# Association of the prevalence and grade of steatosis in patients with chronic hepatitis C with the host and viral factors

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## Abstract

***Aim :*** **The aim of this study was to investigate the prevalence of hepatosteatosis in chronic hepatitis C patients, evaluate the potential impact of some host- and virus-related factors on its occurrence and possible influence of steatosis on the consequences of hepatitis.**

***Patients and methods :*** **The case records of 387 patients with hepatitis C and cirrhosis were studied. The prevalence and grade of steatosis were investigated and evaluated by logistic regression analysis as dependent variable to age, gender, alcohol consumption, body mass index, hepatitis C virus (HCV) genotypes, liver enzymes activity, histological activity index and fibrosis.**

***Results :*** **Steatosis was found in 47.3% of the patients. It was more prevalent in males, alcohol abusers, overweight and obese patients, and in those with HCV genotypes 3 and 2. Multivariate analysis confirmed body mass index as an independent risk factor for steatosis in the overall patient cohort and in those with genotype1 without any correlation with the steatosis grade. The prevalence and grade of steatosis were associated with alcohol consumption and higher fibrosis stage. The age of the patients showed converse association.**

***Conclusions :*** **The male gender, body mass index, alcohol consumption, genotype 2 and 3 were confirmed as risk factors for hepatosteatosis. Older patients had a lesser steatosis grade.**

**The correlation of histological activity index and fibrosis scores with the prevalence and higher grade of steatosis suggested a possibility to worsen the course of hepatitis C and to accelerate disease progression.** (Acta gastroenterol. belg., **2007**, 70, **260-266**).

**Key words** : chronic hepatitis C, hepatic steatosis, steatosis grade, association.

## 1. Introduction

The coexistence of steatosis and chronic hepatitis C (CHC) is a common histological finding with the prevalence of 30-70% (1-5). Although the precise mechanisms leading to hepatosteatosis (HS) in chronic hepatitis C virus (HCV) infection are not completely elucidated, both host and viral factors contribute to the development of steatosis in chronic HCV infection. It is considered now that HCV infection is associated with two types of HS : principally virus induced in genotype 3 and mainly metabolic in the other genotypes (6). The data of the experimental models and clinical investigations confirmed a direct cytopathic effect of genotype 3 HCV infection on hepatocytes and its steatogenous effect. A link between steatosis and body mass index (BMI), HCV genotype 3 and insulin resistance was reported (4,7,8).

Additionally the role of host factors such as age, gender, alcohol intake and presence of diabetes mellitus may also be of importance, though the data presented are controversial (7).

There is also growing evidence of adverse interactions between CHC and HS. The presence of HS is associated with more advanced hepatic fibrosis and the increased risk of hepatocellular carcinoma (3,4,9-12). Nevertheless some studies did not confirm that HS could be a marker and not the cause of fibrosis and disease progression (2,8,13,14).

The presence of HS independently decreases sustained response rate to antiviral therapy, whereas sustained responders have improvement in HS (5,7,15).

## 2. Patients and methods

### 2.1. *Study population*

This retrospective study included case records of 387 patients (219 males and 168 females) with biopsy proven chronic hepatitis C (CHC) or liver cirrhosis (CLC) who were hospitalised and examined between 1995 and 2003 years in the Centre of Hepatology, Gastroenterology and Dietetics of Vilnius University.

Patients were selected according to the following criteria :

- None of them received antiviral therapy at the time of the liver biopsy ;
- None of them were taking hepatotoxic medication causing steatosis (NSAID, salicylates, steroids, tetracyclines, amiodarone, perhexiline maleate, etc.) ;
- All of them were negative for HBV and HIV ;
- All of them had liver biopsy tissue available for the review ;
- alcohol consumption was recorded precisely : intake $\geq$ 40 g/day for males and $\geq$ 20 g/day for females was considered as alcohol abuse ;
- BMI was calculated according to the formula : body weight (kg) / body height ($m^2$). Normal BMI was considered as $\leq$ 25 kg/$m^2$, overweight – 26-30 kg/$m^2$ and obesity > 30 kg/$m^2$.

