Relationship between Serum Iron Indices and Hepatic Iron Quantitation in Patients with Fatty Liver Disease

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Abstract

Fatty liver disease can range from fatty liver alone (steatosis) to fatty liver associated with inflammation (steatohepatitis).

***Purpose**: The study was undertaken to determine the relationships between the histological finding and biochemical measures of hepatic injury with hepatic iron content in patients with chronic liver diseases and steatosis.*

***Methods**: The study of 115 patients with chronic liver diseases and 60 controls, which were examined for frequency and correlation between elevated liver enzymes, ultrasound diagnosed steatosis, liver biopsy, features of metabolic syndrome and indices of iron metabolism. Steatosis was graded by ultrasound as absent or present. Histology was available in 93 patients.*

***Results:** Statistical relationships were found between the histological grading of iron and hepatic iron concentration, whereas histology activity index was related to hepatic grading of iron, but not to hepatic iron concentration. The value of serum iron, serum ferritin and transferrin saturation were significantly higher in patients with chronic liver diseases compared with controls.*

***Conclusion:** Serum measures of iron, total iron-binding capacity and ferritin can be used to monitor hepatic iron content more economically and simply than with hepatic iron concentration measurement on liver biopsy.*

Keywords: Steatosis, Chronic liver diseases, Iron, Histology activity index

Introduction

Fatty liver disease is generally classified into two main clinical categories, each with a different etiology - namely, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD).

Both lead to pathological triglyceride accumulation within hepatocytes (steatosis), and progressive necroinflammatory liver disease (steatohepatitis). ALD refers to the entire spectrum of liver disease due to alcohol abuse (1), while NAFLD is a complex metabolic liver disease that is mainly related to the consequences of insulin resistance and obesity. NAFLD (2) is diagnosed when, after meticulous clinical and laboratory investigations, it is confirmed that patients are not consuming significant amounts of alcohol (typically greater than 10g/day for women and greater than 20g/day for men) (3). ALD and NAFLD share a similar histological spectrum, which starts with 'simple' steatosis and may be accompanied by inflammation. Steatosis is present in 90% of ALD patients and in 100% of patients with NAFLD (4, 5). Alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH) are the progressive forms of ALD and NAFLD, respectively, that evolve into cirrhosis. Alcohol abuse is thought to be responsible for up to 45% of hepatocellular carcinoma and, although most cases arise within a context of alcoholic cirrhosis (6, 7, 8), hepatocellular carcinoma can be seen in non-cirrhotic ALD. It may also arise in NAFLD-related cirrhosis and cryptogenic cirrhosis, although not as frequently as in ALD- or viral-hepatitis-related cirrhosis. Recently, hepatocellular carcinoma development has been reported in non-cirrhotic patients with either NAFLD/NASH or the metabolic syndrome (9, 10, 11)

Alcoholic liver disease (ALD) is commonly associated with iron overload. The two possible mechanisms for iron overload are: taking iron in hepatocytes in specific way through increased levels of transferrin receptor (TfR) 1 and increased intestinal iron absorption from decreasing hepcidin. It is worth examining whether a similar mechanism is present in the development of steatosis and non-alcoholic steatohepatit (NASH) (4). Hepatocytes have several ways of taking iron. Ethanol increases transferrin (Tf)-handed reception by receptor-dependent manner, but regulates down the non-TF associated iron intake.

With immunohistochemical studies was found that TfR1 was increased in hepatocytes in 80% of AFLD liver tissues but not found in normal liver tissues. In patients with AFLD, intestinal iron absorption was increased by oral intake of iron. Chronic infection with the hepatitis C virus (HCV) affects over 170 million individuals worldwide (12). 20% of patients develop cirrhosis after 20 years with possible complications of hepatocellular carcinoma and portal hypertension (13). The major factors known to be associated with fibrosis progression in HCV are older age at infection, male gender and excessive alcohol consumption (14) increased iron stores and hepatic iron content have been suggested to be important in fibrosis progression, however the studies have been inconclusive so far (15) .

Hepatitis C virus-infected chimpanzees fed dietary iron supplements were more prone to iron overload with resulting increased liver enzymes and liver damage than HCV-uninfected animals on the same diet. In humans, serum markers of iron stores (serum iron, transferrin saturation, or ferritin) are elevated more frequently in HCV than other chronic liver diseases (13, 15). Elevated iron stores, and in particular, elevated hepatic iron levels have been associated with a decreased rate of response to interferon treatment in persons with chronic hepatitis C infection. Iron depletion by phlebotomy in patients with HCV reduces serum aminotransferase levels and in combination with interferon, may have improved anti-viral efficacy than interferon alone. However, despite these provocative observations, studies assessing the association between serum iron markers or hepatic iron markers and fibrosis progression still remain controversial. The study was undertaken to determine the relationships between the histological finding and biochemical measures of hepatic injury with hepatic iron content in patients with chronic liver diseases and steatosis.

