ORIGINAL PAPER

Steatosis and serum lipid patterns in patients with chronic viral hepatitis: differences related to viral etiology

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Abstract

Lipid metabolism disorders related to viral etiology are described in chronic viral hepatitis, independent of age, gender and liver synthetic function. Steatosis is present, especially in chronic hepatitis C but also in chronic hepatitis B. Although liver biopsy is the gold standard in determining presence of steatosis, its presence can be determined by ultrasonographic examination as an initial screening test. Our aim in this study was to evaluate the presence of steatosis in chronic hepatitis B and C, to determine its frequency in both hepatitis type, and to explore possible relationships between presence of steatosis, lipid metabolism disorders and viral etiology. Our study lot included 66 patients, 36 subjects with chronic hepatitis C, and 30 with chronic hepatitis B. We only encountered significant levels of steatosis in the chronic hepatitis B sub-group. We found the average age, cholesterol, triglyceride, HDL-C, VLDL-C levels in the group with steatosis to be significantly higher than those in the group without steatosis ($p<0.05$). Ultrasound reports of hepatic steatosis were particularly associated with histological inflammation, as well as fibrosis; however, the sensitivity and specificity of steatosis on ultrasound was poor when compared to steatosis on biopsy. Hepatic steatosis was significantly more frequent in chronic hepatitis C than in chronic hepatitis B. Severe inflammation and advanced fibrosis were more frequently found in HCV-infected patients with steatosis than in patients without steatosis.

***Keywords*:** hepatic steatosis, lipid metabolism disorders, chronic viral hepatitis.

Introduction

Hepatosteatosis is defined as fat deposits in the liver that exceed 5% of the total weight of liver, or with more than 5% of hepatocytes containing fat deposits under light microscopic examination [1].

Chronic hepatitis C (HCV) and steatosis have been shown to often occur together. Hepatic steatosis, detected by histological examination, appears to have a multifactorial etiology in patients with chronic hepatitis C [2–5]. Steatosis may be the result of a direct virologic effect (particularly in patients infected with genotype 3 [6]. Additionally, steatosis in HCV-infected patients may be associated with accepted steatosis risk factors, including obesity, diabetes mellitus and hypertriglyceridemia [7].

The clinical significance of hepatic steatosis in chronic hepatitis B virus (HBV) patients is poorly understood. Not many studies investigate the relationship between chronic hepatitis B and fatty liver and the effect of steatosis on the course of the disease.

The aim of our study was to evaluate the presence of steatosis in chronic hepatitis B and C, to determine its frequency in both hepatitis types, and to explore possible relationships between the presence of steatosis, lipid metabolism disorders and viral etiology.

Material and Methods

The study included 66 patients with chronic hepatitis B or C: 36 HCV-infected patients and 30 HBV-infected patients.

The diagnosis of chronic hepatitis was sustained on elevated transaminase levels for at least six months, positive for HBs antigen or HCV antibodies, and histopathological findings. Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) were measured in all patients.

The following patients were excluded from the study: those receiving antiviral treatment before the study, those on hepatotoxic drug treatment, those consuming alcohol regularly or excessively and those diagnosed as having autoimmune or metabolic liver diseases.

All patients underwent hepatic ultrasonography during their initial evaluation.

Liver biopsies were performed for each patient 2–3 months after the hepatic ultrasound. No liver biopsies

taken six months after ultrasonography have been used in this study. The bioptic material was fixed in formalin, paraffin-embedded and immunohistochemically stained. The stains used the Avidin–Biotin Complex (ABC) technique. The following antibodies were used: CD3 (DAKO) 1:50 dilution and CD20 (DAKO) 1:100 dilution.

Determination of the histological activity index (HAI) and staging of the biopsy materials were done according to the Knodell's classification. According to the degree of fibrosis, staging was set from 0 to 4 (0: no fibrosis; 1: mild; 2: moderate; 3: severe; 4: cirrhosis). In steatosis classification, less than 5% was considered normal, 5–25% as mild, more than 25% as severe fat deposit.

Demographic, clinical and histological characteristics were compared between patients with steatosis on biopsy *vs.* patients without steatosis on biopsy. A multiple logistic regression model, with stepwise backward elimination of non-significant variables, was developed to determine factors that were associated with the detection of steatosis on ultrasound. Steatosis on ultrasound was the dependent variable, with the independent variables including age, gender, histological stage, and histological grade, ALT, AST, TC, HDL-C, LDL-C and TG.

