ELEVATED HEMOGLOBIN INFLUENCES THE RISK OF NONALCOHOLIC FATTY LIVER DISEASE

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Summary

Nonalcoholic fatty liver disease (NAFLD) has become increasingly recognized as a public health problem around the word, which has become endemic in the industrialized countries. The etiology of NAFLD remains unknown, but specialists have established a strong association with age, gender, body mass index (BMI), body iron status and individual components of the metabolic syndrome (MS) such as type 2 diabetes, hypertension and hyperlipidemia. There is evidence supporting the view that hemoglobin may serve as a marker for injuries and diseases associated with glycemia, oxidative stress, hypertension, insulin resistance, obesity, and diabetes. Body iron levels play a critical role in NAFLD and the MS. Elevated hemoglobin levels may cause increased blood viscosity and decreased blood flow to the liver leading to hypoxia-related liver damages. The multivariate analysis showed that hemoglobin level is a good predictor for individuals with suspected NAFLD with or without the MS. Inflammation could lead to anisocytosis via release of immature red blood cells into the peripheral circulation. Increased red blood cell distribution width (RDW) in patients with hepatosteatosis compared to healthy controls is not surprising, because hepatosteatosis is associated with a subclinical inflammation. Body iron levels are important risk factor for NAFLD and incorporation of body iron levels as a predictor may help to prevent liver damages in patients with NAFLD. The dysmetabolic iron overload syndrome (DIOS) is detected in about one third of patients with NAFLD and the MS. All of these settings suggest that hemoglobin test should be considered as a part of clinical evaluation for patients with NAFLD.

Keywords: *nonalcoholic fatty liver disease, iron overload, hemoglobin level, dysmetabolic iron overload syndrome*

Резюме

Влияние высокого уровня гемоглобина на риск развития безалкогольной жировой дистрофии печени

Безалкогольная жировая дистрофия печени является признанной проблемой мирового здравоохранения и особенно актуальна для промышленно развитых стран. Этиология этого заболевания остается неизвестной, но была установлена прочная ассоциация с возрастом, полом, индексом массы тела, уровнем железа в организме и отдельными компонентами метаболического синдрома, такими как сахарный диабет 2 типа, гипертония и гиперлипидемия. Есть доказательства, поддерживающие представление, что гемоглобин может служить маркером повреждения и патологий, связанных с гипергликемией, оксидативным стрессом, гипертонией, резистентностью к инсулину, ожирением и диабетом. Уровень железа в организме играет решающую роль в патогенезе безалкогольной жировой дистрофии печени и метаболического синдрома. Высокий уровень гемоглобина может вызывать увеличение вязкости крови, уменьшая тем самым кровоток, приводящий к связанным с гипоксией повреждениям печени. Мультивариантный анализ показал, что уровень гемоглобина – хороший предиктор безалкогольной жировой дистрофии. Воспаление может провоцировать анизоцитоз, вызванный появлением незрелых эритроцитов в периферической крови. Увеличение распределения эритроцитов по объему у пациентов со стеатогепатитом не удивительно, потому что при этой патологии имеется воспаление. Уровень железа в организме – важный фактор риска для безалкогольной жировой дистрофии печени и оценка состояния метаболизма железа, как предиктора, может помочь предотвратить повреждение печени у пациентов с этой патологией. Синдром дисметаболического избытка железа обнаружен приблизительно у одной трети пациентов безалкогольной жировой дистрофии печени и метаболическим синдромом. Все эти данные позволяют использовать гемоглобин как параметр клинической оценки пациентов с безалкогольной жировой дистрофией печени.

Ключевые слова: *безалкогольная жировая дистрофия печени, избыток железа, гемоглобин, ширина распределения эритроцитов по объему, синдром дисметаболического избытка железа*

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become increasingly recognized as a public health problem around the word, which become endemic in the industrialized countries. Several factors common to the Western lifestyle, such as sedentary behavior, diets rich in saturated fats, and central obesity are shared risk factors for NAFLD.

