Liver steatosis associated with chronic hepatitis C

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Abstract
Hepatic steatosis is a common histological finding in chronic liver diseases. One of the pathological entities in which hepatic steatosis has been found is chronic hepatitis C. The prevalence of steatosis in these patients ranges from 40% to 86%, with an average of 55%, which is two times higher than the steatosis seen in adults uninfected with hepatitis C. Many studies have shown that hepatic steatosis is a medical condition that may progress to steatohepatitis, progressive hepatic failure, hepatic cirrhosis, and is a risk factor for development of hepatocellular carcinoma. We have proposed to evaluate the severity of steatosis in patients with chronic hepatitis C and to correlate it with necroinflammatory processes and fibrosis. We included 259 patients diagnosed with chronic hepatitis C and proven histological steatosis. Age of patients with hepatic steatosis varied from 20 to 69 years. Most cases of steatosis associated with chronic hepatitis C (47.87%) were diagnosed in the age group 50–59 years. Of 259 patients, 141 (54.44%) were female and 118 (45.56%) male. Slight steatosis was identified in 130 cases (50.19%), moderate fatty changes were present in 54 (20.85%) patients and severe steatosis in 75 (28.96%) patients. The appearance of steatosis was macrovesicular and predominantly affected the third zone of the hepatic lobe. Most cases of hepatic steatosis were associated with necroinflammatory activity and low and moderate fibrosis. Cases of marked steatosis associated with intense activity accounted for about 10%, while marked steatosis was associated with severe fibrosis in less than 5% of cases.

Keywords: hepatic steatosis, hepatitis C virus, hepatic fibrosis, liver inflammation, chronic liver disease.

Introduction
Steatosis is defined as the accumulation of fat in hepatocytes. Physiologically, hepatocytes contain small amounts of fat necessary for their metabolism; when liver fat exceeds 5–10% of its weight, we can suspect a diagnosis of steatosis [1, 2].

Hepatic steatosis is a very common condition, its prevalence ranging from 17% in China to 30% in the United States [3–5]. Today it is considered that hepatic steatosis has become a global health problem due to the increasing trend of incidence and its prevalence [6]. The number of patients with steatosis increased due to dietary habits, changes in the content of food and lifestyle with reduced exercise and increased sedentary lifestyle, etiopathogenic factors that affect people from childhood.

Steatosis is more common in Western countries where the incidence may reach 20–30% [7]. It is closely associated with visceral obesity, dyslipidemia, alcohol consumption, type 2 diabetes, sedentary lifestyle, infection with hepatitis viruses and insulin resistance [8–10].

Accumulation of fat in the cytoplasm of hepatocytes is a complex pathological process in which many factors are involved in the altering of fat metabolism [11].

Regarding the relationship between steatosis and chronic hepatitis, numerous authors have shown that hepatic steatosis is a common histological finding in patients with chronic hepatitis C. These lesions were identified in 30 to 70% of patients diagnosed with chronic hepatitis C [12–14]. In our study, we sought to determine the relationship between hepatic steatosis in chronic hepatitis C to liver fibrosis and inflammatory process.

Patients and Methods
We included a sample of 259 patients diagnosed with chronic hepatitis C, hospitalized between 2006 and 2011 in the Clinic of Internal Diseases (Hepatology) of the Emergency County Hospital of Craiova, Romania, for clinical evaluation, biological and histopathological diagnosis in the advent of treatment with interferon and ribavirin. All patients received the same protocol of biologic evaluation, biologic and histopathological diagnosis in the advent of treatment with interferon and ribavirin. All patients received the same protocol of clinical and histopathological investigation. All patients had liver biopsies under ultrasound guidance. We fixed all the tissue in 10% neutral formalin solution and processed it further by histological techniques of classical paraffin inclusion. Histological sections were stained with Hematoxylin–Eosin and trichromic Goldner–Szekely for highlighting lesions steatosis, inflammatory infiltrate and fibrogenesis process.

Results
In our study, we analyzed 259 patients aged 20–70 years who were clinically and histologically diagnosed with chronic hepatitis C and liver steatosis. Analysis of the distribution of fatty changes depending on the age showed that the recorded values increased from 20–29...
to 50–59 years. If in the age group 20–29 years, we only registered 17 (6.56%) patients, in the age group 50–59 years we registered 124 subjects, representing 47.87% of all cases. With the exception of 60–69 years, the number of cases with hepatitis-associated chronic steatosis doubled for each decade.

Regarding the sex of patients, 141 of 259 (54.44%) patients were female and 118 (45.56%) male. Except the decade 20–29 years, when 14 male patients and three females only were diagnosed with chronic hepatitis and steatosis, females predominated in the remaining decades (Figure 1).