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2.2. *Laboratory and virological investigations*

The standard laboratory tests of the liver function were carried out : activity of serum alanine and aspartate aminotransferases (ALT and AST), γ-glutamyltranspeptidase (γGT), alkaline phosphatase (ALP), concentration of bilirubin (Bi), cholesterol, high and low density lipoproteins (HDL, LDL), triglycerides, glucose, total protein and albumin.

Antibody to HCV (anti-HCV) was examined by the second generation enzyme immunoassay (Abbott Laboratories, USA), HCV RNR – by PCR (Amplicor HCV ; Roche Diagnostics, Switzerland), HCV genotype – by PCR followed by line probe assay (Inno-Lipa HCV ; Innogenetics, Belgium), HBsAg (DIAplus ; AxSYM MEJA, Abbott Laboratories, USA).

2.3. *Liver histology*

Ultrasound guided liver biopsy was carried out in all the patients. Biopsy specimens were stained by hematoxyllin-eosin and picrosirius red (16) and examined at the National Centre of Pathology (accredited by the College of American Pathologists). Histological activity index (HAI) was scored according to Ishak *et al.* (1995) (17), while fibrosis (F) according to METAVIR classification system (1994) (18). Liver steatosis was graded on a scale of 0-3 : 0 - absence of steatosis ; grade 1 (mild) - < 30% of hepatocytes containing fat vacuoles, grade 2 (moderate) – 30-70% of hepatocytes affected and grade 3 (severe) - > 70% of hepatocytes affected (Brunt *et al.*, 1999) (19).

2.4. *Statistical analysis*

The descriptive data were shown as percentage or means with the standard deviation (SD). Kolmogorov-Smirnov's test was applied to define the distribution of quantitative variables in groups of patients with and without HS. The data with symmetric distribution were further analysed by Student's t-test, whereas those with non-symmetric distribution by nonparametric Mann-Whitney-Wilkinson test. For the statistical analysis of ranked and nominal variables Mann-Whitney-Wilkinson or Fisher test was applied.

To assess the relationship between HS and the demographic data, biochemical and histological tests, univariate and multivariate binary logistic regression analyses were performed. Steatosis was considered as a dependent variable for both analyses, whilst age, gender, alcohol intake, BMI, ALT, AST, γGT, ALP, HAI, fibrosis were considered as independent variables. The data were computed with SPSS version 11.5 for Windows (SPSS Inc., USA) and SAS system version 9.1 (SAS Institute Inc., USA). A value < 0.05 was considered significant for all the tests.

## 3. Results

3.1. *Baseline demographic data*

Three hundred and eighty seven patients (219/56.6% males and 168/43.4% females, mean age 46.8 ± 14.0 yrs) with CHC (334/86.3%) and CLC (53/13.7%) were enrolled in this study (Table 1). Overall 152/45%

Table 1. — **Characteristics of patients according to steatosis**

| Variables | Overall | | With steatosis | | Without steatosis | | p value with HS *vs* without HS |
|---|---|---|---|---|---|---|---|
| | n | mean ± SD<br>range | n | mean ± SD<br>range | n | mean ± SD<br>range | |
| Age (years) | 387 | 46.8 ± 14.0<br>17 - 83 | 183 | 48.1 ± 13.6<br>21 - 76 | 204 | 45.6 ± 14.3<br>17 - 83 | 0.081 |
| BMI (kg/m$^2$) | 387 | 26.1 ± 4.4<br>18 - 40 | 183 | 27.0 ± 4.5<br>18 - 40 | 204 | 25.2 ± 4.1<br>18 - 38 | < 0.0001 |
| ALT (U/L) | 387 | 135.8 ± 165.9<br>5 - 2500 | 183 | 160.7 ± 209.7<br>5 - 2500 | 204 | 113.5 ± 108.9<br>12 - 700 | < 0.0001 |
| AST (U/L) | 387 | 93.4 ± 108.4<br>8 - 1460 | 183 | 113.1 ± 137.2<br>8 - 1460 | 204 | 75.7 ± 69.4<br>18 - 509 | < 0.0001 |
| γGT (U/L) | 381 | 111.8 ± 174.2<br>5 - 1908 | 183 | 155.1 ± 52.8<br>9 - 1908 | 198 | 72.4 ± 99.8<br>5 - 831 | < 0.0001 |
| ALP (U/L) | 381 | 83.8 ± 47.8<br>21 - 460 | 182 | 89.1 ± 49.9<br>23 - 405 | 199 | 79.0 ± 45.5<br>21 - 460 | 0.012 |
| HAI (scores) | 387 | 6.4 ± 3.1<br>1 - 13 | 183 | 7.25 ± 3.0<br>1 - 13 | 204 | 5.6 ± 2.9<br>1 - 12 | < 0.0001 |
| F (scores) | 387 | 2.3 ± 0.9<br>0 - 4 | 183 | 2.5 ± 0.9<br>1 - 4 | 204 | 2.2 ± 0.9<br>0 - 4 | < 0.0001 |
| Variables | n | ratio | n | ratio | n | ratio | p value |
| HCV genotype 1:2:3 | 146 | 102:14:30 | 81 | 49:8:24 | 65 | 53:6:6 | 0.003 |
| Males:females | 387 | 219:168 | 183 | 116:67 | 204 | 103:101 | 0.014 |
| Alcohol (yes/no) | 387 | 144/243 | 183 | 84/99 | 204 | 60/144 | 0.001 |