Material And Methods

The study of 115 patients with chronic liver diseases 74 men and 41 women from 31 to 77 years (average age 47.3 ± 15.8 years), and 60 healthy subjects to controls, 30 men and 30 women from 29 to 83 age (average age 50.5 ± 11.3 years), which were examined for frequency and correlation between elevated liver enzymes, ultrasound diagnosed steatosis, liver biopsy, features of metabolic syndrome and indices of iron metabolism.

Steatosis was graded by ultrasound as absent or present. Histology was available in 93 patients. Biopsies were fixed, paraffin-embedded, and stained with haematoxylin and eosin, reticulin, periodic acid-schiff, Perls', Van Gieson and Victoria Blue.The liver biopsies were scored using the Modified Ishak scoring system with fibrosis stage scored on a scale from 0 to 6. Histological assessment of hepatocyte and macrophage iron stores were graded by two independent histopathologists on a scale of 0 to 4 on Perls' Prussian blue stained liver sections, as previously described.

The results were statistically processed by variation analysis; t-test
for pair differences; Friedman analysis of 2-test, correlation and regression analysis. Differences were considered significant at the P<0.05 level.

Results

The 115 patients (41 women and 74 men) had a mean age of 47.3 ± 15.8 years. The mean value of ferritin was 499 ± 25 ng/mL and that of serum iron was 46.6 ± 6.3 μg/dL.

HCV infection was detected in 53 patients (46.07%), 35 of whom (30.4%) had NAFLD without overt diabetes, 11 (9.5%) had NAFLD, and 11 had NASH at histology. Finally, 24 patients (20.8%) were classified as having AFLD.

Overall, 92 patients (80.2%) had steatosis on US: 46 moderate and 46 severe. The etiological pattern of the patients with steatosis was as follows: 35 (38%) subjects were infected with HCV, 35 (38%) had NAFLD, 11 (12%) were with NAFLD, and 11 (12%) had a diagnosis of NASH at histology.

HCV infection was detected in 53 patients (46.07%). All these were infected by HCV genotype 1; 36 (68%) had steatosis, nine were detected by US and 27 by liver biopsy.

At liver biopsy, performed in 54 patients out of 115 (46.9%), 27 (50%) had chronic hepatitis C and nine (16.6%) had micronodular cryptogenic cirrhosis. Seven patients (12.9%) had NAFLD (macrovesicular steatosis) and 11 (20.3%), NASH (macrovesicular steatosis and lobular inflammation). Seventeen patients (31.5%) had siderosis: eight - grade 1 and nine - grade 2.

Univariate and multivariate analyses were performed to identify predictors of steatosis. By univariate analysis age (P = 0.06), ferritin (P = 0.0006), GGT (P = 0.03) and anti-HCV positivity (P = 0.02) were associated with steatosis (P < 0.10). Increased hepatic iron deposition was determined on the basis of a positive iron stain using Perls' Prussian blue on liver biopsy. This is graded on a scale from 0 to 4. Overall, 39 patients (33.9%) had grade 1 (minimal deposition of iron), five patients (4.3%) had grade 2 positive staining (intermediate deposition). No patients had massive deposition of iron (stage four).

The highest incidence of elevated serum iron found in cases with non-alcoholic fatty liver disease, alcoholic fatty liver disease, mostly in cases with steatosis hepatitis and HHC (Fig. 1). The highest incidence of elevated ferritin found in cases with alcoholic and non-alcoholic fatty disease, mostly in cases with steatosis hepatitis, followed by cases of HHC (Fig. 2). We also found a relationship between the parameters of iron overload and alcohol consumption (p=0,036-0,019), liver enzymes (p=0,001-0,0001), bilirubin (p=0,0001), prothrombine time (p=0,001), platelets count (p=0,0001), and Ig A (p=0,001), as well as hepatic siderosis (p=0,0003-0,0001). Iron overload was more intensive and correlated with steatosis, steatohepatitis and liver fibrosis (p=0,019-0,0001) (Fig. 3, 4).