NAFLD is defined as fat accumulation in the liver exceeding 5% to 10% by weight in the absence of alcohol abuse (less than 140 ml of alcohol per week). NAFLD symptoms range from simple steatosis to nonalcoholic steatohepatitis (NASH), and to advanced fibrosis and cirrhosis. The etiology of NAFLD remains unknown, but specialist have established a strong association with age, gender, body mass index (BMI), body iron status and individual components of the metabolic syndrome (MS) such as type 2 diabetes, hypertension and hyperlipidemia.

Iron is an essential element for the growth and well-being of almost all living organisms. It is an access to a wide range of redox potentials and can participate in many electron transfer reactions, spanning the standard redox potential range. It is also involved in O2 transport, activation, and detoxification. Trace metals play an important catalytic role with lipids in the formation of reactive oxygen species and oxidative stress. Iron, in particular, is a key component in catalyzing the production of reactive radicals and creating lipid peroxidation and oxidative stress.

There is the evidence supporting the view that hemoglobin may serve as a marker for injuries and diseases associated with glycemia, oxidative stress, hypertension, insulin resistance, obesity, and diabetes.

The liver is a major site for the storage of iron and the metabolism of lipids and is therefore an important site for interaction between these two metabolic pathways.

The present study explored and summarized the evidence and relationships between hemoglobin level and risk of NAFLD.

Effect of iron on lipids metabolism: iron overload and lipid peroxidation

Iron (Fe) is an essential element for all living cells. Most of iron within the body is found in hemoglobin within erythrocytes, contributing to about 50% of the body iron. Heme iron is recycled by macrophages following degradation of senescent red blood cells.

However, Fe tendency to catalyze free-radical formation makes free iron potentially cytotoxic. Iron is a potent catalyst of oxidative stress via the Fenton reaction and can directly cause lipid peroxidation generating malonyldialdehyde, which is capable to activate hepatic stellate cells (HSCs), a major player of fibrogenesis in NAFLD. Iron can also directly induce fibrogenesis, as HSCs can be activated by the generation of reactive oxygen species (ROS) with ascorbate/FeSO4. Reactive oxygen species (ROS) cause peroxidation of polyunsaturated fatty acids and nucleic acids.

Furthermore, the amount of iron in cells is far above the solubility, and it is necessary to con-

centrate the excessive iron and to keep it soluble. Storage of excessive cytoplasmic iron in soluble and nontoxic form is performed in many cell types by ferritin. Another potential source of cellular iron is extracellular ferritin, whose uptake by early erythroid cells is regulated and whose iron can be used for heme synthesis.

The amount of serum ferritin normally reflects the amount of iron stored in the body in healthy individuals, which is about 20-30% of the body iron. However, ferritin is also an acute-phase reactant and elevated serum ferritin levels have been associated with the severity of liver damage in NAFLD subjects. Therefore, under conditions of chronic illness, ferritin levels do not reflect the amount of iron stored in the body. Unlike serum ferritin, hemoglobin level is less affected by the presence of acute inflammation.

Free radicals and oxidants are continuously generated within mammalian cells but are normally neutralized by the body's antioxidant metabolism. Oxidative stress can damage lipids, proteins, and DNA. Lipid peroxidation is a common product of iron-induced oxidation and may be expected to occur at the highest rate when both cofactors are readily available. *Mainous A. et al.* support the hypothesis that iron-mediated oxidation of cholesterol increases oxidative stress [1].

Choi J.W. et al. suggested that iron is directly involved in the lipid metabolism, and low density lipoprotein (LDL) oxidation process may require iron. Girls with severe iron deficiency anemia had lower total serum cholesterol and triglycerides (TG) concentrations, and that these reduced serum lipid levels returned to normal levels following iron supplementation [2]. Recently, it has been shown that LDL is oxidized by iron within the lysosomes of macrophages.

