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Ferritin and increased vs upper reference interval tibc saturation to identify increased iron stores in African Americans

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ABSTRACT

Background: Increased serum ferritin (SF) in combination with increased total iron binding capacity saturation (TS) in the upper reference internal was evaluated to identify African Americans with increased iron stores.
Methods: Among 16,856 primary care-based African Americans screened at Howard University Field Center of the Hereditary Hemochromatosis and Iron Overload Screening (HEIRS) Study, 142 with SF >500 μg/l women or >700 μg/l men and increased TS (>45% women or >50% men; main study) and 146 with similar ferritin increases and upper reference interval TS (30–45% women or 35–50% men; ancillary study) were offered clinical evaluation to confirm increased SF and identify the cause.
Results: Repeat SF remained increased in 83% of 92 participants with increased TS initially (main study) vs 58% of 64 with upper reference interval TS initially (ancillary study) ($P=0.0002$). These persistent SF increases were associated with blood transfusions (treatment for sickle cell disease) in 20% of 76 main study and 11% of 37 ancillary study participants ($P=0.4$). Ninety percent of participants with persistent non-transfusional increased SF in the main study and 85% in the ancillary study had alanine-aminotransferase, aspartate-aminotransferase, C-reactive protein and/or hemoglobin values outside of the reference interval. Increased iron stores were documented by phlebotomy or liver biopsy in 4 of 7 main study and 2 of 2 ancillary study participants with persistent non-transfusional increase in SF.
Conclusion: Increased iron stores occur in African Americans with increased SF in combination with either increased or upper reference interval TS. Limiting clinical evaluation to only those individuals with both increased SF and increased TS will miss individuals with increased iron stores.

1. Introduction

Because the body does not have a physiologic mechanism to excrete excess iron, increased iron stores develop in individuals who receive repeated blood transfusions in the absence of blood loss or who absorb from the diet more iron than is needed to replace the small obligatory losses of 1–1.5 mg/day [1]. Excessive iron absorption occurs because of systemic conditions characterized by ineffective erythropoiesis (increased production and death within the bone marrow of red blood cell precursors) [2] or because of mutations in a variety of genes that transport or regulate the transport of iron across the intestinal mucosa [3]. Increased iron stores are common in rural sub-Saharan African populations that have very high dietary iron content. Studies of pedigrees suggest that, in addition to high dietary iron content, a genetic defect different from mutations in the *HFE* gene frequently found in Caucasians may also be implicated in increased iron stores in Africans, but the putative gene has not been identified [4]. At the same time, a primary increase in iron stores in African Americans has been increasingly recognized in recent years, but the prevalence is not known [5].

The Hereditary Hemochromatosis and Iron Overload Screening (HEIRS) Study is a multi-center, National Institutes of Health-sponsored study designed to determine the prevalence of iron overload in adult primary care patients of the U.S. and Canada of various ethnicities [6]. Over 100,000 participants were screened by testing for *HFE* C282Y and H63D mutations and measuring serum ferritin concentration (SF) and total iron binding capacity saturation (TS) [7]. Those participants with C282Y homozygosity and/or combined increases of SF and TS were invited to return for clinical follow-up to assess whether these findings were indicative of increased iron stores. The main HEIRS study did not investigate further for iron overload participants with an increased SF but TS within the reference interval on screening, but it seems possible that some of these participants may have increased iron stores. The focus of this ancillary HEIRS study was to determine whether, among the predominantly African-American population screened at the Howard

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Table 1
Participation in and biochemical markers of iron status at clinical evaluation (CE).