Results

The chronic hepatitis C patients group consisted of 36 subjects, 12 males and 24 females with a median age of 51±10.9 years (range 30–70 years). We found eight males and 17 females with hepatic steatosis. The difference was not statistically significant.

The chronic hepatitis B group consisted of 30 subjects, eight females and 22 males with a median age of 44.13±12.05 years (range 17–64 years). We found two females and 12 males with hepatic steatosis. The difference was statistically significant ($p<0.05$).

We subdivided the patients into groups with or without steatosis according to the findings of the liver biopsy. We found 25 patients with steatosis HCV-infected (69.4%) and 14 patients HBV-infected (46.66%). The difference according viral etiology was statistically significant ($p<0.05$) (Table 1).

Table 1 – ***Histological characteristics in patients with chronic viral hepatitis***

Parameters	HCV-infected patients with steatosis	HCV-infected patients without steatosis	HBV-infected patients with steatosis	HBV-infected patients without steatosis
High grade of necroinflammation activity	10	2	1	1
Advanced fibrosis	8	2	1	1

HAI was scored as portal inflammation (0–4), lobular degeneration (0–4) and periportal necrosis.

Imunohistochemically, the numerous lymphocytes were CD3-positive (T-cells); the inflammatory infiltrate (portal and lobular) was rich in T-cells. CD20-positive B-cells were found in the portal tract (Figure 1).

In cases with severe steatosis we found fat vacuoles in the perivenular hepatocytes, displacing the nuclei to the edges of the cells (macrovesicular steatosis) and swollen hepatocytes near an efferent venule, containing numerous small vacuoles while the nuclei have a central position (Figure 2).

Two cases presented with mild forms of chronic hepatitis, showing portal changes (lymphocytic infiltrate not extended beyond the margins of the tract). Parenchymal changes were identified by the irregularity of the limiting plate of hepatocytes around the portal tract and mild steatosis (Figure 3).

In five cases, liver biopsy showed enlarged portal tracts and inflammatory infiltrate in association with hepatocellular damage (interface hepatitis), focal necrosis, periportal and lobular inflammation and moderate steatosis (Figure 4).

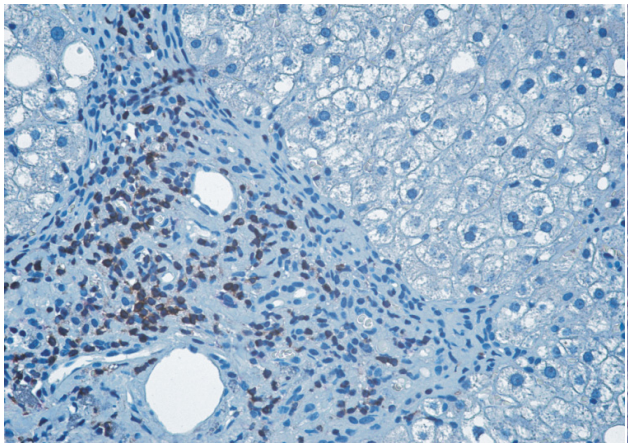

Figure 1 – ***HBV infection: liver biopsy shows a mild steatosis with fibrosis and expansion of the portal zone, which contains a lymphoid aggregate of T-lymphocytes marked with CD3. CD3 (DAKO) 1:50 dilution and CD20 (DAKO) 1:100 dilution immunostaining (ob. ×40).***

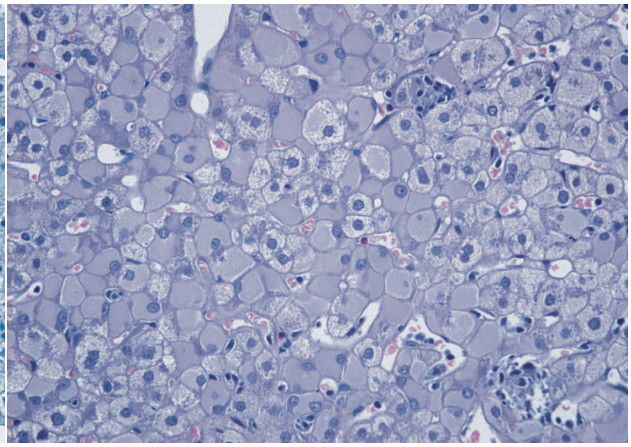

Figure 2 – ***HCV hepatitis: liver biopsy shows the presence of severe macro and micro vesicular steatosis and advanced fibrosis; inflammatory infiltrate in the portal zone (HE stain, ob. ×40).***

