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Original Contribution

COMPARATIVE EVALUATION OF IRON OVERLOAD IN PATIENTS WITH CHRONIC LIVER DISEASE

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ABSTRACT

Objective: To assess and compare the presence and significance of iron overload in patients with chronic liver disease.

Methods: The study of 220 patients with chronic liver diseases, 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.

Results: The value of serum iron (22.3 ± 8.3 vs.17.6 ± 3.5), serum ferritin (225.4 ± 213.8 vs. 132.6 ± 65.2) and transferrin saturation (32.4 ± 9.2 vs. 27.0 ± 3.8) were significantly higher in patients with chronic liver diseases compared with controls. Serum iron and ferritin were significantly higher in non-alcoholic fatty disease compared with controls (p=0.039) and chronic hepatitis B (HHV) (p=0.001), alcoholic fatty disease (AFLD) compared with controls (p=0.0001) and chronic hepatitis B (HHB) (p=0.0001), chronic hepatitis C (HHC) compared with controls (p=0.05).

Conclusion: Iron overload was highest in patients with alcoholic etiology, followed by non-alcoholic fatty liver disease and chronic hepatitis C. In chronic hepatitis B, as well as autoimmune diseases, abnormalities were detected in individual patients.

Key words: steatosis, ferritin, iron, transferrin, chronic liver disease.

INTRODUCTION

There are many forms of diseases associated with iron storage, some - hereditary, others - acquired. The most popular forms is hereditary HFE-related hemohromatozis and this is a disturbance, which is a main focus in most studies to date. The iron in the body is regulated by controlled absorption, and past studies have partly clarified how this regulation works.

From the classic studies of McCann and Widdowson (1) 70 years ago has been found that with the exception of small quantities of iron lost from the body, mostly through scaling of the cells there is no regulated iron excretion. Rather than the iron metabolism is a closed circle in which iron retained in the body is used again. Hemohromatozis is characterized by accumulating excess iron in the body. This is a condition in which iron absorption is unregulated and is absorbed more iron than necessary. But how the body "knows" how much iron is absorbed? Over the past 60 years have been made many attempts to explain the regulation of iron absorption by the existence of "mucosal block" (2, 3). Experimental data shows that the existence of "blocking" iron absorption is not crucial mechanism but rather a "mucosal intelligence. In iron deficiency iron absorption increases and in iron overload - iron absorption reduces. Other factors that regulate iron absorption are: anemia, which increases iron absorption through increased erythropoesis; general inflammation, which reduces the iron absorption and hypoxia, which also increases iron absorption.

The calmness with which iron can distribute or receive electrons is very suitable for a

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variety of chemical reactions and probably for this reason is in fact essential for all life forms. But because of its reactivity iron can also cause damage, and so the life forms must be included in mechanisms for redistribution of the remaining amount of iron - enough iron to meet its basic functions, but not enough to cause damage. All fine-tuned mechanisms must work in harmony to maintain this delicate balance. Iron deficiency and inflammation are the most common causes of anemia. Iron overload is much less widespread, but is also clinically important.

Diseases associated with iron storage can be divided into hereditary and acquired forms. Acquired forms of diseases associated with iron storage are generally associated with hyperactivity of the bone marrow, discovery, suggesting that there may be a direct link between erythropoesis and iron absorption. The mechanism of this effect remains unknown, but possible factor associated with the activity of bone marrow that may regulate (adjust) iron absorption.

Iron can accumulate in the liver in various conditions, including congenital and systemic diseases of iron overload (hereditary hemohromatozis), conditions associated with systemic accumulation of iron from macrophages (transfusions, haemolytic conditions, chronic anemia, etc.). And also in some hepatitis (hepatitis C, alcoholic liver disease and non-alcoholic steatosis hepatitis, cutaneous porphyria later) and specific liver accumulation of iron in cirrhosis of uncertain pathogenesis. Physician is faced with the task to establish whether the accumulation of iron in the liver is important to determine the type of disease, leading to its accumulation (i.e diagnosis). The instruments available for solving this task, including histological examination by staining for iron, quantitative analysis of iron, clinical history, laboratory tests of iron (serum iron and serum ferritin).