	No. screened	No. (%) eligible for CE	No. participating in CE	SF>500 μg/l women, >700 μg/l men at CE			SF 200–500 women, 300–700 men at CE	SF not elevated at CE
				TS>45% women, >50% men	TS 30–45% women, 35–50% men	TS<30% women, <35% men		
A. Main study (CE offered for screening SF>700 μg/l men, >500 μg/l women and TS>50% men, >45% women)								
African American	16,856	142 (0.8%)	92	46 (50.0%)	22 (23.9%)	8 (8.7%)	10 (10.9%)	6 (6.5%)
Males	6139	57 (0.9%)	36	21 (58.3%)	7 (19.4%)	2 (5.6%)	4 (11.1%)	2 (5.6%)
Females	10,717	85 (0.8%)	56	25 (44.6%)	15 (26.8%)	6 (10.7%)	6 (10.7%)	4 (7.1%)
B. Ancillary study (CE offered for screening SF>700 μg/l men, >500 μg/l women and TS 35–50% men, 30–45% women)								
African American	16,856	146 (0.9%)	64	10 (15.6%)	18 (28.1%)	9 (14.1%)	20 (31.3%)	7 (10.9%)
Males	6139	62 (1.0%)	27	2 (7.4%)	9 (33.3%)	3 (11.1%)	11 (40.7%)	2 (7.4%)
Females	10,717	84 (0.8%)	37	8 (21.6%)	9 (24.3%)	6 (16.2%)	9 (24.3%)	5 (13.5%)

University, Washington, DC, Field Center, increased stores exist among participants with SF>97.5 percentile for the population but TS in the upper reference interval rather than increased.

2. Methods

2.1. Main study

The HEIRS study was designed to evaluate the prevalence, genetic and environmental determinants, and potential clinical, personal, and societal effects of iron overload and hemochromatosis in a multicenter, multiethnic sample of 101,168 primary care adults ≥25 years [6,7]. The Howard University Field Center screened 20,924 individuals, including 16,856 African Americans. The study was approved by the Howard University IRB and all participants gave written informed consent. Screening participants with SF>200 μg/l women, >300 μg/l men in combination with TS >45% women, >50% men were invited to participate in a clinical evaluation [6]. Of these individuals who were invited to return for a clinical evaluation in the main study, we focus on a subgroup in this present report: those who had screening SF>500 μg/l women, >700 ug/l men (see next section).

2.2. Ancillary study — screening participants with SF>97.5 percentile and increased vs upper reference interval TS

From the third National Health and Nutrition Examination Survey (NHANES III) data [8], SF of 500 μg/l is >97.5 percentile for women and SF of 700 μg/l is >97.5 percentile for men. The present report focuses on 142 of the Howard University Field Center participants with SF>97.5 percentile (>500 μg/l women, >700 μg/l men) in combination with TS>45% women, >50% men (prospectively defined as increased by the HEIRS Study) who were invited to participate in clinical evaluation as part of the main study. It also focuses on 146 screening participants with SF>97.5 percentile but TS in the upper part of the reference interval (30–45% for women and 35–50% for men) who were eligible for clinical evaluation in an ancillary study. We made an attempt via telephone and/or letter to invite these individuals to participate in a clinical evaluation as part of the present ancillary study, but were not successful in contacting 44 (30%).

2.3. Clinical evaluation

During the clinical evaluation, additional written informed consent was obtained, a medical history was obtained, a physical examination was performed and fasting blood was drawn for laboratory tests. Blood was shipped to the HEIRS Study Central Laboratory for testing. Blood tests performed at the clinical evaluation included the following: iron, total iron binding capacity, TS, SF, DNA isolation for repeat *HFE* testing, complete blood count, serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and C-reactive protein (CRP). Hepatitis B surface antigen and hepatitis C antibody were performed if the ALT was increased; and reticulocyte count, haptoglobin, lactate dehydrogenase (LDH), indirect bilirubin, and hemoglobin electrophoresis with quantitation of hemoglobins A2 and F if the patient was anemic. Those participants suspected of having primary iron overload and/or liver disease were recommended to have consideration of diagnostic liver biopsy or quantitative phlebotomy and the data from these procedures were collected. The participant was informed via a letter and the participant's primary doctor was also informed if so agreed to by the participant.