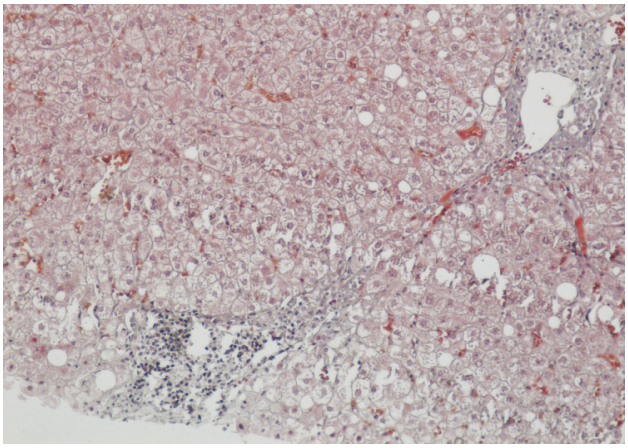

Figure 3 – ***HCV infection: liver biopsy shows minimal interface hepatitis and mild portal inflammation, minimal portal and periportal fibrosis, mild steatosis, enlarged sinusoids (GS stain, ob. ×20).***

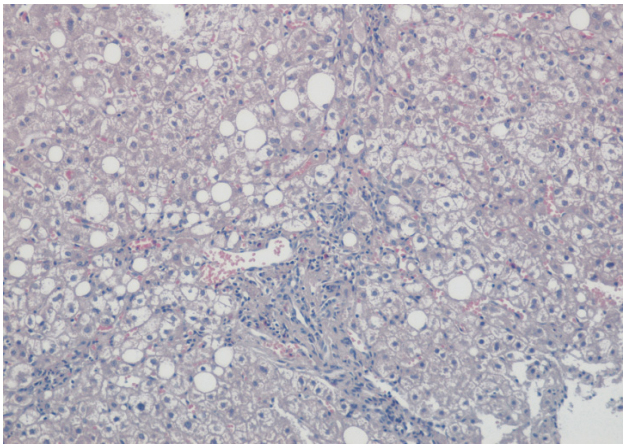

Figure 4 – ***HCV hepatitis: liver biopsy shows moderate portal and periportal fibrosis, moderate inflammation, minimal interface hepatitis and moderate steatosis (HE stain, ob. ×20).***

In 18 cases, we found severe inflammation, periportal or intralobular focal necrosis and bridging necrosis. Bile ducts presented irregularities of the epithelial wall, vacuolation and lymphocytic infiltrate, bridging fibrosis and severe steatosis (Figure 5).

In HBV cases, we found ground-glass cells. These cells are typically scattered throughout the parenchyma in this stage of infection. Their name derives from the finely granular appearance of the central part of the cytoplasm, which is rich in endoplasmic reticulum and hepatitis B surface material. Other organelles often appear to be separated from the ground-glass area by a pale hallo (Figure 6).

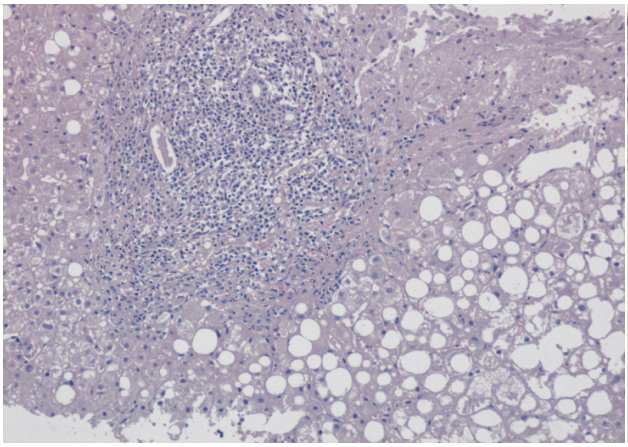

Figure 5 – ***HCV hepatitis: liver biopsy shows prominent interface hepatitis, severe steatosis, bridging fibrosis, severe inflammation, injury of the interlobar bile ducts (vacuolization and lymphocytic infiltration) (HE stain, ob. ×20).***

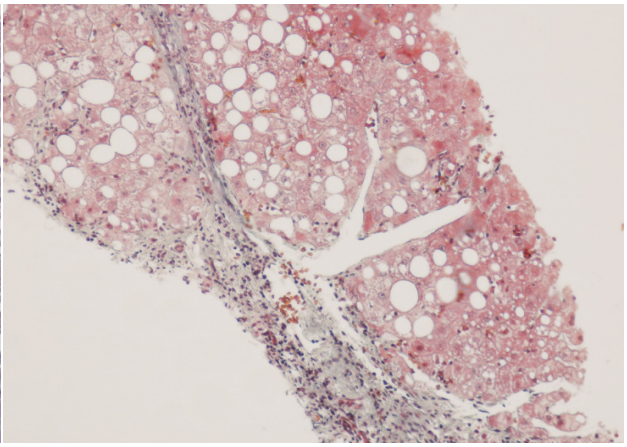

Figure 6 – ***HBV infection: liver biopsy shows enlarged cells with finely granular cytoplasm ground glass hepatocytes (GS stain, ob. ×20).***