Deposition of hemosiderin in the liver may occur in many diseases of different genetic hemohromatozis and in most cases the exact mechanism remains unclear. In general, using a careful clinical evaluation and histological examination, these conditions can be distinguished from genetic hemohromatozis,although in doubtful cases it may be necessary to be made biochemical study of tissue for iron in the liver or genotyping.

Hematologic disorder

Transfusions of blood and chronic haemolysis usually leads to postponement of hemosiderin in the liver. Iron, which is brimming with blood is ready to accumulate in the cells of Kupfer, and that is why these cases are easy to distinguish from hepatocellular iron for much of the genetic forms hemohromatoza. Iron, following haemolysis tends to be delayed in hepatocytes and Kupfer cells and in this case the proof of hemolysis by laboratory methods is very effective in distinguishing hemosideroza caused by haemolysis of genetic hemohromatoza. Anemia of chronic disease in the same way may lead to deposition of iron in Kupfer cells.

Hemosideroza related cirrhosis. In cases of cirrhosis with deposition of iron, easily can be established whether it is homozygous inbred hemohromatoza by using traditional means of assessment. In suspicious cases can help genetic research. Etiology of iron deposition remains unclear, but it is known that patients with bile (biliary) cirrhosis are less prone to accumulate iron (70-20%) than other patients with cirrhosis (no bile) (22-67%). In conclusion, to summarize, some cases of cirrhosis with iron deposition may have low penetration of homozygous or heterozygous hereditary hemohromatoza hereditary, but iron deposition may occur in cirrhosis as a secondary phenomena, and may be morphologically hepatocellular located like in hemohromatoza.

Alcohol

Alcoholic liver disease (AFLD) 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 heptsidin. 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. Regulating hormone for iron homeostasis - hepcidin adjust downward in the livers of mice placed under conditions of ethanol abuse. The general mechanism for depositing hepatic iron, fats and Triggering role of iron may be present as a primary mechanism for the development of AFLD and non-alcoholic fatty liver disease (NAFLD).

Chronic viral hepatitis

Chronic viral hepatitis is often accompanied by the presence of hemosiderin in the liver, which usually is poorly expressed and unlikely to be due to hereditary hemohromatoza. Landfills with hemosiderin may be present in Kupfer cells, hepatocytes (with a gradient in zone 1-3) and / or portal areas. Depletion of iron through flebotomiya, reduces levels of serum transaminases, reduces inflammation and may slow the progression of hepatitis C (5, 6, 7). There is no clear evidence whether the amount of stained iron in the liver affects viral elimination by therapy with interferon and ribavirin (8, 9).

Purpose and objectives:

The aim of this study was to assess and compare the presence and significance of iron overload in patients with chronic liver disease.

Tasks:

1. To determine the incidence and features of the syndrome of iron saturation in patients with various chronic liver diseases.
2. To determine the incidence and features of the syndrome of iron saturation in healthy subjects.
3. To undertake comparative characteristics of serum markers of iron metabolism between healthy subjects and chronic liver diseases.
4. To undertake comparative characteristics of serum markers of iron metabolism and iron content in liver tissue in various chronic liver diseases.
5. Seek warning values of individual clinical and laboratory parameters to identify patients at high risk of iron overload in the liver.
6. To assess the dynamics of serum indicators of iron metabolism.
7. To assess the significance of the syndrom of iron overload on the clinical course and evolution of chronic liver disease.

MATERIAL AND METHODS

Tested a total of 220 persons, divided into seven comparable groups including 160 patients with chronic liver disease, 104 men and 56 women from 23 to 77 years (average age 49.9 ± 12.8 years), and 60 healthy subjects to controls, 30 men and 30 women from 29 to 83 age (average age 50.5 ± 11.3 years).

