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Hepatic Iron in African Americans Who Underwent Liver Biopsy

James C. Barton, MD, Luigi F. Bertoli, MD, Thomas J. Alford, MD, J. Clayborn Barton, BS and Corwin Q. Edwards, MD

Abstract: *Background:* Primary iron overload in African Americans has been reported predominantly from autopsy studies. *Methods:* We characterized hepatic iron phenotypes in 83 African Americans who underwent liver biopsy during the interval 1990 to 1995. We tabulated pathology report form data, iron grades in hepatocytes (0–4) and Kupffer cells (0–3) and abnormal liver histology. Increased iron was defined as hepatocyte or Kupffer iron grades ≥2, respectively. Heavy iron was defined as hepatocyte iron grade 3 or 4. Primary iron overload was defined as the presence of grade 3 or 4 hepatocellular iron in the absence of evidence of chronic alcohol effect, viral hepatitis, steatosis, unexplained inflammation, chronic erythrocyte transfusion or chronic ingestion of iron supplements. *Results:* There were 37 men and 46 women (mean age: 53 ± 15 [SD] years). We observed heavy ethanol consumption, 12.0%; viral hepatitis, 26.5%; steatosis without heavy ethanol consumption, 43.4%; inflammation, 45.6%; fibrosis, 26.2% and bridging fibrosis/cirrhosis, 29.4%. Logistic regression on bridging fibrosis/cirrhosis revealed positive associations with heavy ethanol consumption (P = 0.0410) and viral hepatitis (P = 0.0044). The 22 patients (26.5%) with increased iron had greater mean age, proportion of men and heavy ethanol consumption. Five patients had heavy iron staining, among whom were 3 women (mean age: 54 years) with primary iron overload. Two of the 3 women had cirrhosis and diabetes mellitus. *Conclusions:* Among 83 adult African Americans who underwent liver biopsy, 3.6% had hepatic iron phenotypes consistent with primary iron overload.

Key Indexing Terms: African Americans; Hemochromatosis; Hepatocyte; Iron overload; Liver. **[Am J Med Sci 2015;349(1):50–55.]**

BACKGROUND

Primary iron overload in African Americans is a heterogeneous group of disorders. DNA analysis as an aid to diagnosis of some primary iron overload disorders became possible after the discovery of the *HFE* gene (chromosome 6p21.3) in 1996.[1] In whites, for example, *HFE* genotypes, especially p.C282Y homozygosity, account for approximately 90% of hemochromatosis phenotypes.[1,2] In contrast, hemochromatosis-associated *HFE* genotypes are uncommon in African Americans with high-iron phenotypes.[3,4] Deleterious mutations in non-*HFE* iron-related genes have been described in few African Americans with primary iron overload unassociated with anemia.[5–7] Population screening to identify primary iron overload in African Americans has been performed using an elevated transferrin saturation or serum ferritin criterion like that used in screening whites.[4,8] The prevalence of possible or confirmed cases was very low[4,8] in part because mean transferrin saturation is lower and mean serum ferritin is higher in African Americans than whites.[4] Thus, the pretreatment diagnosis of primary iron overload in African Americans depends predominantly on demonstration of high-iron phenotypes detectable in the liver.

Grading stainable iron in hepatocytes and Kupffer cells is a basic hepatic iron phenotyping method. There is a significant positive correlation of hepatocyte iron grade with hepatic iron concentration (HIC) measured using atomic absorption spectrometry.[9] The hepatic iron index (HII) is HIC adjusted by age (μmol Fe/g dry weight/y).[10,11] HII ≥1.9 confirms the diagnosis of iron overload and has been used as a diagnostic criterion of primary iron overload in African Americans.[12,13]

Most high-iron phenotypes in African Americans without anemia have been communicated as case reports,[3,14,15] small case series[12,13] or autopsy studies.[13,16–18] We performed a retrospective study of consecutive diagnostic liver biopsy specimens of African American adults in a large suburban medical center in central Alabama during the interval 1990 to 1994, a period during which DNA-based diagnosis of primary iron overload disorders was not available. We identified and characterized hepatic iron phenotypes using hepatocyte and Kupffer cell iron grades. We discuss our results in the context of the overall prevalence of high-iron phenotypes in African Americans and acquired factors that may contribute to their development.