Assessing the association between steatosis and inflammatory activity, we found that most patients (114, representing 44%) had mild steatosis and mild and moderate inflammatory activity (Figure 2). Mild inflammatory activity characterized by the presence of a reduced lymphoplasmocytary infiltrated in one or more porto-biliary spaces was diagnosed in 97 cases, accounting for 37.45% of all cases. Moderate inflammatory activity characterized by the presence of an inflammatory infiltrate rich in round mononuclear cells in most areas was detected in 101 patients, representing 39%. An intense inflammatory activity (Figure 3), characterized by the presence of inflammatory infiltrate in porto-biliary spaces with isolated presence of lymph follicles was diagnosed in 61 patients, accounting for 23.55% (Figure 4).

Distribution of inflammatory cells varied from case to case, but all cases were characterized by the presence of mononuclear cells in the portal areas, and intra-lobularly at times. The inflammatory infiltrate was composed mainly of lymphocytes and plasma cells, but also macrophages and rare granulocytes. Lymphocytic infiltrate of the Kiernan space presented in severe cases of lymphoid aggregates, forming lymphoid follicles with germinative centers.

Light steatosis, characterized by the presence of lipid vacuoles in less than 33% of the hepatocytes, was observed in 130 (50.19%) cases. Moderate steatosis (Figure 5) characterized by the presence of lipid vacuoles in less than 33% of the hepatocytes was present in 54 (20.85%) patients. Severe steatosis (Figure 6), characterized by the presence of lipid in more than 66% of the hepatocytes was present in 75 (28.96%) patients. The appearance of steatosis was of macrovesicular type, but there have been cases where macrovesicular steatosis was associated with microvesicular steatosis. We have not identified any case of pure microvesicular steatosis. Hepatic steatosis occurred mainly at the periphery of the hepatic lobe and rarely around the central vein.

Another parameter was the relationship of steatosis with fibrosis. Liver fibrosis is the result of abnormal growth of the synthesis of collagen and other extracellular matrix constituents, particularly in the Kiernan area, chronic secondary damage of hepatocytes.

When we studied cases, hepatic fibrosis was quantified F1 to F4 and has been correlated with the presence of various degrees of steatosis.

Mild fibrosis (F1) characterized by the presence of fibrous expansion in porto-biliary spaces was present in 60 patients. It was correlated with mild steatosis in 33 cases, moderate steatosis in 19 cases and severe in eight cases.

Septal fibrosis (F2) characterized by the presence of fine septa of collagen fibers that started in perivascular spaces and penetrated the vein and hepatic lobule (Figure 7) was present in 91 patients. It was correlated with mild steatosis in 53 cases, moderate steatosis in 23 and severe in 15 cases.

Grade 3 fibrosis (F3), characterized by the formation of thick fibrous septa, arranged in porto-central septae, centrolobular veins joining either portal adjacent spaces or other spaces adjacent to the central vein, was found in 80 cases and was accompanied by light steatosis in 32 cases, moderate in 23 and severe in 27 cases (Figure 8).

We found severe fibrosis (F4), characterized by forming of cirrhotic nodules and pseudo nodules in 28 cases (Figure 9). It was associated with mild steatosis in eight patients, moderate in nine patients and severe in 11 patients (Figure 10).
Liver steatosis associated with chronic hepatitis C

Figure 3 – Mild steatosis associated with abundant inflammatory infiltrate. HE staining, ×200.

Figure 4 – Correlation between necro-inflammatory activity and the type of steatosis in cases with associated chronic viral hepatitis C.

Figure 5 – Image of moderate steatosis, both macro- and micro-vesicular. HE staining, ×200.

Figure 6 – Severe steatosis, predominantly of macro-vesicular type. HE staining, ×200.

Figure 7 – Microscopic image of chronic hepatitis associated with moderate micro- and macro-vacuolar steatosis and porto-portal and intralobular fibrosis. Trichromic Goldner–Szekely staining, ×200.

Figure 8 – Microscopic image of moderate steatosis associated with porto-portal and porto-central fibrosis and abundant inflammatory infiltrate. Trichromic Goldner–Szekely staining, ×200.
Discussion

The occurrence of fatty liver is a complex, multifactorial, process, which is not completely known. In developing this disease, diet is an essential element, many researchers showing that steatosis results from an imbalance between the accumulation and metabolism of triglycerides [15]. The amount of triglycerides within the liver is primarily dependent on the amount of fat ingested. Some experimental studies on laboratory animals have shown that 20% of ingested fats are for the liver, where they are metabolized to obtain energy or other metabolic synthesis or, alternatively, can accumulate as triglyceride vacuoles. The diet rich in carbohydrates increases hepatic lipids, carbohydrates promote the synthesis of fatty acids from acetyl-coenzyme A [15].

