Liver cell steatosis in hepatitis C infected patients

Joanna Cielecka-Kuszyk¹,², Joanna Jabłonska³, Joanna Siennicka², Paulina Godzik², Marcin Komorowski², Kazimierz Madalinski²

¹ Department of Pathology
Children’s Memorial Health Institute
Warsaw, Poland

² Department of Virology
National Institute of Public Health – National Institute of Hygiene
Warsaw, Poland

³ Department of Hepatology and Acquired Immunological Deficiencies Syndromes
Institute of Infectious and Parasitic Diseases, Medical University
Warsaw, Poland

Abstract

Liver cell steatosis is a frequent histological feature in chronic hepatitis caused by hepatitis C virus (HCV) and is found in approximately 50% of patients. This prevalence is suggesting a direct role of HCV in the pathogenesis of fatty liver. Presence of steatosis in hepatitis C may increase the risk of fibrosis progression. This retrospective analysis includes 176 liver needle biopsies obtained from HCV infected patients. Steatosis was found in 77 patients (43.75%). Usually fat droplets were seen in the cytoplasm of hepatocytes in zone 1 near the portal tracts or along fibrous septa, independently from the inflammatory infiltrates. It was associated with other features of cellular degenerative changes such as ballooning or acidophilic degeneration. We have found positive correlation between steatosis and fibrosis in HCV infected patients.

Key words: hepatitis C, liver biopsy, steatosis, inflammation, fibrosis

Introduction

Liver cell steatosis is a frequent histological feature in chronic hepatitis caused by hepatitis C virus (HCV). Most studies have reported approximately 40% to 50% prevalence of steatosis among patients undergoing liver biopsy because of HCV infection independently of the viral genotype. This prevalence is suggesting a direct role of HCV in the pathogenesis of fatty liver [9], however steatosis is more frequent and more severe in patients with HCV genotype 3 [11], correlates with the level of HCV replication in serum and in the liver [1], and disappears in sustained virological responders after antiviral therapy [10]. In comparison, among patients with autoimmune hepatitis and hepatitis B, steatosis is observed only up to 18% of patients [3]. The association of steatosis with liver fibrosis is not clear because of contradictory published data. The presumably viral steatosis detected in 71% of the patients with genotype 3 was not associated with accelerated fibrosis [7], which was confirmed among patients with genotype 1 [7, 14]. In patients with steatosis, increased apoptosis was associated with the activation of hepatic stellate cells and with the increased stage of fibrosis [13]. The presence of steatosis in patients with HCV has prognostic implications and it is associated with a more rapid progression of fibrosis and poorer response to antiviral treatment [3].
Studies performed in experimental models have demonstrated that HCV core protein can induce accumulation of triglyceride rich droplets in hepatocytes and can inhibit microsomal triglyceride transfer protein activity. Through multiple interferences with intrahepatic lipid metabolism, HCV induces steatosis and may theoretically represent a mechanism favoring the entry of the virus into hepatocytes [3].

Material and methods

Patients
The study group consisted of 176 patients – 87 females (mean age 45.2 yrs) and 89 males (mean age 45.4 yrs) with chronic hepatitis C, evaluated by liver biopsy and confirmed by serological (anti-HCV antibodies positive) and virological markers (HCV RNA positive). HCV antibodies were detected in patients serum by Microparticle Enzyme Immunoassay (MEIA), using IMx System HCV version 3.0 (Abbott). This assay has been designed to detect antibodies to putative structural and non-structural proteins of the HCV genome. The presence of anti-HCV indicates that an individual may have been infected with HCV, may harbour infectious HCV and/or may be capable of transmitting HCV infection. The presence of HCV genetic material (RNA) in patients serum confirms infectious HCV existence. HCV RNA was detected in patients serum by RT-PCR method, using Perkin Elmer GeneAmp PCR System 9600.

The liver biopsy specimens obtained from 176 HCV infected patients were examined retrospectively. The age and gender of the patients was as follows: 87 females (mean 45.2 yrs ±11.7) and 89 males (mean 45.4 yrs ±13.6).

Liver histology
Liver biopsy specimens were fixed in 8% neutral buffered formalin, routinely processed (dehydration, clearing, infiltration) and embedded in paraffin. Four µm thick sections were stained with haematoxylin-eosin (H&E), chromotrope 2R-anilin blue and impregnated with silver according to Gomori method. Chromotrope 2R-anilin blue and impregnated with silver according to Gomori method. Gomori method was used for the demonstration of collagen, reticular fiber, muscle fibers, collagen and nuclei. Chromotrope 2R-anilin blue method was used for demonstration of collagen, reticular fiber, intracellular fibrils and Mallory bodies.

All histological features were finally scored using the five degree scale for the grade (inflammatory activity) and stage (fibrosis) of the disease. The grade was assessed as: 0 – no fibrosis, normal connective tissue; 1 – portal fibrosis with fibrous portal expansion; 2 – perportal fibrosis, perportal or rare portal-portal septa; 3 – septal fibrosis, fibrous septa with architectural distortion; 4 – cirrhosis.

Steatosis connoted small, large or mixed-sized vacuoles within the cytoplasm of hepatocytes. Clusters of at least 10-15 hepatocytes containing fat were required for the designation. Mild steatosis was diagnosed if isolated clusters with <10% fat-containing hepatocytes were present. Moderate steatosis was diagnosed if 10-20% of fat containing hepatocytes were present. Severe steatosis was graded if diffuse distribution of >20% of fat containing hepatocytes was present [5].

