Iron Deficiency in Patients With Nonalcoholic Fatty Liver Disease Is Associated With Obesity, Female Gender, and Low Serum Hepcidin

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BACKGROUND & AIMS: **Iron deficiency is often observed in obese individuals. The iron regulatory hormone hepcidin is regulated by iron and cytokines interleukin (IL) 6 and IL1β. We examine the relationship between obesity, circulating levels of hepcidin, and IL6 and IL1β, and other risk factors in patients with nonalcoholic fatty liver disease (NAFLD) with iron deficiency.**

METHODS: **We collected data on 675 adult subjects (>18 years old) enrolled in the Nonalcoholic Steatohepatitis Clinical Research Network. Subjects with transferrin saturation <20% were categorized as iron deficient, whereas those with transferrin saturation ≥20% were classified as iron normal. We assessed clinical, demographic, anthropometric, laboratory, dietary, and histologic data from patients, and serum levels of hepcidin and cytokines IL6 and IL1β. Univariate and multivariate analysis were used to identify risk factors for iron deficiency.**

RESULTS: **One-third of patients (231 of 675; 34%) were iron deficient. Obesity, diabetes, and metabolic syndrome were more common in subjects with iron deficiency ($P < .01$), compared with those that were iron normal. Serum levels of hepcidin were significantly lower in subjects with iron deficiency (61 ± 45 vs 81 ± 51 ng/mL; $P < .0001$). Iron deficiency was significantly associated with female gender, obesity, increased body mass index and waist circumference, presence of diabetes, lower alcohol consumption, black or American Indian/Alaska Native race ($P \leq .018$), and increased levels of IL6 and IL1β (6.6 vs 4.8 for iron normal, $P \leq .0001$; and 0.45 vs 0.32 for iron normal, $P \leq .005$).**

CONCLUSIONS: **Iron deficiency is prevalent in patients with NAFLD and associated with female gender, increased body mass index, and nonwhite race. Serum levels of hepcidin were lower in iron-deficient subjects, reflecting an appropriate physiologic response to decreased circulating levels of iron, rather than a primary cause of iron deficiency in the setting of obesity and NAFLD.**

Keywords: NASH CRN; BMI; NAFLD; Nutrition; Ferroportin; Inflammation.

Obesity and iron deficiency (ID) are considered the two most common nutritional disorders worldwide.[1] The association between obesity and iron status was first described by Wenzel and coworkers[2] in 1962, who noted that obese adolescents had lower serum iron compared with nonobese adolescents. A diet rich in carbohydrates and fats and poor in nutrients, such as iron, combined with a greater iron requirement in obese individuals may play a role.[3] However, Menzie and coworkers[4] evaluated the role of dietary factors in obese ID individuals and did not find an association between iron intake and ID. More recently, research has focused on the role of systemic, obesity-related, low-grade inflammation leading to ID by increased hepcidin expression.[5–7]

In response to increased iron stores, hepcidin, the iron regulatory hormone, binds and internalizes the cellular iron export protein ferroportin, thus downregulating iron efflux from the enterocyte, macrophage, and hepatocyte.[8] Conversely, decreased or deficient iron stores downregulate hepcidin to restore iron balance to

Abbreviations used in this paper: AI/AN, American Indian/Alaska Native; ALT, alanine transaminase; BMI, body mass index; DM, diabetes mellitus; ID, iron deficiency; IL, interleukin; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NASH CRN, NASH Clinical Research Network; TS, transferrin saturation.

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appropriate physiologic levels. Hepcidin expression is also increased in chronic inflammation, by inflammatory cytokines interleukin (IL) 6 and IL1β by signal transducers and activators of transcription 3.[9] Hepcidin is predominantly expressed in the liver, but also in subcutaneous and visceral adipose tissue, albeit at such a low level it may not contribute to systemic hepcidin levels.[6,10] Thus, the impact of obesity-induced hepcidin upregulation and the relationship between liver versus adipose-derived hepcidin and iron regulation in the setting of obesity is not well understood.