2.4. Follow-up with liver biopsy and quantitative phlebotomy

An effort was made to monitor compliance with these recommendations, but liver biopsy and/or phlebotomy was done at the discretion of the personal physicians. Information about liver biopsies and phlebotomies was obtained from these physicians with informed consent from participants. Liver biopsies were graded for hepatocellular iron according to the 0–4+ scale of Scheuer [9]; grades of 0 and 1+ are generally considered to be within the reference interval. The total amount of iron mobilized by a patient's phlebotomy program was calculated in the following manner. The amount of iron removed at each phlebotomy from the first phlebotomy until the phlebotomy that resulted in SF<50 ng/ml was added as long as the subsequent phlebotomy was performed <56 days after the preceding phlebotomy. The amount of iron removed at a particular phlebotomy was calculated on the basis of the volume of blood removed, the hemoglobin concentration, and an assumed 3.46 mg iron per gram of hemoglobin.

2.5. Laboratory tests

2.5.1. Serum ferritin concentration

SF was analyzed on a Hitachi 911 analyzer using a turbidimetric antibody method (Roche Diagnostics, Indianapolis, IN). In this assay, ferritin antibody bound to latex forms an antigen–antibody complex with ferritin in the sample. Turbidity measured at 700 nm is directly proportional to the concentration of ferritin.

2.5.2. Total iron binding capacity saturation

Serum iron concentration and unbound iron binding capacity (UIBC) were analyzed by a ferrozine based colorimetric iron and unsaturated iron binding capacity method on a Hitachi 911 analyzer (Roche). Total iron binding capacity was derived as the sum of the iron and unsaturated iron binding capacity values. TS was the quotient of iron divided by total iron binding capacity expressed as percent.

2.5.3. HFE polymorphism detection

HFE C282Y and H63D genotyping was performed using a modification of the Invader assay (Third Wave Technologies, Inc. Madison, WI). In this assay, 2 oligonucleotides (wild type or mutant probe and Invader oligo) hybridize in tandem to a specific region of DNA generating a structure that is recognized and cleaved by the Cleavase VIII enzyme. This structure includes an unpaired "flap" on the 5' end of the wild type or mutant probe. Cleavage releases the 5' flap, which serves as the Invader oligo in the second cleavage reaction on a FRET oligonucleotide probe, which is tagged with a fluorophore quenched by an internal dye. Upon cleavage, the 5'-fluoresein labeled product is detectable using a fluorescence plate reader. The assay was modified to include 12 cycles of allele-specific PCR reaction to increase the amount of DNA available for the reaction.

2.5.4. Other tests

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed on a Hitachi 911 analyzer using colorimetric methods (Roche). C-reactive protein (CRP) was analyzed on a Hitachi 911 analyzer using a turbidimetric immunoprecipitation method (Roche). Hemoglobin concentration was determined using a Beckman Coulter GenS (Beckman/Coulter, Fullerton, CA). Hepatitis B surface antigen (HBsAg) and hepatitis C antibody (anti-HCV) were analyzed using immunometric assays on a Vitros ECi (Ortho-Clinical Diagnostics, Inc., Raritan, NJ).

2.5.5. Statistical analysis

If participants with increased repeat SF had received >10 blood transfusions lifetime, they were placed in a category of increased body iron stores because of blood transfusions. The remaining patients were assessed as to whether or not liver function tests were increased (ALT>40 U/l men, >31 U/l women and/or AST>37 U/l men, >31 U/l women), CRP was increased (CRP>5.0 mg/l) and/or hemoglobin was low (hemoglobin ≤13.2 g/dl men or ≤11.6 g/dl women). The reference intervals for the laboratory tests were those of the HEIRS Study Central Laboratory. Proportions were compared with the Fisher exact test. Continuous variables were compared with the student's *t* test.

3. Results

3.1. Screening results and participation in clinical evaluation (Table 1)

One hundred and forty-two African Americans had SF>500 μg/l for women or >700 μg/l for men in combination with TS>45% in women or >50% in men on initial testing, and 92 participated in a