• Group I - 35 patients with primary non-alcoholic fatty disease (NAFLD) - NAS. (n=22) and NASH (n=13). Standard criteria, ultrasound and histology (n = 30) demonstrated.

• Group II - 35 patients with alcoholic liver disease - fatty (AS, n=12) or alcoholic steatosis hepatitis (ASH, n=23). With absolute alcohol intake of 40g or 80g/daily. The diagnosis was confirmed histologically.

• Group III - 35 patients with chronic hepatitis B. Serological and histological (n=30) confirmed diagnosis - METAVIR - F1-F2 (n=24) and F3 (n=6). With proven viral replication.

• Group IV - 35 patients with chronic hepatitis C. Serological and histological (n=30) confirmed diagnosis - METAVIR - F1-F2 (n=27) and F3 (n=3). With proven viral replication.

• Group V - 10 patients with chronic hepatitis B and D. Serological and histological confirmed diagnosis - METAVIR - F1-F2 (n=9) and F3 (n=1). With proven viral replication (HBV).

• Group VI - 10 patients with chronic autoimmune liver disease. Primary biliary cirrhosis (PBC) - 5 women.

• Chronic autoimmune hepatitis (n=5). The diagnosis was confirmed histologically and immunologically.

• Group VII - 60 healthy controls. Average age was 35.39 ± 20.66 years (from 18 to 80 years). Studied for correlation between frequency and elevated liver enzymes, ultrasound diagnosed steatosis, features of metabolic syndrome and indices of iron metabolism.

Methods:

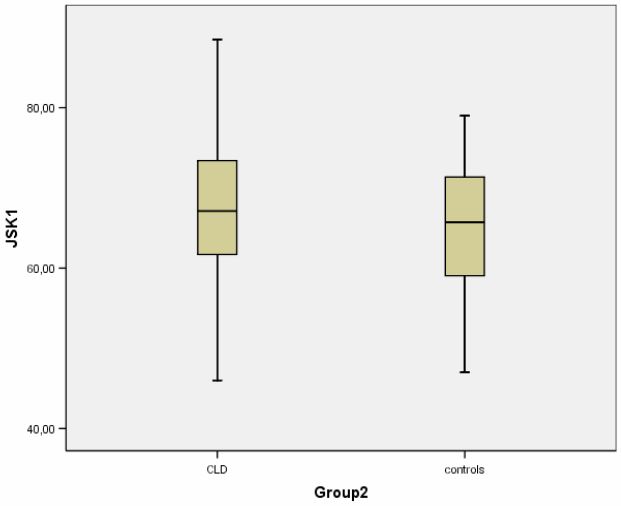
1. History, physical examination and demographic data.
2. Standard and specific disease according to laboratory tests of blood and urine, immunological and virological investigations.

3. Laboratory tests for assessment of iron metabolism. Serum iron (men: 12.5 to 26 mmol /L; women: 10.5 to 23 mmol/L). Total Iron binding capacity (44-66 mmol/L). Transferrin saturation (Fe ÷ Total Iron binding capacity × 100% -20-40%). Serum ferritin (males: 20-280 mg/L Women: 10-140 mg/L).
4. Percutaneous or surgical liver biopsy with histological assessment of activity and stage of disease, staining with Haematoxylin-eozin, van-Gieson and Masson. Iron content in liver tissue - evaluation of Ishak and Perl's Prussian-blue and determining the extent of delay.
5. Fibroesofagogastroduodenoskopy (FGDS) - for the screening of esophageal varices, portal hypertensive gastro-and/or duodenopaty at the end of the observation, or in any other clinical suspicion.
6. Conventional abdomen and pelvis ultrasound Honda Electronics 2000 and Aloka SSD 500 and 3,5 MHz sector transducer. Degree of steatosis.
7. Abdomen CT and MRI.
8. Statistic methods-variation analysis; t-test for pair differences; Friedman analysis of 2-test, correlation and regression analysis.