METHODS

Selection of Study Subjects

The performance of this study was approved by the Institutional Review Board of Brookwood Medical Center. We performed a computerized and manual search of the database of the Medical Center's surgical pathology department to identify all pathology report forms of liver specimens obtained by percutaneous or intraoperative biopsy from adults (age ≥18 years) during the 5-year study interval 1990 to 1994. Thereafter, we selected patients whose pathology report forms identified them as either African American or black. One hundred African Americans underwent liver biopsy during the study interval. We retrieved their liver biopsy slides and tabulated the succinct demographic data, clinical history and pathologist's interpretation of the biopsies. Using hospital or clinic charts or blood bank records was not part of the present study.

Performance of Liver Biopsies

Liver specimens were obtained by biopsy as part of routine medical care. No biopsy specimen was obtained as a sequel to population or family screening to detect iron overload

From the Southern Iron Disorders Center (JACB, JClB), Birmingham, Alabama; Department of Medicine (JACB), University of Alabama at Birmingham, Birmingham, Alabama; Department of Medicine (JACB, LFB), Brookwood Medical Center, Birmingham, Alabama; Brookwood Biomedical (LFB), Birmingham, Alabama; Department of Pathology (TJA), Brookwood Medical Center, Birmingham, Alabama; and Department of Medicine (CQE), Intermountain Medical Center and University of Utah, Salt Lake City, Utah.

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Correspondence: James C. Barton, MD, Southern Iron Disorders Center, Suite 626, 2022 Brookwood Medical Center Drive, Birmingham, AL 35209 (E-mail: ironmd@isp.com).

phenotypes. Radiologists or gastroenterologists obtained specimens using percutaneous technique, 18-gauge needles and ultrasound or CT guidance. Surgeons obtained specimens during open cholecystectomy or other intraperitoneal operations.

Selection of Evaluable Liver Biopsy Specimens

We excluded biopsy specimens less than 10 mm long. Thus, the specimen of 1 woman was inevaluable because there was insufficient liver for interpretation. We excluded 15 other cases because the biopsies were performed to confirm the diagnosis of malignancy and not to evaluate parenchymal liver disease. Furthermore, none of these biopsy specimens had sufficient liver parenchyma for analysis. The biopsy specimen of 1 man was excluded because his pathology report indicated that he had transfusion iron overload consequent to treatment for acute leukemia. Altogether, there were 83 specimens evaluable for the present analyses.

Histology Technique

Liver specimens were fixed in 10% neutral buffered formalin. Triplicate sections of paraffin-embedded liver were routinely prepared. One section was stained with hematoxylin and eosin, another with Perls' acid ferrocyanide technique to demonstrate nonheme ferric iron and a third with Masson's trichrome technique to assess collagen fibrosis. In some cases, reticulin stains were also used to assess fibrosis. Other special stains were used in some cases, as appropriate. Appropriate positive and negative control specimens were prepared and reviewed with each staining batch.

Liver Morphology

Interpretation of liver histology reported herein represents consensus opinions of the surgical pathologist and at least 2 of the authors. Steatosis unassociated with excessive ethanol consumption, inflammation and bridging fibrosis/cirrhosis was assessed as described elsewhere.[19] The abnormality was graded as absent or present. The presence or absence of fibrosis, bridging fibrosis or hepatic cirrhosis was determined using Masson's trichrome-stained specimens with or without reticulin stains as described previously.[19] Fibrosis without bridging or cirrhosis was graded as either present or absent. We defined that bridging fibrosis and cirrhosis were equivalent; their presence or absence was analyzed as a dichotomous variable. The presence of abnormalities characteristic of viral hepatitis was based on a combination of information obtained from pathology request forms and histologic features of the biopsy specimens.