Discussion

In non-alcoholic fatty liver disease, lipid peroxidation promotes the transition from steatosis to steatohepatit involving multiple cellular adaptations and induces biomarkers of oxidative stress that occurs when it is modified fatty acid metabolism. An increasing number of non-invasive tests for diagnosing NASH have emerged in recent years (4, 5). However, liver biopsy is still considered the gold standard for confirming or excluding the diagnosis of NASH in a patient with chronically elevated liver enzymes, imaging-detected steatosis and other relevant clinical features that are mainly related to the presence of the metabolic syndrome (4, 9, 11),

In ALD, as treatment decisions are not usually based on histology, liver biopsy is not generally necessary for patient management. However, it is required for confirmation of the diagnosis of steatohepatitis before inclusion into therapeutic protocols and for evaluation of concurrent liver disease. In addition, liver biopsy in ALD can provide prognostic information that is not only related to the severity of the histopathological lesions, but is also - given the presence of histological features such as mixed steatosis, perivenular fibrosis and megamitochondria - associated with progression to advanced liver disease and an increased rate of cirrhosis development (6, 7, 8), In ALD, mildly increased iron stores are common.

Iron deposition (grade 1+ or 2+) is detected in hepatocytes and Kupffer cells with a non-zonal distribution. When higher grades of siderosis (3+, 4+) with a predominantly periportal hepatocellular distribution are observed in non-cirrhotic ALD, the possibility of hereditary hemochromatosis should be excluded. In ALD, stainable hepatic iron is positively correlated with fibrosis (7, 15). In NAFLD, iron deposition - when present - is mild (1+ or 2+), and is usually observed in periportal/periseptal hepatocytes and/or reticuloendothelial cells (sinusoidal lining cells, portal macrophages and endothelium of larger vessels) (4, 16, 17). Studies of the relationship of abnormal iron indices, iron genetics and iron tissue deposition with the development of liver fibrosis and pathogenesis of NAFLD have shown conflicting results (3, 15, 18). Relationship that we found between ferritin and moderate / severe steatosis is also supported by the concept that serum ferritin is a risk factor for fatty liver.

This hypothesis is supported by additional data D'Souza (10), showing that non-alcocholic fatty liver disease is an important determinant of increased levels of serum ferritin. Moreover, they show that the relationship between ferritin and insulin resistance is much more - evident in nonalcoholic group with fatty liver disease. In our study, the iron overload exists in about 1/3 of the patients with chronic liver disease. The prevalence and intensity of this syndrome were more higher in patients with NAFLD, ALD and CHC. The parameters of iron overload correlate with liver enzymes, some liver function test and hepatic siderosis, as well as steatosis, steatohepatitis and fibrosis.

In our study statistical relationships were found between the histological grading of iron and hepatic iron concentration, whereas histology activity index was related to hepatic grading of iron, but not to hepatic iron concentration. The value of serum iron, serum ferritin and transferrin saturation were significantly higher in patients with chronic liver diseases compared with controls. In conclusion: Serum measures of iron, total iron-binding capacity and ferritin can be used to monitor hepatic iron content more economically and simply than with hepatic iron concentration measurement on liver biopsy.

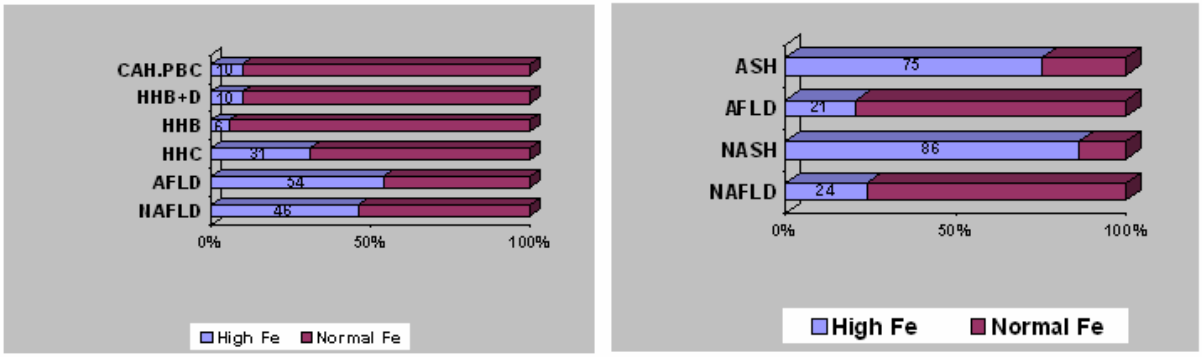


Fig. 1 Percentage of increase in serum iron in different groups chronic liver diseases.