Hepatic steatosis is a common finding in many chronic liver diseases. Steatosis has long been regarded as an unimportant pathological lipid accumulation. Studies in recent decades have shown that hepatic steatosis is a medical condition that may progress to steatohepatitis, progressive liver failure and even liver cirrhosis [7]. More recently, steatosis was identified as a risk factor for development and progression of liver fibrosis and even extensive risk factor for development of hepatocellular carcinoma [16–18].

Hepatic steatosis has been noted in chronic hepatitis C. According to some authors fatty prevalence in patients with chronic hepatitis C range from 40% to 86%, with an average of 55% [19–22]. If the prevalence of steatosis in the adult population in Western countries is 30–20% in patients with chronic hepatitis C prevalence of steatosis reaches about 55% [23].

In our study, we showed that steatosis can be seen from 20 years to the elderly in patients with chronic hepatitis C. Other researchers [5] noted that the incidence of steatosis greatly increased with age. Basically, the number of patients doubles every decade. The presence of steatosis in young people who are unlikely to consume alcohol and in which obesity and dyslipidemia are rare, indicate that hepatitis C might be directly involved in developing this liver damage.

Some authors [24] showed that in people infected with genotype 3 hepatitis virus, achieving a sustained virological response after treatment has led to the disappearance of steatosis, which demonstrates the ability for steatogenesis of genotype 3 hepatitis C [25]. Other studies have shown that the core protein of HCV genotype 3 has the ability to inhibit the secretion of very low-density lipoproteins in the liver and, consequently, to induce steatosis [26–28].

Data on the steatogenic capacity of HCV genotype 1 are more scarce and inconsistent. While some authors have shown that in patients infected with C virus genotype 1 virus eradication has no effect on fatty [24], others have shown that in an animal model hepatitis virus core protein overexpression interferes with the secretory activity of hepatocytes and in particular the secretion of very low density lipoproteins. This contributes to the occurrence of hepatic steatosis [29]. We believe that steatosis appearing in most of the patients with chronic hepatitis C is a multifactorial process, in which the emergence of viral infection contributes to high fat diet, chronic alcohol consumption, dyslipidemia, obesity, chronic consumption of medication, diabetes, etc. Some patients may have had infection hepatitis C prior to steatosis. Therefore, we and other authors [19, 30] believe that it is difficult to determine a precise relationship between steatosis and infection with hepatitis C.

Regarding sex distribution, our study showed that steatosis associated with chronic hepatitis C was more frequent in females (54.44%). AlQaraawi et al. [31], analyzing a sample of 116 patients with steatosis and chronic hepatitis C found a prevalence similar to us, in that 56.9% of patients were female. Other authors [32] analyzing a sample of 286 patients found a higher prevalence of steatosis in men (57.2%). We believe that these data were influenced by the combination of several potentially steatogenic factors.

It is difficult to assess the impact of steatosis on necroinflammatory activity and the process of liver fibrosis in patients with chronic hepatitis C, since these processes are multifactorial. However, several clinical and experimental studies have shown that hepatic steatosis accelerates the development and progression of
fibrosis in chronic hepatitis C [21, 33]. We have shown that most patients had mild to moderate steatosis, which were associated with necroinflammatory activity and moderate fibrosis. A relatively small percentage of patients (10.42%) had severe steatosis and intense activity; as small (4.25%) percentage was that of patients with severe steatosis and marked fibrosis.

In chronic hepatitis, fibrosis is a consequence of chronic inflammation [34]. There are a number of factors that stimulate the synthesis of extracellular matrix in the inflammatory processes, mainly collagen fibers being involved. Cells involved in the fibrillogenesis found in chronic hepatitis are represented by the fibroblasts of the Kien space [35] and the liver stellate cells that can migrate into the areas of liver injury, proliferate, and synthesize connective matrix in the sinusoidal capillaries [36]. Hepatic stellate cells (Ito cells), considered to be the major cells responsible for the production of collagen, gain myofibroblasts properties in response to signals received from the neighboring cells [37], from apoptotic fragments generated by adjacent hepatocytes or oxygen free radicals (ROS) [38, 39]. Other important stimuli that occur in hepatic steatosis and induce the synthesis of extra-cellular matrix conjunctiva are the resulting products of lipid peroxidation [40].

We believe that both the inflammatory process and the collagen fibrillogenesis from hepatic steatosis associated with chronic hepatitis C are only partially deciphered. Their deeper knowledge will lead to new therapeutic options.

**Conclusions**

Hepatic steatosis in patients with chronic hepatitis C was of macrovesicular type and mainly affected zone 3 of the hepatic lobe. It occurs more frequently in females and had a high prevalence over 55 years. Most cases of hepatic steatosis were associated with necroinflammatory activity and low and moderate fibrosis. Cases of marked steatosis associated with intense activity accounted for about 10%, while marked steatosis was associated with severe fibrosis in less than 5% of cases.

**Contribution Note**

All authors have contributed equally in preparing this manuscript and thus share first authorship.

**References**


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