Hepatic steatosis associated with hepatitis C virus infection

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Abstract

Hepatic steatosis (including microvesicular and macrovesicular fat) is a significant histologic feature associated to the chronic hepatitis C (HCV). The purpose of this paper was to analyze the incidence of hepatocyte steatosis in patients with HCV and the potential role of some the known variables as risk factors possibly involved in the occurrence of steatosis: age, sex, obesity, biological parameters, diabetes mellitus, degree of necroinflammation (NI) and stage of fibrosis. 96 of 125 (76.8%) patients had hepatic steatosis (mild 76%, moderate and severe 24%); in comparison with patients without steatosis, those with HCV and steatosis were more frequently women (males/ females: 1/1.9) of older age (49.97 vs. 47.7 years), with a greater ICM (index of corporal mass) (26.55 sqm vs. 23.52 sqm), with raised glycemic values (13 of the 14 patients with HCV and diabetes mellitus had steatosis), an average value of serum ALT significantly raised (95.38 U/l vs. 78.96 U/l), and an average score of NI activity significantly higher (9.39 vs. 6.75).

Keywords: hepatic steatosis, chronic hepatitis C virus (HCV).

Introduction

It is well known that patients with alcoholic hepatic disease (AHD) present a significant risk for contracting a subsequent infection with hepatitis C virus (HCV), often developing some form of disease with evolution towards chronicization, and the infection accentuates pre-existing hepatic injuries due to chronic ethylism. It is well known that alcohol abuse amplifies the lesions of hepatitis C, favoring a more rapid progression towards hepatic cirrhosis and hepatocellular carcinoma.

Mild or moderate hepatic steatosis frequently identified in hepatitis C (HCV), with an average of 50% of cases, neither suggests nor excludes a concomitant AHD [1].

When a severe panlobular steatosis is present, an AHD should be suspected, especially in the case of a steatohepatitis (degenerative-vacuolar lesions of hepatocytes, accompanied by neutrophilic infiltrates) or of perivenular and pericellular fibroses. Actually, fibrosis with this distribution, even in the absence of hepatocyte steatosis, should be suspected as having alcoholic etiology.

The concomitance of HCV- and AHD-type histological alterations should warn the clinician on a risk of rapid evolution of the disease, with a significantly smaller rate of survival. Possible mechanism involved in producing these effects could be immune-mediated lesions secondary to altered hepatocytic membrane, to inhibition by alcohol of hepatocyte regeneration, to intensifying viral replication under the influence of alcohol, with concomitant immune suppression.

Hepatic steatosis constitutes a significant histopathological feature associated to the infection with HCV (approximately 50% of affected individuals have a fatty liver), and its causes and significance in chronic infection with HCV continue to undergo further research. Some of the possible factors associated with a hepatic steatosis, with a probable contribution to its development, are obesity, alcohol abuse, diabetes mellitus type 2, hyperlipidemias, drugs, and concomitant infections.

These risk factors with possible implications in the occurrence of non-alcoholic steatohepatitis are also frequently mentioned in the history of HCV-infected patients and with concomitant hepatic steatosis. As it is well known, hepatic steatosis was generally attributed to alcohol abuse.

The purpose of this paper was to analyze (on a relevant number of cases) the incidence of the above mentioned risk factors in patients included in the study group, to assess the participation of hepatocyte steatosis in achieving the histopathological picture of chronic hepatitis, the interrelation of steatosis with the other lesions and especially with fibrosis (in whose installment and progression it seems to contribute), and to prospect the possible association of steatosis to increasing the evolutive risk of the disease.

Material and method

We evaluated the frequency and amplitude of hepatic steatosis in 189 patients, distributed in two groups:

- group A, including 125 patients (46 males and 79 females, with ages between 21 and 67 – average age of 47.5) with chronic infection with HCV confirmed through the presence of anti-HVC serum antibodies, and demonstrated histopathologically on hepatic biopsy samples;
- group B, containing 64 patients, positive for HBs
antigen (35 males and 29 females with ages between 17 and 68, and an average age of 39.9).

The biopsy sample material was fixed in 10% formaldehyde, included in paraffin, and the sections were stained with Hematoxylin–Eosin (HE), Blue chromotrope–Aniline, van Gieson, Gordon–Sweet silver method; Scharlach staining performed on cryostat sections allowed observation of lipids in red-orange. For iron identification we used Perls method.