Fe overload in mammals has been often associated with injury, fibrosis, and cirrhosis in the liver followed by cardiac disease, endocrine abnormalities, arthropathy, osteoporosis and skin pigmentation.

Thus, alterations in Fe metabolism should be carefully analyzed before evaluating cellular responses to either damaging agents or xenobiotics of biomedical or ecological impact since Fe is a double-faced element that can be either good or bad to the cell, depending on whether it serves as a micronutrient or as a catalyst of free radical reactions.

Effect of lipids on heme synthesis and iron metabolism

The liver is an important organ for iron and lipid metabolism. Deregulation of fat metabolism in the fatty liver is associated with overproduction of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides. LDL oxidation plays a pivotal role in the development of atherosclerosis.

Phospholipids were divided into three groups in dependence of effect on heme synthesis. The first is the choline-containing group (lecithin and sphingomyelin) with inhibitory or slightly accelerating action on heme synthesis. The second group is the acid phospholipids namely phosphatidylethanolamine, cardiolipin, phosphatidic acid and phosphatidylenositol, were strong activators and the intensity of activation was in the order of the acidity of the phospholipids. The third group contains the lysophospholipids, namely lysolecithin, lysophosphatidylethanolamine and sphingosylphosphorylcholine, activated the heme sintesis most effectively.

An activation mechanism of phospholipids was proposed in which the hydrophilic anionic part of lipid in the lipoprotein enzyme molecule attract ferrous iron. After being removed from solvation water, the ferrous iron is transferred to the hydrophobic part of the enzyme molecule to be inserted into porphyrin. *Yoneyama et al.* [3] reported that more acidic phospholipids including phosphatidylethanolamine, are strong activators of the mitochondria enzyme protohemeferrolyase which transfers ferrous iron to protophorphyrin forming heme.

In addition, the synthesis of heme may be enhanced by the presence of the acidic phosphonoethanolamine. The resistance of this compound to some types of phospholipase hydrolysis, the presence of the unique fatty acid and the general physical properties of the phosphonoethanolamine contribute to the membrane properties of the cellular and subcellular membranes. Alterations in membrane permeability may also contribute to glyconeogenic activity of the cells. *Shug et al.* (4) suggested that the increased heme synthesis might be related to increased glyconeogenesis.

Green and Fleischer et al. [5] show the relationship of phospholipids participation on the mitochondria respiratory chain (phospholipid plays an important role in electron transport, oxidative phosphorylation and the energy-linked transport of ions across mitochondria membranes). *Bertoli et al.* [6] reported that a pattern exists between respiratory activity and cellular phospholipid content. This correlation may be due to the role of phospholipids in synthesis of heme. In 1971, *Peng YM and Elson E* observed increased synthesis of phospholipids in *Tetrahymena pyriformis* grown in medium supplemented with iron [7].

Iron in fatty liver and in the metabolic syndrome

Body iron levels play a critical role in NAFLD and the MS. Serum hemoglobin α and β subunits have been identified as biomarkers for biopsy-proven NAFLD in adults. A proteomic study has shown that

free hemoglobin (Hb) α and β subunits in serum were significantly increased from normal controls to steatosis and to NASH, suggesting free Hb subunits in serum could be a biomarker for liver lesions.

Hemoglobin level as a predictor closely associated with NAFLD

Yilmaz et al. show that hemoglobin is the only predictor closely associated with NASH and fibrosis for the NAFLD patients without the MS. Elevated hemoglobin levels may cause increased blood viscosity and decreased blood flow to the liver leading to hypoxia-related liver damages [8]. *Yu et al.* followed-up 6,944 initially NAFLD-free Chinese subjects for 3 years and found hemoglobin to be a strong predictor for NAFLD [9]. A 5-year follow up study on 5,562 lean Chinese adults who were initially free of NAFLD found that hemoglobin and platelet counts were significantly associated with the development of NAFLD [10]. *Bai et al.* reported that distribution of hemoglobin levels by decade of age showed strong correlation with the prevalence of suspected NAFLD using gender-specific cut-points. The multivariate analysis showed that hemoglobin level is a good predictor for individuals with suspected NAFLD with or without the MS. In conclusion, adults with high hemoglobin levels of 14.4 μg/dl for males and 13.2 μg/dl for females are at the greatest risk for developing abnormal liver function. Hemoglobin test should be considered as a part of clinical evaluation for patients with NAFLD [11].