Statistical analysis
The statistical analysis was performed using GraphPAD InSTAT. One-way analysis of variance ANOVA for evaluation of fibrosis level and age differences, and χ² test for evaluation the differences between groups presented different grade of inflammation, steatosis, fibrosis and lymph follicles presence were used. As criterion of significance p<0.05 (significant) was applied.

Results
All 176 liver biopsies were examined retrospectively by light microscopy. Mild inflammatory infiltrates were observed in 155 patients (88.1%). Moderate inflammatory infiltrates were observed in 19 patients (10.8%). Two patients did not show any inflammation in the liver (1.1%). Marked inflammatory infiltrates were not observed in any patient. The presence of lymphoid follicles was found in the portal tracts in 52 patients (29.5%). We found correlation between the age of infected patients and fibrosis in the liver (p<0.05). Degree of fibrosis was higher in older patients.

Lymphoid follicles occurred independently from the grade of inflammation (p=0.92). In the presence of lymphoid follicles we have found bile duct damage in the form of vacuolisation, stratification, crowding of epithelial cells and infiltration by lymphocytes. Our patients showed different stages of fibrosis: 19 patients (10.8%) did not have any fibrosis, 131 (74.4%) patients showed mild fibrosis, 18 (10.2%) patients had advanced fibrosis and 8 patients (4.5%) showed cirrhosis.

Steatosis was found in 77 patients (43.75%): 71 patients had mild, 5 patients moderate and 1 patient severe steatosis. Usually, large fat droplets were seen in the cytoplasm of hepatocytes in zone 1 near the portal tracts or along fibrous septa, independent of the inflammatory infiltrates (Fig. 1). Fat droplets seen near the central vein in the zone 3 were more dispersed in the cytoplasm of hepatocytes. It was associated with other features of cellular degenerative changes such as ballooning or acidophilic degeneration. We found positive correlation between steatosis and fibrosis (p=0.021). Steatosis more frequently occurred in patients...
with high stage of fibrosis (S3 and S4). We did not found any correlation between steatosis and the degree of inflammation (Tab. 1).

**Discussion**

Our study indicates that steatosis in HCV infected patients is a frequent histological finding and correlates with the fibrosis of the liver. Chronic hepatitis C is associated with steatosis more often than chronic hepatitis of other etiology. The presence of large droplet fatty change helps also to distinguish hepatitis C with autoantibodies from autoimmune hepatitis. The cause of steatosis in HCV infection is not clear, but the interference of the viral core component with lipid metabolism has been suggested [12].

Laboratory studies have shown that HCV core protein can induce hepatic steatosis in transgenic mice and the virus can elicit free radical-mediated lipid peroxidation and increased glutathione turnover, possibly by affecting iron storage within the hepatocyte, which may promote fat disposition [8]. However, steatosis observed in the liver in chronic hepatitis C patients should not be considered to be viral in origin. Frequent causes of fatty liver are high alcohol consumption, obesity, diabetes type II, hyperlipidaemia which is attributed to the insulin-resistant state that underlies the metabolic syndrome [2, 6].

Quantitative assessment of steatosis by automated computerized procedures, in liver biopsies confirmed the morphological findings that steatosis is focally distributed and is seen more frequently in zone 1 in patients with chronic hepatitis C than in those with alcoholic liver disease (ALD). The grade of fat globules was also different. The proportion of macroglobules was significantly higher in patients with ALD than those with HCV infection. The proportion of microglobules was higher in chronic hepatitis C [15]. We confirmed also the focal presence of steatosis in the lobular periportal zone of the liver biopsy specimens, however we found macro and microglobules in the same proportion.

Large meta-analysis of more than 3000 patients with chronic hepatitis C found in databases in Europe, Australia and the USA confirmed the role of steatosis as significantly and independently associated with fibrosis in these patients. In this large study, steatosis was independently associated with genotype 3 as well as fibrosis, hepatic inflammation, alcohol use, BMI and age. Thus, the presence of steatosis may sensitize the liver to inflammation and injury such as apoptosis. The recent meta-analysis demonstrated that the association between steatosis and fibrosis was dependent on a simultaneous association between steatosis and hepatic inflammation [3]. The presence of hepatic steatosis may connote cytopathic-predominant processes, whereas the absence of hepatic steatosis may reflect immune-predominant processes [4]. In our study we did not obtain the information on HCV genotype of the patients, but it should be considered that genotype 1 dominates in the Polish population [16]. We observed the correlation between fibrosis and steatosis. Positive correlation was found between the stage of fibrosis, liver cell steatosis and the age of patients. Our results showed that inflammatory activity in HCV infection was usually mild and did not correlate neither with fibrosis nor with steatosis.

**Table 1**

<table>
<thead>
<tr>
<th>Number of biopsies</th>
<th>Number of biopsies in the different stage of fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>S0  S1  S2  S3  S4</td>
</tr>
<tr>
<td>G0 (2)</td>
<td>2    0    0    0    0</td>
</tr>
<tr>
<td>G1 (155)</td>
<td>17   67   51   12   8</td>
</tr>
<tr>
<td>G2 (19)</td>
<td>0    8    5    6    0</td>
</tr>
<tr>
<td>Steatosis + (77)</td>
<td>8    30   20   14   5</td>
</tr>
<tr>
<td>Steatosis – (99)</td>
<td>11   45   36   4    3</td>
</tr>
</tbody>
</table>

**Fig. 1** Focal steatosis in the periportal zone (A). Periportal steatosis with portal inflammation (B). Steatosis in the cirrhotic nodule (C).
References