patients were overweight or obese. Alcohol consumption was revealed in 144/37.2% patients (132/91.7% males and 12/8.3% females).

Mean values of biochemical parameters – ALT, AST and γGT – were elevated and exceeded the upper normal level (UNL) – 3.4, 2.3 and 2.7 times, respectively, while ALP activity was normal.

Genotype 1 prevailed and was found in 102/69.9% patients, genotype 3 – in 30/20.5% and genotype 2 – in 14/9.6%.

Two hundred and twenty (56.8%) patients had fibrosis stage 1-2 and 146/37.7% - F 3-4 ; histological activity index was mostly minimal (1-3 scores) and mild (4-8 scores) : 81/20.9% and 186/48.1%, respectively.

### 3.2. *Comparison of demographic, biochemical, histological data and HCV genotypes between groups of patients with and without HS*

Overall HS was found in 183/47.3% patients : mild (I°) – in 117/63.9%, moderate (II°) – in 52/28.4% and severe (III°) – in 14/7.7%. HS was more prevalent in males than in females (116/63.4% vs 67/36.6%, $p = 0.014$) and in alcohol abuse patients ($p < 0.0001$) (Table 1).

Mean value of BMI was higher ($p < 0.0001$) and obesity two times more prevalent in patients with HS than in those without HS (40/21.9% *vs.* 22/10.8% ; $p < 0.05$), while their age did not differ considerably.

Values of ALT, AST and γGT activity as well as HAI and F scores were significantly higher in HS group ($p < 0.0001$). ALP activity was normal in both groups (Table 1).

Genotype 3 was more prevalent in patients with HS overall irrespective on BMI (24/29.6% vs 6/9.2%, $p = 0.003$) as well as in patients with HS and normal (< 25) BMI ($p = 0.0021$).

In CHC setting overall and in patients with genotype 1 with normal (< 25) BMI HAI and F scores were higher in those with HS and statistically significantly increased depending on HS grade (correlations : in CHC setting overall HS and HAI – Spearman coef. = 0.19976, $p = 0.0156$, HS and F – Spearman coef. = 0.21104, $p = 0.0106$ ; in patients with genotype 1 and BMI < 25 HS and HAI – Spearman coef. = 0.42816, $p = 0.0058$ ; HS and F – Spearman coef. = 0.61682, $p < 0.0001$), but no HS correlation with HAI and fibrosis scores was observed in patients with genotype 3.

Low correlation between HS and BMI was found in CHC setting overall (Spearman coef. = 0.19199, $p = 0.0001$), but not in patients with genotype 1 and BMI < 25.

### 3.3. *Univariate analysis of factors associated with HS*

HS was significantly associated with male gender (OR = 1.698), alcohol consumption (OR = 2.036), genotype 3 (OR = 4.326) and to a lesser extent, genotype 2. HS was also associated with the higher liver enzymes activity, HAI (OR = 1.198), fibrosis stage (OR = 1.458) and BMI, but not with the age of the patients (Table 2). Slight association between BMI and HS was found in all the patients of the CHC cohort as well as in those with genotype 1 and 3 (OR = 1.103, $p < 0.001$ ; OR = 1.107 $p = 0.004$ ; OR = 1,139 $p = 0.0134$, respectively).