In HCV-infected patients, steatosis was associated with a high grade of necroinflammation in 10 cases and with advanced fibrosis in eight cases. In the chronic hepatitis C group without steatosis the association with advanced fibrosis and severe inflammation was lower. In the HBV group there was no notable difference regarding the association with severe inflammation or advanced fibrosis, between those with and without steatosis.

We found the average age, cholesterol, triglyceride, HDL-C, VLDL-C levels in the group with steatosis to be significantly higher than those in the group without steatosis ($p<0.05$). The differences in the average AST, ALT and viral etiology showed no statistical significance.

Mean serum cholesterol level was lower in HCV-infected patients compared to HBV-infected patients (172.97±36 mg/dL *vs.* 185.32±28 mg/dL, $p<0.00001$). For all other parameters, excepting LDL-C, the values were lower in HCV-infected patients than in HBV-infected patients (Table 2).

Ultrasound reports of hepatic steatosis were particularly associated with histological inflammation, as well as fibrosis. All HBV patients as well as 16 out of the 18 HCV-positive patients having a high grade of necroinflammation activity and advanced fibrosis showed heaptic steatosis in the ultrasound exam. However, the sensitivity and specificity of steatosis on ultrasound was poor when compared to steatosis on biopsy. For example, 12% (8/66) of patients with normal ultrasound had more than 25% steatosis on biopsy.

Table 2 – *Lipid parameters in patients with chronic viral hepatitis*

Lipid parameters	Chronic hepatitis C	Chronic hepatitis B	*p*
Cholesterol [mg/dL]	172.97	185.32	<0.00001
Cholesteryl esters [mg/dL]	117.99	122.45	
HDL-C [mg/dL]	40.49	46.07	
LDL-C [mg/dL]	107.94	106	
VLDL-C [mg/dL]	24.29	27.53	
Triglycerides [mg/dL]	127.82	137.83	

Discussion

Our aim in this study was to evaluate the presence of steatosis in chronic hepatitis B and C, to determine its frequency in both hepatitis type, and to explore possible relationships between presence of steatosis, lipid metabolism disorders and viral etiology.

Some studies reported that steatosis in chronic hepatitis B is independently associated with body mass index and fasting glucose level, risk factors for metabolic steatohepatitis, and is not correlated with the degree of fibrosis [1, 8, 9].

Lipoproteins are closely connected to the process of hepatitis C virus infection and the association of chronic hepatitis B virus infection and decreased levels of high-density lipoprotein cholesterol has been documented [10–12].

In our study, steatosis was significantly more frequent in chronic hepatitis C than in chronic hepatitis B. The presence of steatosis on liver biopsy in patients with hepatitis C is more frequent when compared to other chronic liver diseases such as chronic hepatitis B and autoimmune hepatitis [13–16]. Steatosis is also 2.5 times more prevalent in patients with HCV when compared to the general population [5, 15].

Steatosis in chronic hepatitis C has been postulated to be associated with the effects of the viruses rather than an immunological response [17–19]. The reported prevalence of steatosis in patients with chronic hepatitis C varies between 40% and 80%, depending on the features of the studied population in terms of alcohol consumption, prevalence of obesity, diabetes and other risk factors of fatty liver. If all known factors of fatty liver are excluded, the prevalence of steatosis in chronic hepatitis C is still about 40%. This evidence alone suggests that HCV may directly cause steatosis, at least in some patients [20].

In chronic hepatitis B, steatosis seems to be a result of metabolic causes attributed to the host rather than the effect of the virus. In chronic liver diseases other than chronic hepatitis C, no study showing the relationship between steatosis and the disease is available. In these diseases, steatosis has been thought to be a different independent pathological entity. A study reports that chronic hepatitis B occurred concurrently with steatosis in 27% of the studied group [21]. In another study published by the same authors, patients with hepatitis C and B were compared in terms of risk factors, and steatosis was found to occur more frequently in chronic hepatitis C [22].