On HE stained sections we assessed the degree and stage of disease according to significant criteria of grading and quantifying (applying the Knodell score), assessing the intensity and extension of alterations present in the portal spaces – lymphocytary infiltrate, lymphoid follicles, alteration of biliary canals, “interface hepatitis” aspects, porto-portal and porto-centrolobular necroses, intralobular degenerative lesions (clear intumescence of hepatocytes, acidophil necroses, lymphocyte infiltrates in interhepatocyte spaces and sites).

We graded micro-macrovacular steatosis according to the following:

- score zero = minimal or absent steatosis;
- score 1 = steatosis flecking <30% of hepatocytes;
- score 2 = moderate steatosis, affecting 30–60% of hepatocytes;
- score 3 = severe steatosis, affecting >60% of hepatocytes.

**Results**

In postviral chronic hepatitis, hepatocyte steatosis consisting of accumulation of lipids in the form of cytoplasmatic vacuoles, usually mixed, macrovacuolar usually has a non-systematized distribution.

In the lot of patients studied, the incidence of hepatic steatosis was of 72.48%, with a somewhat significantly different distribution in the two groups of patients: 77% in HCV and 64% respectively in HBV. In most cases, hepatocyte steatosis had a mixed macrovacuolar character (Figures 1 and 2), with variable topography, either predominantly in the acinar 1 or 2 areas (Figures 3 and 4), or most frequently diffuse, panlobular, non-systematized and sometimes with a degenerative character (Figure 5).

We encountered dispersed foci of microvacuolar steatosis (hepatocytes with central nucleus surrounded by small, clearly defined vacuoles) in only eight (5.83%) of the 137 cases of steatosis, interesting <30% of hepatocytes.

In the 125 patients selected with HCV we investigated the potential role of some of the known variables known as risk factors possibly involved in the occurrence of steatosis.

Among the epidemiological factors we considered age, gender, and source of infection (blood transfusions, intravenous drugs, etc.).

Obesity was estimated calculating the index of corporeal mass (ICM) as weight (kg) related to height (sqm). According to established limits through a consensus of experts, we defined as overweight ICM >25 kg/sqm, and obesity ICM >30 kg/sqm.

We determined ICM in 96 (76.8%) of the 125 patients with HCV, which had an average value of 25.85 kg/sqm.

From biological parameters, we analyzed the alanine aminotransferase (ALT), gamma-GTP, alkaline phosphatase (AF), glycemia, serum level of free lipids – triglycerides (TG), and cholesterol (C). Then we assessed the relationship between steatosis and hepatic lesions (mainly, the degree of necroinflammation and stage of fibrosis).

In Table 1 we present characteristics of the 125 patients with HCV with and without steatosis. In comparison with patients without steatosis, those with steatosis were more frequently women (males/females rate = 1/1.9) of older age (49.97 years, as compared with 47.7 years), with a greater ICM (26.55 sqm as compared with 23.52 sqm), with raised glycemic values (13 of the 14 patients with diabetes mellitus had steatosis), and an average value of serum ALT significantly raised (95.38 U/l as compared with 78.96 U/l).

| Table 1 – Characteristics of 125 patients with chronic hepatitis C, with and without hepatic steatosis |
|-------------------------------------------------|---------------------------------|---------------------------------|
| Variables                                      | Patients with steatosis (n = 96) | Patients without steatosis (n = 29) |
| Gender M/F                                     | (S0 + S1 + S2)                  | (S0)                             |
| Average age                                    | 49.97                          | 47.7                             |
| ICM (kg/sqm)                                   | 26.55                          | 23.52                            |
| DM                                             | n = 13                         | n = 1                            |
| ALT (UI)                                       | 95.38                          | 78.96                            |
| Cholesterol (mg%)                              | 177.4                          | 167.8                            |
| Triglycerides (mg%)                            | 101.66                         | 92.23                            |
| Hepatic siderosis                              | n = 8                          | n = 5                            |

The level of total cholesterol and of serum triglycerides was slightly raised in patients with steatosis (177.4 mg% vs. 167.8 mg%, 101.66 mg% vs. 92.23 mg%). Hepatic siderosis (increase of iron deposits especially in the Kupffer cells) was more frequently encountered in patients with steatosis as compared with those without steatosis (eight cases as compared to five), even though the differences do not seem significant.