Iron Grading

All slides were reviewed by a surgical pathologist and at least 2 of the authors. The iron grades in each case represent consensus opinions. Hepatocellular iron was graded according to these criteria: grade 0—no visible iron; grade 1—iron visible in very few hepatocytes; grade 2—iron visible in 5% to 10% of hepatocytes; grade 3—iron visible in ≥40% of hepatocytes and grade 4—abundant iron visible in most hepatocytes.[12] Kupffer cell iron was graded according to these criteria: grade 0—no visible iron in Kupffer cells; grade 1—iron visible in ≥one-third of Kupffer cells; grade 2—iron visible in one-third to ≤two-thirds of Kupffer cells and grade 3—abundant iron visible in more than two-thirds of Kupffer cells.[12] Hepatocyte or Kupffer cell iron of grade 0 or 1 was defined as normal.

Increased iron was defined as hepatocyte and/or Kupffer cell iron grade ≥2.[12] Heavy iron staining was defined as hepatocyte iron grade 3 or 4, regardless of Kupffer cell iron grade. Primary iron overload was defined as the presence of grade 3 or 4 hepatocellular iron in the absence of evidence of chronic alcohol effect, viral hepatitis, steatosis, unexplained inflammation, chronic erythrocyte transfusion or chronic ingestion of iron supplements.

Hepatic Iron Concentration

The reference range for HIC measured by atomic absorption spectrometry is 200 to 2400 μg Fe/g dry weight (3.6–43.0 μmol Fe/g dry weight). In only 1 patient was the HIC measurement requested by the interpreting pathologist and displayed in the report form. Measuring HIC on the biopsy specimens as an addendum to the data on pathology report forms was beyond the scope of the present study.

Other Conditions

These were tabulated from information on the pathology request forms and included the following: reports of heavy ethanol consumption (usually not otherwise specified), positive serologic reactions or quantitative or qualitative RNA assessments for viral hepatitis B or C, elevated blood iron measures (defined as serum iron concentration, transferrin saturation or serum ferritin concentration), history of erythrocyte transfusion, consumption of iron supplements, numbers of pregnancies and histories of thalassemia and other heritable or acquired types of anemia. Some conditions we tabulated were taken from the pathologist's histologic interpretations of the liver biopsy specimens.

Statistical Analyses

The present data set consisted of observations in 83 consecutive adult African Americans whose liver biopsy specimens were evaluable. Analyses were performed with a computer spreadsheet (Excel 2000; Microsoft Corp, Redmond, WA) and a statistical program (GB-Stat version 10.0, 2003; Dynamic Microsystems, Inc, Silver Spring, MD). Descriptive data are displayed as enumerations, percentages and mean ± 1 SD. Frequency values were compared using Pearson's χ^2 analysis or Fisher's exact test, as appropriate. Mean values were compared using 1-tailed student *t*-test. Some data were analyzed using Pearson's correlation coefficient. We performed logistic regressions on increased stainable iron and cirrhosis. All independent variables except age (continuous variable) were dichotomous. Values of $P < 0.05$ were defined as significant.

RESULTS

General Characteristics of 83 Patients

There were 37 men (44.6%) and 46 women (55.4%) (Table 1). Liver biopsy was performed using percutaneous technique in 64 patients (77.1%) and intraoperative technique in the remaining 19 patients (22.9%). The mean ages of men and women were similar (52 ± 16 years and 54 ± 14 years, respectively; $P = 0.5201$). There were reports of heavy ethanol consumption in 10 patients (12.0%), viral hepatitis in 18 patients (26.5%), steatosis unassociated with a report of heavy ethanol consumption in 36 patients (43.4%), inflammation in 31 patients (45.6%), fibrosis in 16 patients (26.2%) and bridging fibrosis/cirrhosis in 20 patients (29.4%) (Table 1). Elevated blood iron measures were reported in 4 patients (4.8%). No patient had a history of anemia treated with erythrocyte transfusion.

Comparisons of Patients With and Without Increased Stainable Liver Iron

Twenty-two patients (26.5%) had increased stainable iron (Table 1). Their mean age, the proportion of men and the reports of heavy ethanol consumption were greater than in patients