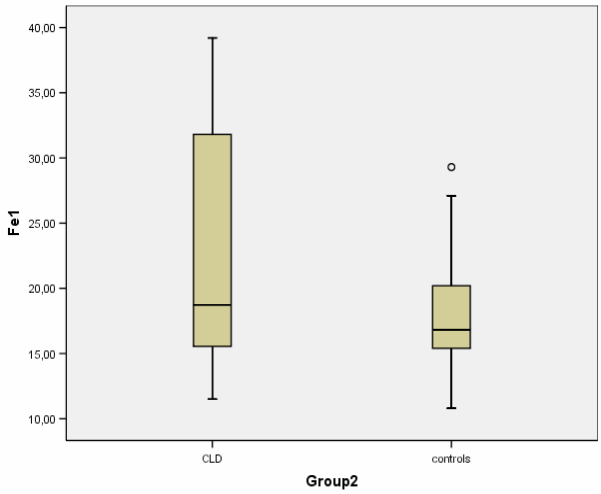
RESULTS

The obtained results are represented below in figures and tables.

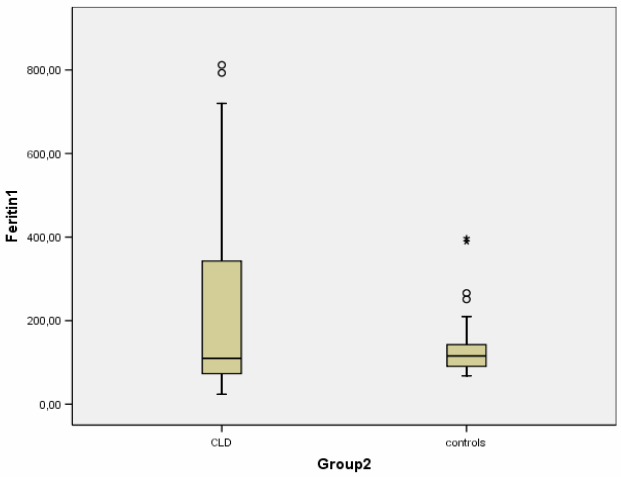
The value of serum iron (22.3 ± 8.3) was significantly higher in patients with chronic liver diseases compared with controls (17.6 ± 3.5). Serum ferritin (225.4 ± 213.8 vs. 132.6 ± 65.2) and transferrin saturation (32.4 ± 9.2 vs. 27.0 ± 3.8) were significantly higher in patients with chronic liver diseases compared with controls **Fig. 1.**



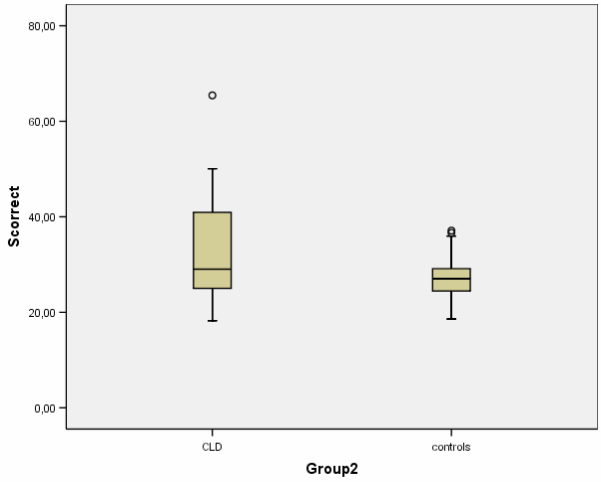
ANOVA: F =17.755, p=0.0001



ANOVA: F =3.115, p=0.079 - n.s



ANOVA: F =10.89, p=0.001



ANOVA: F =19.02 , p=0.0001

Fig. 1 Values of serum iron (mmol/l), Iron binding capacity, ferritin (mg/l), transferrin saturation (%) in healthy individuals and patients with chronic liver diseases.

Values of serum iron were significantly higher in: NAFLD compared with controls (p=0.039) and HHB (p = 0.001); AFLD compared with controls (p =0.0001), HHB (p=0.0001), HHC compared with controls (p = 0.050). Iron binding capacity values were significantly higher in: AFLD compared with controls (p=0.0001), NAFLD (p=0.046), HHC (p = 0.002), HHB (p=0.0001) **Fig. 2**.