96 (76.8%) of the 125 patients had hepatic steatosis graded as mild in 74 cases (76%), and moderate and severe in 22 cases (24%) (Figure 6).

In HBV the incidence and severity of steatosis (Figure 7) were more attenuated in comparison with HCV, and deposits of lipids in hepatocytes were more frequently noted in males; 41 (64%) of the 64 patients infected with HBV presented hepatic steatosis which we estimated as mild in 32 cases (78%) and moderate or severe in nine cases (32%).

In patients with steatosis associated to HCV we estimated and quantified the histological pattern of hepatic lesions (degree of inflammatory and necroinflammatory alterations, and stage of fibrosis).

Based on the results obtained, we described (Figures 8–10) the relationship between steatosis and the histological score for inflammatory lesions, necroinflammation, and for the stage of fibrosis.
Hepatic steatosis associated with hepatitis C virus infection

Figure 1 – Hepatitis with C virus (HCV). Hepatic steatosis micro-macrovesicular (HE staining, ×200)

Figure 2 – Hepatitis with C virus (HCV). Hepatic steatosis micro-macrovesicular (Scharlach staining, ×400)

Figure 3 – Hepatitis with C virus (HCV). Hepatic steatosis in the acinar 1 and 2 areas (PAS staining, ×200)

Figure 4 – Hepatitis with C virus (HCV). Hepatic steatosis in the acinar 1 and 2 areas (Blue chromotrope–Aniline staining, ×200)

Figure 5 – Hepatitis with C virus (HCV). Panlobular steatosis with a degenerative character (HE staining, ×200)

Figure 6 – Incidence of steatosis in HCV and HBV

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<th>H/C</th>
<th>H/B</th>
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<tr>
<td>mild</td>
<td>74</td>
<td>32</td>
</tr>
<tr>
<td>moderate and severe</td>
<td>22</td>
<td>9</td>
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</table>
Figure 7 – Hepatic steatosis in HBV (HE staining, ×200)

Figure 8 – Relationship between steatosis and histological score of inflammation

Figure 9 – Relationship between steatosis and average score of necroinflammatory activity (NI)

Figure 10 – Relationship between steatosis and stage of fibrosis

Figure 11 – Fibrosis surrounding the central vein. Stage 2 (Gordon-Sweet staining, ×400)

Figure 12 – Fibrosis of perisinusoidal spaces. Stage 2 (Gordon-Sweet staining, ×400)
Histological score for inflammation and necroinflammatory activity were independently and significantly associated with the presence of steatosis; we found an average score of necroinflammatory activity significantly higher in patients with steatosis (9.39) as compared with those without steatosis (6.75).

Based on the hypothesis that in the infection with HCV hepatic steatosis contributes to the occurrence and progression of fibrosis through a steatohepatitic-like mechanism, with activation of stellate cells and perisinusoidal fibrosis, we assessed, on 40 of the 125 biopsy samples, the presence and extension of acinar fibrosis, also evaluating its association with the degree of steatosis and other histological traits. In order to evaluate the acinar fibrosis, we applied the Gordon–Sweet staining method and graded independently the fibrosis surrounding the central vein (Figure 11) and fibrosis of perisinusoidal spaces (Figure 12).

Hepatocyte steatosis was present in 70% of cases, and was associated in 55% of cases with fibrosis of various degrees of the central vein and of perisinusoidal spaces.

We observed a correlation between severity of subsinusoidal fibrosis, older age of the patients, degree of hepatic steatosis, and raised ICM. In most cases, subsinusoidal fibrosis was also concordant with the degree of portal inflammation.

Following the relationship between portal and acinar fibrosis, we observed that subsinusoidal fibrosis, as well as central vein fibrosis seems to be concordant with the degree of portal fibrosis (Table 2).