Though statistically significant association of HS with BMI, the activity of liver enzymes HAI and F score values was found, the OR was rather low. Additional univariate analysis for these variables showed that the risk of HS in overweight patients (BMI > 25) was twice higher than in those with normal BMI, while in obese ones (BMI ≥ 30) it increased 1.6 times as compared with those having BMI < 30 (Table 3). Elevated liver enzymes (ALT, AST, γGT) were two- and threefold, HAI > 7 threefold (OR = 2.846) and F III-IV° twofold (OR = 2.067) more prevalent in patients with HS, indicating that steatosis may promote HCV-mediated liver injury.

### 3.4. *Multivariate analysis of factors associated with HS*

After adjusting gender, age, BMI, liver enzymes, HAI, F and HCV genotypes to steatosis, only five variables were corroborated as independent risk factors for HS : male gender, BMI, HAI, F and HCV genotypes 3

Table 2. — **Factors associated with HS (data of univariate analysis)**

| *Variables* | $n_{with\ HS}/n_{without\ HS}$ | *OR* | *95% CI* | | *p value* |
|---|---|---|---|---|---|
| Gender M *vs* F | 183/204 | 1.698 | 1.130 | 2.551 | 0.0108 |
| Age | 183/204 | 1.013 | 0.998 | 1.028 | 0.0811 |
| BMI (overall) | 183/204 | 1,103 | 1,051 | 1,158 | < 0,0001 |
| BMI (genotype 1) | 49/53 | 1,107 | 1,056 | 1,164 | 0.0040 |
| BMI genotype 3) | 24/6 | 1,139 | 1,027 | 1,263 | 0.0134 |
| ALT | 183/204 | 1,003 | 1,001 | 1,005 | 0,0042 |
| AST | 183/204 | 1,006 | 1,002 | 1,009 | 0,0006 |
| ALP | 179/199 | 1,005 | 1,000 | 1,009 | 0,0444 |
| γGT | 180/198 | 1,005 | 1,002 | 1,007 | < 0,0001 |
| HAI | 183/204 | 1,198 | 1,117 | 1,284 | < 0,0001 |
| F | 183/204 | 1,458 | 1,167 | 1,823 | 0,0009 |
| Alcohol (yes *vs* no) | 183/204 | 2,036 | 1,340 | 3,095 | 0.0009 |
| Genotype 2 *vs* 1 | 81/65 | 1.442 | 0.467 | 4.453 | 0.0128 |
| Genotype 3 *vs* 1 | 81/65 | 4.326 | 1.631 | 11.473 | 0.0128 |

n – number of patients, OR – odds ratio, CI – confidence interval.

Table 3. — **Overweight, obesity, elevated liver enzymes, higher HAI and F association with HS. (data of univariate analysis)**

| *Variables* | *OR* | *95% CI* | | *p value* |
|---|---|---|---|---|
| BMI > 25 vs BMI ≤ 25 | 1.959 | 1.306 | 2.939 | 0.0012 |
| BMI ≥ 30 vs BMI < 30 | 1,669 | 1,020 | 2,730 | 0,0415 |
| ALT > 40 vs ALT ≤ 40 | 3,130 | 1,647 | 5,948 | 0,0005 |
| AST > 37 vs AST ≤ 37 | 2,572 | 1,530 | 4,322 | 0,0004 |
| ALP > 127 vs ALP ≤ 127 | 1,822 | 0,954 | 3,479 | 0,0693 |
| γGT > 41 vs γGT ≤ 41 | 2,704 | 1,761 | 4,151 | < 0,0001 |
| HAI > 7 vs HAI ≤ 7 | 2,846 | 1,867 | 4,338 | < 0,0001 |
| F III-IV vs F I-II | 2,067 | 1,373 | 3,111 | 0,0005 |