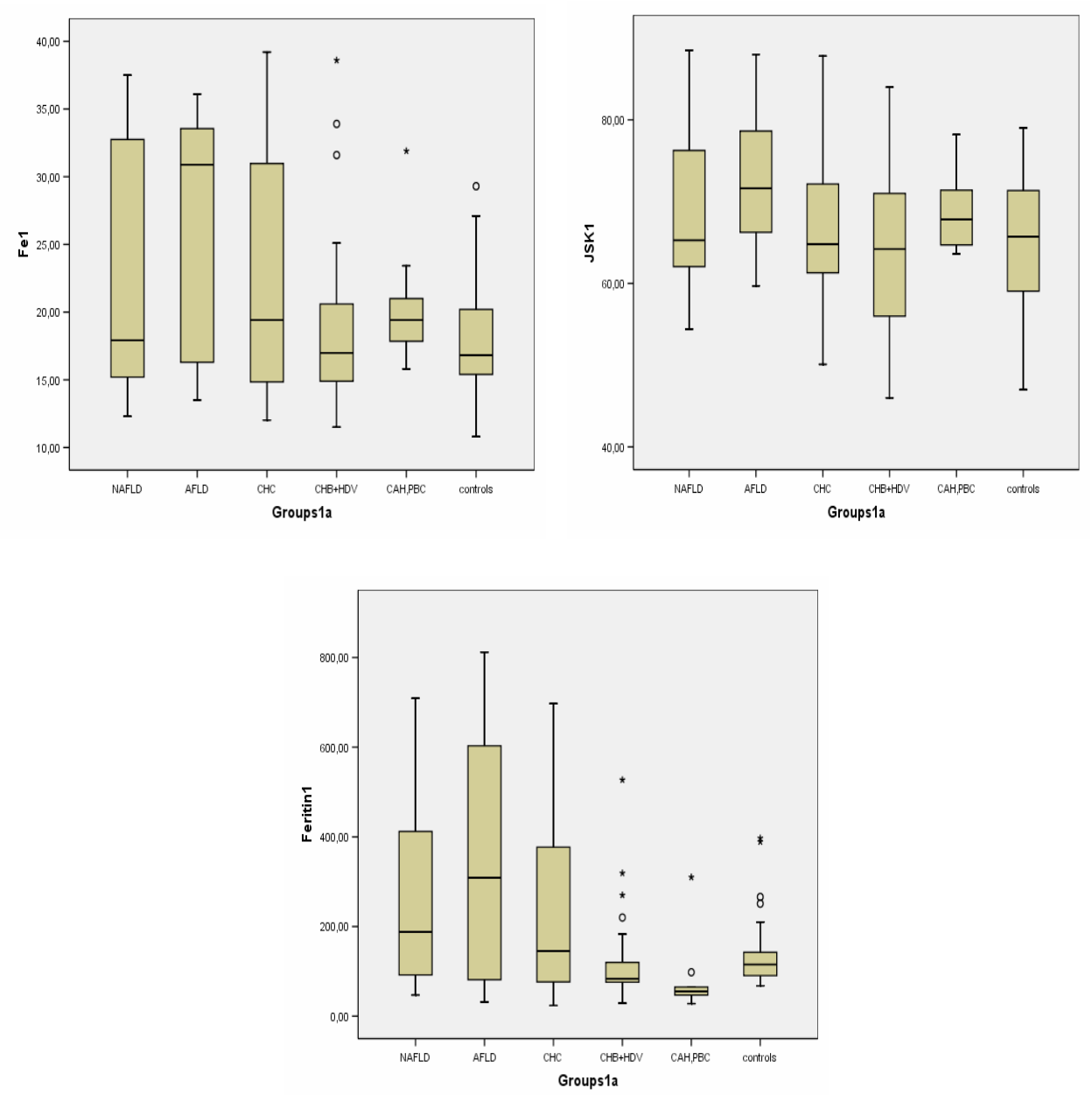


Fig. 2 Values of serum iron (mmol / l), Iron binding capacity, ferritin (mg/l) in different groups of patients with chronic liver diseases.

