. Kumar D, Farrell GC, Fung C et al (2002) Hepatitis C virus genotype 3 is cytopathic to hepatocytes: reversal of hepatic steatosis after sustained therapeutic response. Hepatology 36:1266–1272
27. Gervain J, Simon G, Papp I et al (2001) Determination of the type and subtype of the hepatitis virus C in chronic viral hepatitis patients in Hungary. Orv Hetil 142:1315–1319
28. Tornai I, Dalmi L, Gervain J et al (2005) Treatment of patients with chronic hepatitis C, using pegylated interferon alfa-2a and ribavirin — the first experiences in Hungary based upon a multicentric, open, prospective study. LAM 15:807–813
29. Ishak K, Baptista A, Bianchi L et al (1995) Histological grading and staging of chronic hepatitis. J Hepatol 22:696–699
30. Brunt EM, Janney CG, Di Bisceglie AM et al (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474
31. Jármay K, Karácsony G, Nagy A et al (2005) Changes in lipid metabolism in chronic hepatitis C. World J Gastroenterol 11:6422–6428
32. Abid K, Pazienza V, de Gottardi A et al (2005) An in vitro model of hepatitis C virus genotype 3a-associated triglycerides accumulation. J Hepatol 42:744–751
33. Barba G, Harper F, Harada T et al (1997) Hepatitis C core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Nat Acad Sci USA 94:1200–1205
34. Shi ST, Polyak SJ, Tu H et al (2002) Hepatitis C virus NS5A colocalizes with the core protein on lipid droplets and interacts with apolipoproteins. Virology 292:198–210
35. Perlemuter G, Sabile A, Letteron P et al (2002) Hepatitis C core protein inhibits microsomal triglycerid transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. FASEB J 16:185–194
36. Fartoux L, Chazouilleres O, Wendum D et al (2005) Impact of steatosis on progression of fibrosis in patients with mild hepatitis C. Hepatology 41:82–87
37. Day CP, James OFW (1998) Steatohepatitis: a tale of two "hits"? Gastroenterology 114:842–845
38. Lee KS, Buck M, Houglum K et al (1995) Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by

oxidative stress through c-myb expression. J Clin Invest 96:2461–2468
39. Bedossa P, Houglum K, Trautwein C et al (1994) Stimulation of collagen alpha 1(1) gene expression is associated with lipid peroxidation in hepatocellular injury: a link to tissue fibrosis? Hepatology 19:1262–1271
40. Parola M, Pinzani M, Casini A et al (1993) Stimulation of lipid peroxidation or 4-hydroxynonenal treatment increases procollagen alpha 1 (1) gene expression in human liver fat-storing cells. Biochem Biophys Res Commun 194:1044–1050
41. Farinati F, Cardin R, De Maria N et al (1995) Iron strorage, lipid peroxidation and glutathion turnover in chronic anti-HCV positive hepatitis. J Hepatol 22:449–456
42. Paradis V, Mathurin P, Kollinger M et al (1997) In situ detection of lipid peroxidation in chronic hepatitis C: correlation with pathological features. J Clin Pathol 50:401–406