The major finding of *Takemi Akahane* study is that a high Hb level is an independent predictor of NAFLD in Japanese women. The authors found that Hb level was higher in subjects with NAFLD than in those without. The multivariate analysis showed that Hb level correlated with both ferritin level and insulin resistance assessed by HOMA, indicating a high probability that Hb level is associated with iron stores and insulin resistance [12].

In another population study, a higher free serum Hb has been associated with a higher prevalence rates of NAFLD. The source of free Hb in serum was not identified, but probably it results from oxidative stress-induced hemolysis. On the other hand, *Liu et al.* [13] reported that Hb was expressed in hepatocytes and it was increased in NASH. They suggested that elevated oxidative stress in NASH could induce Hb expression and suppression of oxidative stress by Hb could be a mechanism to protect hepatocytes from oxidative damage.

Red cell distribution width in hepatosteatosis

Red blood cell distribution width (RDW), an automated measure of the variation of red blood cell sizes (i.e. anisocytosis), is routinely performed as a part of a standard complete blood count.

Several studies have reported that RDW was associated with adverse prognosis in patients with various conditions, including diagnosed cardiovascular diseases, cancer, chronic lower respiratory diseases, stroke and celiac disease [14-17].

Kim et al. have attempted to present the association of RDW with the degree of fibrosis in NAFLD [18]. In their retrospective cross-sectional analysis, study subjects with NAFLD were selected based on abdominal ultrasonography and history of alcohol intake from a large cohort of individuals who presented for a routine health check-up. The degree of fibrosis was determined according to the noninvasive fibrosis scoring systems including the BARD score and the FIB-4 score, and the relationship between RDW and the degree of fibrosis was determined. Their results revealed a stepwise-increase in RDW with increasing level of liver fibrosis. They concluded that higher RDW was associated with advanced fibrosis in NAFLD patients. The main limitation of this study was absent of liver biopsy. Biopsy-based prospective longitudinal studies should be aimed at validating the diagnostic performance of RDW.

Some authors suggest that RDW should be an inflammatory marker in certain conditions [19, 20]. RDW has been found to be associated with disease activity in inflammatory bowel disease, another inflammatory disease. Chronic inflammation and oxidative stress leads to an elevation in RDW. Proinflammatory cytokines have been shown to inhibit erythropoietin-induced erythrocyte maturation. Thus, inflammation could lead to anisocytosis via release of immature red blood cells into the peripheral circulation. Oxidative stress increases the fragility of red blood cells, decreases the rate of erythroid maturation and erythrocyte lifespan.

Gülali Aktaş et al. suggest that increased RDW in patients with hepatosteatosis compared to healthy controls is not surprising, because hepatosteatosis is associated with a subclinic inflammation [21]. This study are indicating increased RDW in patients with hepatosteatosis compared to controls even both groups had similar Hb levels. Likewise, the association with chronic inflammation and oxidative stress are believed to be proximate mediating mechanisms of more advanced fibrosis in NAFLD.