Table 2 – Relationship between portal and acinar fibrosis

<table>
<thead>
<tr>
<th>Subsinoidal fibrosis (FSS)</th>
<th>Portal fibrosis</th>
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<tr>
<td>0 (n = 18)</td>
<td>8  7 2 1</td>
</tr>
<tr>
<td>1 (n = 13)</td>
<td>1  5 4 3</td>
</tr>
<tr>
<td>2 (n = 9)</td>
<td>0  1 4 4</td>
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<tr>
<th>Central vein fibrosis (FVC)</th>
<th>Portal fibrosis</th>
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<tr>
<td>0 (n = 18)</td>
<td>7  6 3 2</td>
</tr>
<tr>
<td>1 (n = 15)</td>
<td>2  5 5 3</td>
</tr>
<tr>
<td>2 (n = 7)</td>
<td>0  2 2 3</td>
</tr>
</tbody>
</table>

Table 2 – Relationship between portal and acinar fibrosis

Discussions

One of the traits of the infection with HCV seems to be its association with hepatic steatosis (lipid accumulation in the cytoplasm of hepatocytes) and the causes and significance of hepatic steatosis in chronic infection with HCV continue to undergo numerous studies [1].

Among the conditions that might contribute to the development of hepatic steatosis during chronic hepatitis, the following are mentioned: obesity, alcohol abuse, type 2 diabetes mellitus, and hyperlipidemias [2].

Hourigan et al. (1999), the first to research the role of alcohol in HCV hepatic steatosis, reported no association between alcohol ingestion and the severity of steatosis or fibrosis [3].

The results noted by these authors are contradictory to the generally accepted hypothesis, according to which alcohol consumption plays an important role in progression of fibrosis. Similarly to Hourigan’s observations, Asselah et al. (2003) find no significant relationship between the presence of steatosis or fibrosis and alcohol consumption; however, the authors observe the frequency of genotype 3 of HCV in patients with steatosis over 60% [2].

Since both alcohol consumption and the infection with HCV may produce, independently, hepatic steatosis, Monto et al. (2002) presume that association of these two risk factors could induce severe steatosis; moreover, they consider that in patients with chronic infection with HCV and excessive alcohol consumption, steatosis might stimulate the mechanisms of hepatic fibrogenesis [4, 5].

Insufficient data regarding alcohol consumption in the 125 patients with HCV studied did not allow us an evaluation regarding the role of alcohol ingestion in the occurrence of hepatic steatosis. We could study, however, the frequency and severity of hepatic steatosis, at the same time confronting the severity of steatosis with the degree and stage of hepatic disease. At the same time we evaluated the potential role of some of the factors known as causes of HCV-associated hepatic steatosis.

In the first group of patients studied, comprising 125 cases with HCV, hepatic steatosis was observed in 96 cases (77%) with a significantly high frequency in females (females/males rate = 1.9/1) of older age (in females, average age = 49.97; in males = 47.7), with raised ICM (26.55 kg/sqm as compared with 23.52 sqm), with high values of glycemia and a significantly high value of serum ALT (93.58 U/l vs. 78.96 U/l).

Our results are concordant mostly with Asselah’s observations, which noted, in the studied group of patients with hepatitis C, an independent relationship between steatosis and raised ICM, between the high score of necroinflammation and genotype 3 of HCV, without finding any relation between the degree of steatosis, stage of fibrosis, and alcohol consumption [2].

Brandt (2000) suggested that hepatic overcharging with iron could be involved in steatogenesis through the intracellular transport mediated by lipid peroxidase [6]; in our study, only eight patients with steatosis presented concomitantly hepatic siderosis.

Most publications regarding HCV-associated hepatic steatosis note the relationship between steatosis and obesity, some studies suggesting the impact of obesity on steatosis in comparison with other known predictors of fatty liver [4, 5].

While Hourigan (1999) and Monto (2002) showed an independent relation between raised ICM and steatosis [3, 4], Adinolfi et al. (2001) noted only an association with visceral obesity. In patients with HCV and steatosis we found a raised ICM with an average of 26.55 kg/sqm (as compared with 23.52 in those without steatosis) [7].

Regarding the role of diabetes mellitus in the development of hepatic steatosis in patients with HCV, literature data are scarce, in most epidemiological studies performed to the present day (in relation with
HCV infection-associated hepatic steatosis), diabetic patients, as well as alcoholics, being excluded or included in reduced number [4, 7].

Our observations suggest that diabetes mellitus may play a promoting role in the development of HCV-associated steatosis, but more reduced as compared with that of obesity; in the studied material, 13 of the 14 cases with diabetes mellitus presented HCV-associated hepatic steatosis.

The role of raised ICM as a risk factor in promoting diabetes mellitus type 2 is well known, and the connection between these two variables is so strong that there can be no relationship between diabetes and steatosis, independently of ICM.