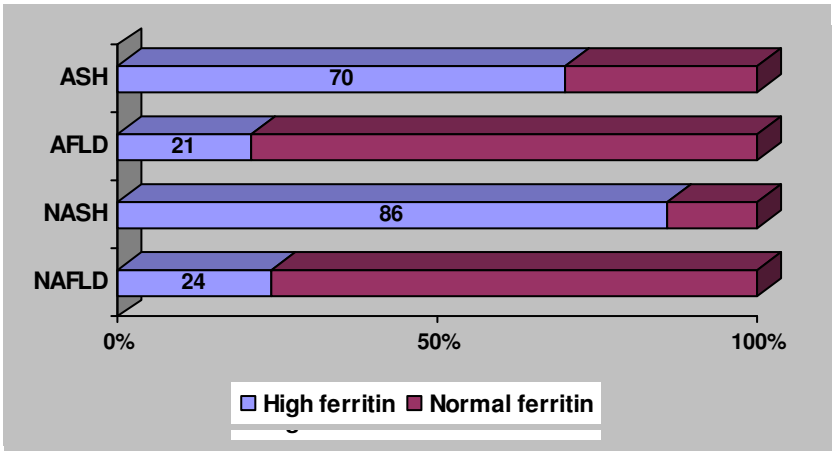


Fig. 2 Percentage of increase in serum ferritin in different groups chronic liver diseases.

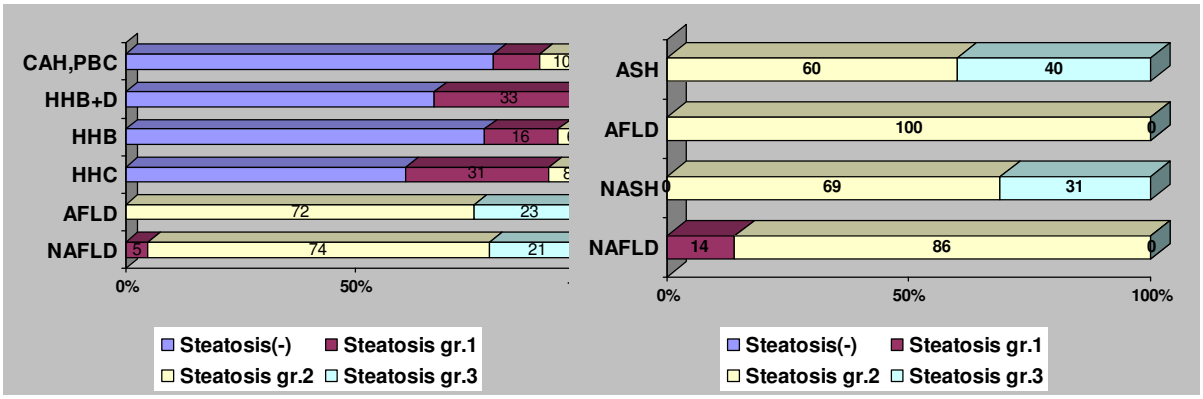
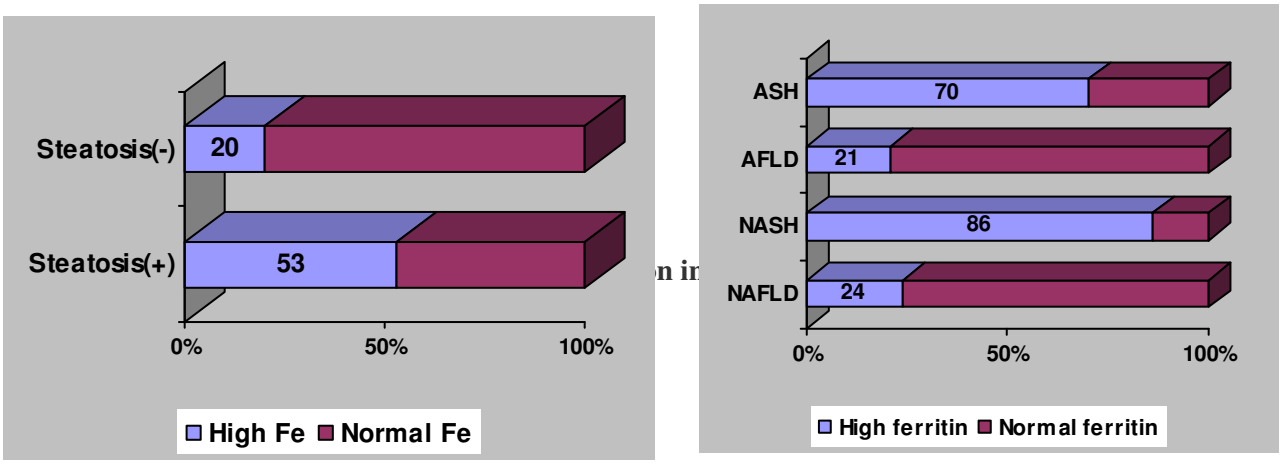


Fig. 3 Histological features of steatosis in different groups chronic liver diseases.



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