"Metabolic" hyperferritinemia associated with NAFLD

Patients with NAFLD often have a high serum ferritin level. Zelber-Sagi *et al.* [22] demonstrated that NAFLD is a major determinant of increased serum ferritin level and the association between serum

ferritin and insulin levels is much more evident in patients with NAFLD than in those without. Many studies have suggested that serum ferritin level is a marker for insulin resistance [23-25]. Furthermore, increased serum ferritin levels have been reported as an independent predictor of liver damage (severe hepatic fibrosis/ NASH) in patients with biopsy-proven NAFLD. A specific receptor for ferritin has been demonstrated on activated HSCs, and it has been proposed that ferritin acts as a cytokine with pro-inflammatory activity regulating fibrogenesis via nuclear factor kappa B (NFjB) -regulated signaling in HSCs. Probably, co-existing liver injury or nutritional/ genetic factors, and in particular the coexistence of steatosis, may compromise the ability to mount an effective antioxidant defense, and thus predispose to fibrogenesis.

Serum level of Fe and Iron overload

Body iron levels are important risk factor for NAFLD and incorporation of body iron levels as a predictor may help to prevent liver damages in patients with NAFLD.

Mild hepatic iron overload is frequently observed in NASH and advanced fibrosis and cirrhosis. However, the role of hepatic iron in the progression of NASH remains controversial. While some studies found that 20% to 62% of individuals with fatty liver disease had evidence of iron overload, other studies failed to show such a relationship. There are two factors, with associated mechanisms, to explain the increased hepatic iron overload: (1) transferrin receptor 1 (TfR1), which takes up iron bound to transferrin; and (2) hepcidin, which controls intestinal iron absorption and regulates the cellular iron export protein ferroportin-1 (Fpn1).

The frequent association between hepatic steatosis and body iron overload is known as insulin resistance-associated hepatic iron overload. Insulin stimulates ferritin synthesis and facilitates iron uptake, and conversely, iron influences insulin signaling, reduces hepatic extraction and metabolism of insulin, which leads to peripheral hyperinsulinemia, and may increase cellular oxidative stress, which inhibits the internalization and actions of insulin.

Hepatic genes involved in iron metabolism

Mitsuyoshi et al. studied hepatic genes involved in iron metabolism in patients with NAFLD and reported hepatic iron score increased as the stage progressed. The stage progression is associated with increased TfR1 genes and decreased hepcidin gene expression [26]. Decreased hepatic Fpn1 levels and increased serum hepcidin levels were seen in patients with biopsy proven NAFLD.

The dysmetabolic iron overload syndrome

The dysmetabolic iron overload syndrome (DIOS) is detected in about one third of patients with NAFLD and the MS. DIOS patients have mild hepatic iron excess with a predominantly mixed sinusoidal pattern, with macrophage iron retention and an iron recycling defect, which influence the natural history of liver disease by inducing oxidative stress in hepatocytes and activation of hepatic stellate cells. *Nemeth E. et al.* have been reported that in DIOS iron absorption is decreased and hepcidin (the hormone that decreased intestinal iron absorption and recycling from macrophages [27]) is increased compared to healthy controls. Excessive body iron plays a causal role in insulin resistance through mechanisms that probably reduce theability to burn carbohydrates. DIOS may facilitate the progression of cardiovascular disease by contributing to the recruitment and activation of macrophages within arterial lesions.

Definition of DIOS has been based on the presence of two or more MS components, steatosis, normal transferrin saturation, and mild hepatic iron overload, with typical involvement of the sinusoidal compartment. The pathogenesis is related to altered regulation of iron transport associated with steatosis, insulin resistance, and subclinical inflammation, often in the presence of predisposing genetic factors.

In conclusion, the growing prevalence of obesity and metabolic syndrome, diabetes mellitus and dyslipidemia in the society provokes the rising prevalence of NAFLD. Iron overload secondary associated with NAFLD may be one of the pieces of the complicated puzzle that will help understand the pathogenesis and treatment of the disease.

The recent description of new condition associated with liver iron overload, that seem to be related with NAFLD, open new perspectives on the possible link between iron excess and atherosclerotic and cardiovascular disorders and raised questions about the mechanism of parenchymal iron loading in the presence of normal transferrin saturation.

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