Ferritin values were significantly higher in: NAFLD compared with controls (p=0.05), HHB (r=0.001) and CAH, PBC (p=0.0001); AFLD compared with controls (p=0.005) HHC (p=0.001), HHB (p=0.001) and CAH, PBC (p=0.0001) compared with HHC HHB (p=0.024) and CAH, PBC (p=0.003) against HHB controls (p=0.001) and CAH, PBC (p=0.007) .

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. 3**.

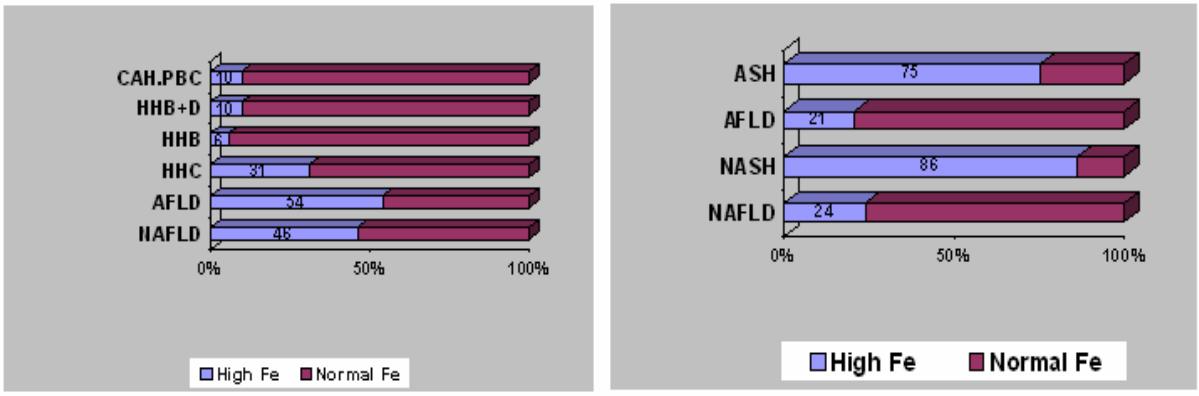


Fig. 3 Percentage of increase in serum iron in different groups chronic liver diseases.

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 .

With increasing alcohol consumption increases the incidence of patients with elevated levels of iron, ferritin and saturation transferrin (p = 0.01-0.001) **Table . 1**.

Table 1. *Indicator values of iron metabolism in different alcohol consumption.*

Chronic liver diseases	***Serum iron***	***Serum Ferritin***	***Saturation transferrin***
	Mean±SD	Mean±SD	Mean±SD
NAFLD - alcohol consumption of 40 grams	17.9±6.9	143.3±133.8	27.5±7.9
Moderate alcohol consumption 40-80 g	17.3±5.4	180,6±127,9	26.6±6.2
AFLD - high alcohol consumption above 80 g	31.4±6,3	486.6±269.5	37.2±8.5

DISCUSSION

Patients with alcoholic liver disease often show iron overload (3). Even mild to moderate alcohol consumption has shown an increase in stored iron (1). Suzuki (2) demonstrated increased levels of transferrin receptor-1 in patients with alcoholic liver disease by imunnohisto-chemical analysis of samples from liver biopsy. Kupfer cells isolated from experimental animal models of alcoholic liver disease also show increased iron content (1). Well-known fact that both iron and alcohol oxidase cause stress and lipid peroxidation (2). So alcohol induced iron overload by increasing production of free radicals and proinflamatory cytokines, tumor necrosis factor alpha (TNF-α). Our study confirms the higher proportion of patients with iron overload in moderate and high alcohol consumption.