Unfortunately, our study was conducted on a small patient group, hence it is difficult to conclude from our results that HCV infection is related with steatosis and HBV infection is not related. In our study, genotyping of the virus could not be conducted due to the combined economic constraints, inadequate laboratory facilities and the fact that this procedure was not yet performed routinely.

In our study, severe inflammation and advanced fibrosis were more frequent in HCV-infected patients with steatosis than in patients without steatosis, so it seems that steatosis could be a promoting factor for liver damage in chronic hepatitis C. Furthermore, it has also been shown that steatosis negatively influences the rate of response to antiviral treatment, as confirmed by large clinical trials [20].

Because the number of HBV-infected patients with steatosis included in this study was very small we could not established any relationship between steatosis and histological damage in chronic hepatitis B. The effect of steatosis on histological damage in chronic hepatitis B is not very clear. Steatosis in chronic hepatitis B is mainly associated with the presence of host-related metabolic factors, such as high body mass index and diabetes and does not seem to be associated with the severity of liver histological lesions [23, 24].

Portal and lobular CD3-positive T-cell inflammatory infiltrate, accompanied by CD20-positive B-cells were found in the portal tract. This represents a common feature of hepatosteatosis. Fat vacuoles in the perivenular hepatocytes represent an indicator for severe steatosis. Cell nuclei are displaced to the edges of the cells; swollen hepatocytes containing numerous small vacuoles are found near an efferent venule. These findings are similar to results published by Rubbia-Brandt L *et al.*, who studied the particularities liver steatosis has in chronic genotype 3 HCV [25].

The two cases that presented with mild forms of chronic hepatitis showed portal changes. Lymphocytic infiltrate did not extend beyond the margins of the portal tract, the parenchymal changes being identified by the irregularity of the limiting plate of surrounding hepatocytes. As shown by other studies, a decrease in the severity of HCV infections (expressed by serum values and viral loads) also results in lower grades of inflammation [26].

Irregularities of the epithelial wall of intrahepatic bile ducts accompanied by vacuolation and lymphocytic infiltrate, bridging fibrosis and severe steatosis were found in severe steatosis. This correlates with the specific HCV-genotype involved, as well as with metabolic factors, such as obesity [27].

HCV-infection is associated with a lower level of lipidic parameters, excepting LDL-C, when compared with HBV-infected patients. Different serum lipid values between the two groups could be related with differences in the mechanisms of liver damage in hepatitis C in comparison to hepatitis B. Further studies are necessary in order to define the pathophysiology of the relationship between lipid metabolism and HCV or HBV-infection [28].

The detection of steatosis on ultrasound in a clinical setting appears to be generally associated with steatosis

and biopsy, but also with hepatic inflammation and fibrosis. The ability of ultrasonography to accurately detect hepatic steatosis is questionable outside of a controlled research setting, as both the sensitivity and specificity of this imaging technique were very low in some studies [29].

Increased echogenicity consists the main ultrasonographic finding that identifies hepatic steatosis. A loss of definition of the hemi-diaphragm and decreased detail of the intra-hepatic architecture (particularly the portal veins) may be supportive findings [30].

Steatosis was reported during screening ultrasonography in some patients with chronic viral hepatitis. In our study, steatosis on ultrasound is not consistently correlated with steatosis on biopsy. We observed a substantial number of false-negative results: we found more than 25% steatosis on the liver biopsy in 12% (8/66) of the patients with normal ultrasound. Lack of ultrasonographic evidence of steatosis does not definitively exclude the presence of steatosis as shown on biopsy. Routine hepatic ultrasonography does not provide an accurate non-invasive diagnostic tool for steatosis in chronic viral hepatitis. Liver biopsy remains the optimal diagnostic procedure for determining steatosis in patients with chronic viral hepatitis [26].

Conclusions

Hepatic steatosis was significantly more frequent in chronic hepatitis C than in chronic hepatitis B.

Severe inflammation and advanced fibrosis were more frequently found in HCV-infected patients with steatosis than in patients without steatosis. It seems that steatosis is a promoting factor for liver damage in chronic hepatitis C. The effect of steatosis on histological damage in chronic hepatitis B is not very clear. In our study, no relationship was established between liver damage and steatosis in HBV-infected patients.

Serum lipid levels were lower in HCV-infected patients than in HBV-infected patients. That suggested the existence of some different mechanisms of liver damage in hepatitis C in comparison to hepatitis B.

Ultrasonography is a weak diagnostic method for steatosis. Steatosis found on ultrasound does not consistently correlate with steatosis on biopsy. Clinicians should interpret a report of steatosis on hepatic ultrasonography with caution. Liver biopsy remains the optimal diagnostic procedure for determination of steatosis.

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