Table 4. — **Factors independently associated with steatosis (data of multivariate analysis)**

| *Variables* | $n_{with\ HS}/n_{without\ HS}$ | *OR* | *95% CI* | | *p value* |
|---|---|---|---|---|---|
| Gender M *vs* F | 178/195 | 2.091 | 1.002 | 4.365 | 0.0495 |
| BMI (overall) | 178/195 | 1,104 | 1,049 | 1,162 | 0,0002 |
| BMI (genotype 1) | 49/53 | 1,082 | 1,007 | 1,163 | 0,0191 |
| HAI | 178/195 | 1,144 | 1,062 | 1,234 | 0,0004 |
| F | 178/195 | 2.083 | 1.304 | 3.326 | 0.0021 |
| Genotype 2 vs 1 | 77/63 | 2.094 | 0.632 | 6.933 | 0.0054 |
| Genotype 3 vs 1 | 77/63 | 5.443 | 1.908 | 15.523 | |

Table 5. — **Factors associated with HS grade (data of univariate analysis)**

| *Variables* | $n_{HS\ grade1}/n_{HS\ grade\ 2}/n_{HS\ grade\ 3}$ | *OR* | *95% CI* | | *p value* |
|---|---|---|---|---|---|
| Gender M *vs* F | 117/52/14 | 2,401 | 1,235 | 4,666 | 0,0098 |
| Age | 117/52/14 | 0,970 | 0,948 | 0,993 | 0,0101 |
| Age >50 *vs* ≤ 50 | 117/52/14 | 0.406 | 0.214 | 0.771 | 0.0059 |
| BMI | 117/52/14 | 1.016 | 0.951 | 1.085 | 0.6362 |
| ALT | 117/52/14 | 1.000 | 0.999 | 1.002 | 0.6897 |
| AST | 117/52/14 | 1.001 | 0.999 | 1.004 | 0.1537 |
| ALP | 114/51/14 | 1.005 | 0.999 | 1.010 | 0.1192 |
| γGT | 114/52/14 | 1.002 | 1.001 | 1.003 | 0.0052 |
| HAI | 117/52/14 | 1.063 | 0.962 | 1.174 | 0.2311 |
| F | 117/52/14 | 1,586 | 1,122 | 2,243 | 0,0091 |
| Alcohol yes *vs* no | 117/52/14 | 3,440 | 1,845 | 6,416 | < 0.0001 |

and 2 (Table 4). The prevalence of HS in men was two times higher (OR = 2.091) than in women. HCV genotype 3 and 2 enhanced risk of HS five- and twofold with respect to genotype 1 (Table 4). HS was also associated with higher scores of histological parameters (HAI and F).

Slight association of HS with BMI was proven (OR = 1.104) in all the patients and in those with genotype 1 (OR = 1,082), whereas age, alcohol use and liver enzymes activity were not confirmed as independent risk factors for HS.

### 3.5. *Association of HS grade with clinical, biochemical and histological parameters*

According to univariate and multivariate logistic regression analyses only the male gender, γGT activity, F score and alcohol consumption were significantly associated with a higher HS grade (Table 5).

The patients over 50 had a lower HS grade in comparison with those under 50 and this finding was confirmed by the data of multivariate analysis (Table 6) : the older the patient, the lesser risk of a higher HS grade (OR = 0.961).

Multivariate analysis also revealed alcohol abuse and F score as independent factors associated with a higher HS grade.

## Discussion

The association between chronic hepatitis C infection and hepatic steatosis has been extensively studied, and evidence of its multifactorial origin was confirmed. However the relative importance of host- and viral-related factors on the occurrence of HS is controversial. The aim of this study was to investigate the prevalence of HS in CHC patients, evaluate the potential impact of some host- and virus-related factors on its occurrence and possible influence of HS on the consequences of chronic hepatitis C.

HS was detected in 183/47.3% of the Lithuanian cohort of CHC patients : mild (I°) – in 63, 9%, moderate (II°) – in 28, 4% and severe (III°) in 7, 7% of biopsies with HS. The data on the prevalence of HS and its grades (mild HS prevailed) are similar to those reported by the other authors : the mean prevalence of HS in patients with CHC infection (calculated by summing up the

Table 6. — **Factors associated with HS grade (data of multivariate analysis)**

| *Variables* | *nHS grade 1/nHS grade 2/nHS grade 3* | *OR* | *95% CI* | | *p value* |
|---|---|---|---|---|---|
| Age | 117/52/14 | 0.961 | 0.937 | 0.986 | 0,0028 |
| Alcohol yes vs no | 117/52/14 | 2.682 | 1.369 | 5.255 | 0.0040 |
| F | 117/52/14 | 1,802 | 1,234 | 2,632 | 0,0023 |

results of 25 studies which included 6400 patients) was 55,54% (range 34,8% - 81,2%) (2,20-22). Differences in the prevalence of HS depend, probably, on the characteristics of the patients studied, on the local peculiarities of their life style and, consequently, risk factors (23).