Diabetes mellitus probably induces steatosis by means of obesity, and this, in turn, promotes development of fibrosis. In addition, it is presumed that fibrosis itself may result because of incomplete glucose metabolism.

The few observations regarding lipids in chronic infection with HCV have reported a correlation between the high level of serum cholesterol and triglycerides, on one hand, and hepatic steatosis on the other hand [4].

In the subgroup of patients studied with chronic hepatitis, for which we had available data, steatosis was correlated with a slightly raised average value of triglycerides and serum cholesterol, as compared with patients without steatosis.

Recent research reports a concordance between severity of steatosis and advanced stage of fibrosis, suggesting that steatosis could accelerate the progression of hepatic fibrosis [7].

The observations presented by various authors remain yet contradictory, some of them having failed to find any connection between hepatic steatosis and hepatic fibrosis in HCV infection [4].

Similarly to the observations of Asselah (2003), in the group of our patients we noted a significant relation between steatosis and the increased score of necroinflammatory (patients with HCV and hepatic steatosis had an average score of necroinflammatory activity of 9.39), as compared with the score of 6.75 in those without steatosis.

Necroinflammation is a dynamic process with fluctuations over time, which would accelerate progression of fibrosis. Since the degree of necroinflammatory activity has been associated with the subsequent progression of fibrosis, there are reasons to believe that necroinflammation is involved in fibrogenesis, inasmuch as hepatic stellate cells are activated around necroinflammatory lesions [8].

Brandt (2000) suggests that steatosis is not a reversible phenomenon, and it can lead to organization of inflammation or hepatocyte necrosis (similarly to the sequence of processes in nonalcoholic steato-hepatitis), with installment of hepatic fibrosis whose progression seems therefore to be independent of ICM [6].

Steatosis together with the inflammatory process seems to be the most important predictor of fibrosis, and the fact that genotype 3 induces steatosis explains the correlation of HCV infection with fibrosis [4].

Other authors consider hepatic steatosis in HCV infection as being the morphological expression of the direct cytopathic effect of HCV – genotype 3, based upon the observation that degree of steatosis was significantly higher in individuals affected with genotype 3 as compared with those infected with other viral genotypes.

Brandt et al. (2000) bring the first direct evidences of the correlation between the level of RNA-HCV and the severity of steatosis. Examining the expression of hepatic steatosis in patients treated with α-IFN, the authors observe the association between viral response to treatment, with disappearance of steatosis, showing that in patients infected with genotype 3 of HCV steatosis is directly related to the level of RNA-HCV; therefore, specific sequences of the HCV-genotype 3 genome should be responsible for the fatty accumulation [6].

In nonalcoholic steatohepatitis and alcoholic hepatic disease, steatosis has been associated with the development of acinar inflammation and progression of fibrosis. Distinct morphological traits of fibrosis include pericellular localization (characterized by collagen depositing along hepatic sinusoids), as well as around terminal hepatic venules (compressing and even obliterating their lumen) [9].

Moragas et al. (1998) demonstrated the presence of subsinusoidal fibrosis in cirrhotic patients either with chronic HCV infection, with a pattern similar to that observed in alcoholic steatohepatitis, without establishing a connection with steatosis, or with concomitant alcohol consumption [10].

Following various hepatic injuries, hepatic stellate cells in the Disse space are activated – the main source of extracellular matrix, this type of cells being observed in inflammations associated with fibrosis (including HCV infection) [11].

In 55% of HCV cases, we observed the presence of central vein fibrosis and/or a fine subsinusoidal fibrosis, with histological traits resembling those observed in steatohepatitis.

Conclusions

In conclusion, the study of 125 patients with HCV proves the relatively high frequency of hepatic steatosis, present in 76.8% of cases, graded as mild in 76% of cases, and moderate and severe in 24% of cases.

The presence of steatosis was noted more frequently in women with a high ICM, with raised glycemic values, with an average value of serum ALT significantly higher, and with a slightly raised level of serum cholesterol and triglycerides.

Hepatic lesions were more severe in patients with steatosis, having an average score of necroinflammatory activity significantly higher than those without steatosis.

We also noted an association between severity of subsinusoidal fibrosis, the degree of hepatic steatosis and portal inflammation, old age, and raised ICM, and also a gradual concordance between subsinusoidal fibrosis, central vein fibrosis, and portal tract fibrosis.
References


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