Chronic hepatitis C with or without cirrhosis, is often characterized by abnormal iron indices, particularly elevated levels of ferritin, which does not always mean iron overload (5,8,9). Several hypothetical mechanisms explaining altered iron indices. These include excess oxygen free radicals, increased by activation fibrogenic stelat cells and the occurrence of immune response by the host. Among our patients with chronic liver disease caused by HCVgenotip 1b which biopsy was done, only 30% have sideroza. Theoretically, serum ferritin may be elevated as an acute phase reaction associated with the necro-inflamatory process of chronic hepatitis C, but the average increase in ALT and degree of activity typically observed in these patients denied this interpretation, even if our HCV infection. Analysis was independently associated with higher levels feritin. But it is difficult to separate the role of HCV that of steatosis, which is a common finding in HCV infection, even when caused by HCV genotype 1. In our study HCV patients also showed moderate steatosis. Non-alcoholic fatty liver disease is itself strongly associated with metabolic syndrome, which may

explain the strong relationship between HCV infection and diabetes (1,2,3,4). The relationship between insulin resistance and moderate / severe steatosis in chronic hepatitis C is well supported. Indeed, insulin resistance can lead to fatty liver in patients with HCV infektion, which makes them prone to diabetes.
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.

Induction is a hemoksigenaza adaptive response against oxidative damage caused by lipid peroxidation and can be critical in disease progression. 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, 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 non-alcoholic group with fatty liver disease.

CONCLUSION

• Increased rate and the degree of deviation of serum iron, ferritin and transferrin saturation and iron deposition in liver tissue were highest in alcoholic etiology, non-alcoholic fatty liver disease, mostly in cases with steatosis hepatitis and chronic hepatitis C.
• In chronic hepatitis B, alone or in combination with co-infection with hepatitis D, as in autoimmune diseases abnormalities were detected in individual patients.
• Serum indicators of iron metabolism were more pronounced at high alcohol consumption, obesity, more severe liver damage that show relation with liver enzymes, indicators for assessing liver function, metabolic parameters.

- There is a connection between iron deposition in liver tissue and the deviation of serum markers, more frequent and more pronounced changes in the positive reaction of iron in the liver.
- Variation in serum parameters of iron metabolism correlate with the histologic changes in the liver - steatosis and fibrosis.

REFERENCES

1. Ishak K.G., Chronic hepatitis: morphology and nomenclature. *Mod Pathol,.* 7: 690-713, 1994.
2. James O.F. and Day C.P., Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol.*, 29: 495-501, 1998.
3. Siegelman E.S. and Rosen M.A., Imaging of hepatic steatosis. *Semin Liver Dis,* 21: 71-80, 2001.
4. Younossi Z.M., Gramlich T, Bacon B.R, Matteoni C.A, Boparai N, O'Neill R. and McCullough A. J., Hepatic iron and nonalcoholic fatty liver disease. *Hepatology*., 30: 847-850, 1999.
5. Adinolfi L.E, Gambardella M, Andreana A, Tripodi M.F, Utili R. and Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology.,* 33: 1358-1364, 2001.
6. Cammà C, Bruno S, Di Marco V, Di Bona D, Rumi M, Vinci M, Rebucci C, Cividini A, Pizzolanti G, Minola E, et al., Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis C. *Hepatology.*, 43: 64-71, 2006.
7. Hwang S.J, Luo J.C, Chu C.W, Lai C.R, Lu C.L, Tsay S.H, Wu J.C, Chang F.Y. and Lee S.D. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. *J Gastroenterol Hepatol.*, 16: 190-195, 2001.
8. D'Souza R.F, Feakins R, Mears L, Sabin C.A. and Foster G.R., Relationship between serum ferritin, hepatic iron staining, diabetes mellitus and fibrosis progression in patients with chronic hepatitis C. Aliment Pharmacol Ther21: 519-524, 2005.
9.Lonardo A, Adinolfi L.E, Loria P, Carulli N, Ruggiero G and Day C.P., Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. *Gastroenterology.*, 126: 586-597, 2004.