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the United States, with an estimated prevalence of 30% among US adults, and is associated with obesity, type 2 diabetes mellitus (DM), and metabolic syndrome (MS).[11] Up to a third of all patients with NAFLD progress to the more severe form called nonalcoholic steatohepatitis (NASH), characterized by hepatocellular ballooning, inflammation, and variable fibrosis.[12] We have previously shown, using a cutoff of serum ferritin 1.5 times the upper limit of normal, that there is an inverse relationship between body mass index (BMI) and serum iron studies in patients with NAFLD (ie, subjects with low transferrin saturation [TS] and low serum ferritin had significantly higher BMI).[13] We have also previously shown that subjects with hepatic iron staining had higher levels of serum hepcidin.[14] The goal of this study was to examine the relationship between circulating hepcidin level, obesity-induced systemic inflammation, and ID and to identify the prevalence of and associated risk factors for ID in patients with NAFLD.

Subjects and Methods

Subjects

A total of 675 adult (age >18 years) subjects enrolled in NASH Clinical Research Network (CRN) studies between October 2004 and February 2008, with biopsy-proved NAFLD (defined as >5% steatosis) and serum iron studies within 6 months of the liver biopsy were studied. The NASH CRN Database and PIVENS Trial inclusion/exclusion criteria have been reported elsewhere.[15,16] Demographic information including age, gender, ethnicity, and race and a detailed medical history including comorbidities, such as history of DM, hypertension, and hyperlipidemia, and menstrual history in women were obtained from patient interviews during screening. Dietary consumption of iron, vitamin C, tea and coffee, and supplemental vitamin C were determined from the Block 98 food frequency questionnaire (NutritionQuest, Berkeley, CA); alcohol consumption was determined from the Alcohol Use Disorders Identification Test Consumption questionnaires.[17] A complete physical examination including measurement of weight, height, and waist and hip circumference was obtained. Subjects with a BMI of $\geq$30 were defined as obese. ID was defined as TS (serum iron/total iron binding capacity) <20%, indicative of ID.[18,19] We also investigated the presence of ID anemia in our cohort using the criteria of serum ferritin <30, TS <20, and hemoglobin <12 in females and <13 in males; only 15 subjects met this criteria and therefore we did not analyze this subset separately. The prevalence of MS in this cohort was defined using the World Health Organization criteria. All subjects gave written informed consent and the study was approved by the institutional review board at each local site of the NASH CRN.

Serologic Data

Clinical laboratory data including hematologic, hepatic, and metabolic, lipid, and serum iron assessments were analyzed for subjects with values collected within 6 months of the liver biopsy. Serum hepcidin levels, available in 558 subjects, were determined by enzyme-linked immunosorbent assay (Intrinsic LifeSciences, San Diego, CA).[20] The lower limit of detection in this assay is 5 ng/mL. Eight subject values were below this limit and a value of 5 ng was imputed for the analysis. Plasma IL6 and IL1β levels, available in 371 and 242 subjects, respectively, were determined using Luminex technology and the human cytokine LINCOplex kit (Catalog number HCYTO-60K; Millipore, St. Charles, MO). The lower limit of detection for the assays was 0.79 and 0.19 pg/mL, respectively. Two subject IL6 values were below this limit and a value of 0.79 pg/mL was imputed for the analysis. Fifty-two IL1β values were below this limit including 13 ID subjects. A value of 0.19 pg/mL was imputed for the analysis.

HFE Genotyping

We examined the relationship between ID and the presence of mutations in the hemochromatosis gene *HFE*, which influence hepcidin production. Genotyping for the two common *HFE* mutations C282Y (rs1800562) and H63D (rs1799945) was performed using a real-time genotyping assay as previously described.[14] *HFE* genotyping data were available in 500 subjects.

Liver Histology

All patients underwent a liver biopsy, which was stained for hematoxylin-eosin, Masson trichrome, and Perls' iron stain. Histologic features of NAFLD and iron accumulation were assessed by the pathology committee of the NASH CRN in a centralized consensus review format, as previously described.[21] NAFLD activity score (range, 1–8) was tabulated by summing scores for steatosis, lobular inflammation, and ballooning degeneration.