The male gender statistically significantly prevailed in patients with HS. The results of logistic regression analysis showed that the risk of HS for men is almost twice higher than that for women ; moreover, the male gender is an independent predictor for HS. A higher grade (II-III°) of HS is found 2.4 times more often in men too.

The results concerning the association between gender and HS in CHC are controversial. Some authors did not find any significant relationship, while others reported the higher prevalence of non-alcoholic fatty liver in men (24-26). Moreover, the men in the same steatosis condition were 10 years younger than the women, therefore the protective effect of estrogens was hypothesized (27). Finally, the data before the year 2000 and the recent report from Brazil indicated the female gender as a predictor for HS (26). Thus, further studies on the relationship of gender and HS in CHC patients – male and female of comparable age and disease characteristic (without metabolic or other steatogenous factors) would be reasonable.

Similarly, the controversial data were reported on the association between age and HS. Some reports indicated older age as a predictor for HS (5,7,28), whereas other reports did not find any relationship (4,29,30). Our data did not confirm age as a predictor for HS, and what is more, converse association between age and HS grade was observed : a higher HS grade (II-III°) was found 2.5 times more often in the patients under 50 years of age than in those over 50. Additionally, the older age of the patients was independently associated with a lesser HS grade. We did not find data on the statistically significant converse association between age and HS grade and there is no trustworthy explanation of this finding (31). The only presumption we have is that older patients may have longer duration of the disease and a more advanced liver damage, which may force the changes in the patient's life style (a stricter diet, less alcohol intake, fewer calories and BMI reduction). We consider that this finding should be confirmed by an additional study of a larger cohort of patients with severe (III°) grade of steatosis (only 14/7.7% patients with HS grade III constitute this group at present). Also, it has been observed that the presence of cirrhosis is rarely associated with HS, while non-cirrhotic patients have a four-fold probability of having steatosis *vs* cirrhosis (22).

Liver steatosis increases with the progression of fibrosis, however when the liver disease progresses to decompensation, the risk of HS falls down (30). Whether this depends on the reduced calorie intake, systemic shunts of portal blood or sinusoidal capillarization remains unclear (32).

Our data regarding BMI and HS correspond to the results of the researchers who validated an important role of increased BMI in the pathogenesis of HS in patients with CHC (4,8). Patients with HS had a higher mean value of BMI and higher prevalence of obesity than those without HS. Multivariate analysis confirmed BMI as an independent risk factor for HS in CHC setting overall and in patients with genotype 1 HCV infection, but not in patients with genotype 3. No correlation of BMI and the grade of steatosis in patients with genotype 1 or with genotype 3 was found.

Nevertheless, some recent studies did not find a relationship between BMI and HS in CHC patients overall, but showed a clear correlation between the grade of steatosis and BMI in genotype 1 HCV infection (8,20). There are considerations that conditions associated with metabolic syndrome (visceral obesity, insulin resistance and diabetes mellitus type II), rather than BMI itself, contribute to steatosis. Since very few patients in our study could be considered as having metabolic syndrome, we did not analyze the correlation of HS and metabolic syndrome.

The distribution of HCV genotypes in our cohort of CHC patients was as follows : genotype 1 was found in 69.9%, genotype 2 – in 9.6%, genotype 3 – in 20.5% of the patients. The last one presented a strong association with HS (8,21), as well as genotype 2a/2c (21,30). Logistic regression analysis confirms this finding : genotype 3 enhances the risk of HS in comparison with genotype 1 more than five times. Patients with genotype 2 are also under the twice higher risk of HS than those with genotype 1.

Different mechanisms of steatosis in HCV genotypes are discussed ; however the specific pathways remain unclear. HS in genotype 1 is more likely to be metabolic and depends on leptin level, insulin resistance, BMI, and visceral obesity (33), while in the case of HCV genotype 3 it is the result of virus specific cytopathic effect. Experimental models showed that the core protein may cause HS by interaction with apoA2, reduction of microsomal triglyceride transfer protein, mitochondrial toxicity, production of reactive oxygen species,

down-regulation of the expression of lipid metabolism-associated genes and peroxisome proliferator-activated receptor (34). Additionally, NS5A together with the core protein may play a certain role in the derangement of lipid metabolism through interaction with apoA1 and contribute to liver steatosis (35). However, these experimental data were obtained mostly as a result of the studies on HCV genotype 1 core and NS5A proteins. Therefore investigations on specific structural differences between those proteins in genotype 1 and 3 could highlight their relation to HS pathogenesis (36,37). The grade of steatosis in genotype 3 correlates with the high quasispecies heterogeneity, level of intrahepatic HCV replication and serum viral load (26,30,37).

The lack of the correlation of HCV genotypes and BMI with the grade of steatosis (also reported by other authors) is in accordance with this hypothesis (8, 23).

In sum, consideration that HS in the genotype 3 HCV infection is a purely viral effect, while in the other genotypes host effect related to insulin resistance is probably an oversimplification of the problem. Steatosis seems to be due to a combination of these factors, with the relative importance of each varying with the genotype (22,38).

The possible impact of the other factors on the occurrence of HS, particularly alcohol, is also important. Studies to date presented conflicting data regarding the contribution of alcohol intake to steatosis in CHC patients (2,9,30). According to our data alcohol consumption increased the risk of HS twice and showed strong association with the higher (II-III°) HS grade.

The concomitant presence of liver steatosis and HCV infection has several important consequences on the liver, including accelerated hepatic fibrosis and disease progression, decreased virologic response rate to antiviral therapy and, possibly, an increased risk of HCC (1,8,39-41).

Multivariate regression analysis confirmed the association of histological activity index and fibrosis score with the prevalence of HS irrespective of genotypes. In addition, a higher grade of hepatic steatosis independently associated with a higher stage of fibrosis in CHC patients' cohort overall and in patients with genotype 1.

The association between HS and fibrosis in patients with CHC was corroborated by many studies (14,42-45) and identified steatosis as the risk factor for progression of fibrosis. Some studies also showed that fibrosis severity is associated with steatosis grade only in patients with HS of metabolic causes, i.e. patients with genotype 1, while steatosis associated with genotype 3 is not related to the grade of fibrosis (20,46).

Nevertheless some studies did not confirm these findings (20). Moreover, an alternative viewpoint exists according to which HS correlated with fibrosis only in patients with genotype 3 infection (2,13,43).

The mechanisms of the pathogenetic link between steatosis and fibrosis remain unknown, however oxidative stress associated with insulin resistance syndrome and stellate cells activation may contribute to fibrosis (38). Alternative explanation for the correlation between steatosis severity and hepatic fibrosis may involve an increased susceptibility of the steatotic liver to the effects of the antiviral inflammatory response (47).

Association between necroinflammation and HS was also found (2,13,48,49) and it was postulated that HS is an important independent cofactor increasing liver necroinflammatory activity and accelerating fibrosis in CHC (8,49). According to the converse point of view, steatosis is a marker rather than the cause of fibrosis progression (50,51).

So there are discrepancies between the results and interpretations, and even the causality of HS and fibrosis is questionable (46). Whether or not steatosis determines fibrosis progression per se and what pathogenetic mechanisms play some role in this process is still to be proven in future studies (52).

In conclusion : the high prevalence of hepatosteatosis in CHC patients is associated with some host and viral factors : the male gender, BMI, genotype 3 and to a lesser extent genotype 2, which might be considered as risk factors for this concomitant disease. The male gender and alcohol consumption are independently and directly associated with a higher grade of steatosis, while the age of patients, conversely, with a lesser grade.

The independent association of the prevalence of HS with necroinflammation (HAI) and fibrosis score and, additionally, the correlation of a higher grade of HS with higher HAI and fibrosis score in patients with genotype 1, suggest that HS may accelerate fibrosis progression and worsen the course of CHC.

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