Iron Status and Indicators of Human Immunodeficiency Virus Disease Severity among Pregnant Women in Malawi

Richard D. Semba,1 Taha E. Taha,1 Newton Kumwenda,1 Laban Mtumvalye,2 Robin Broadhead,2 Paolo G. Miotti,2 and John D. Chiphangwi1

1Departments of Ophthalmology and Epidemiology, Johns Hopkins University Schools of Medicine and Hygiene and Public Health, Baltimore, Maryland; 2National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; 3Departments of Obstetrics and Gynaecology and of Paediatrics and Child Health, College of Medicine, University of Malawi, Blantyre, Malawi

The relationships among hemoglobin, ferritin, and transferrin receptor levels and 2 markers of human immunodeficiency virus (HIV) disease severity—HIV load and CD4+ lymphocyte count—were characterized among 483 pregnant women in Malawi, Africa. The only significant correlation was an inverse correlation between hemoglobin level and plasma HIV load ($r = -0.104; P < 0.03$). The prevalence of iron deficiency anemia was not significantly different across quartiles of HIV load or CD4+ lymphocyte count. In contrast to previous studies, these data suggest that iron status is not related to markers of HIV disease severity in pregnant women in Africa.

Recently, several studies have suggested that a relationship exists between iron status and progression of HIV disease [1, 2]. Increased deposition of iron-ferritin and/or hemosiderin has been noted in the organs of patients with AIDS [1]. Progression of HIV disease was more rapid in patients with thalassemia major who were treated with inadequate doses than in those treated with optimal doses of desferrioxamine, an iron chelator [3]. High serum ferritin concentrations were associated with more rapid progression of HIV disease, which suggests that iron excess may have an adverse influence [4]. In a clinical trial of prophylaxis against *Pneumocystis carinii* pneumonia, a lower likelihood of survival was noted among those who received dapsone than among those who received aerosolized pentamidine [5]. The dapsone tablet also contained 60 mg of iron, and the daily doses of iron were thought to have increased oxidative stress and enhanced HIV replication [6].

A study of bone marrow macrophage iron grades in aspirates obtained from HIV-infected adults suggests that high macrophage iron grades were more common in patients infected with *Candida, Pneumocystis*, and *Mycobacterium* species, and high macrophage iron grade was associated with shorter duration of survival [7]. Haptoglobin, a plasma antioxidant with hemoglobin-binding capacity, was associated with altered iron metabolism and mortality in HIV-infected adults [8]. It has been hypothesized that iron loading in tissues is harmful because of direct cytotoxicity to immune effector cells, predisposition to neoplasia, and enhancement of some infections because of iron overload of macrophages [1]. The concern that iron supplementation may be harmful for HIV-infected patients has important implications, because daily oral iron supplementation is recommended for pregnant women and is used widely to treat iron deficiency anemia. The purpose of our study was to characterize the relationship between iron status and markers of HIV disease progression in pregnant women in Africa.

Subjects and Methods. Pregnant women who were at 18–28 weeks of gestation were seen at the antenatal clinic of the Queen Elizabeth Central Hospital in Blantyre, Malawi, from November 1995 through December 1996. Pregnant women received instruction in prenatal care, AIDS education, HIV testing, pre- and posttest HIV counseling, physical examination, and treatment for sexually transmitted diseases and malaria. Height and weight were recorded, and gestational age was estimated from the last reported menstrual period. Venous blood samples were drawn at screening and 1 week later at enrollment. The cross-sectional study of iron status and HIV load was done within the context of a clinical trial of vitamin A supplementation during pregnancy to improve maternal and infant health outcomes [9]. More detailed demographic information regarding this study population has been published elsewhere [10]. The study included only pregnant women who were HIV positive. None of the women were taking multivitamins or iron...
supplements at the time of enrollment, and no women received antiretroviral medications.

Serum was tested for the presence of HIV-1 antibody by use of EIA (Wellozyme, Wellcome Diagnostics and Genetic Systems EIA, Genetic Systems). Results of both EIAs were required to be positive for a woman to be considered HIV-1 positive. Immunoblotting (Bio-Rad Laboratories) was used to confirm HIV-1 status in women with equivocal results of HIV-1 testing by use of EIA. One week later, blood samples were drawn by means of venipuncture, and plasma was separated immediately and frozen at −70°C. A complete blood count, including determination of hemoglobin level, was done by use of an automated cell counter (Coulter). Anemia was defined as a hemoglobin concentration <110 g/L, according to standard criteria [11].

Plasma was kept frozen at −70°C until time of the analyses. Pooled human plasma was analyzed in each run of the laboratory analyses, to assess between-assay coefficient of variation (CV). Plasma ferritin was measured by means of ELISA (Human Ferritin Enzyme Immunoassay Test Kit, American Laboratory Products), with a CV of 5.7%. A plasma ferritin concentration of <12 μg/L was considered to be consistent with iron deficiency [12]. Because some recent studies suggest that a ferritin concentration of <30 μg/L may be a better indicator for iron storage [13, 14], this cutoff point also was included in the analyses. Plasma transferrin receptor was measured by use of ELISA (Quantikine IVD, Human sTfR Immunoassay, R & D Systems), with a between-assay CV of 5.8%. A plasma transferrin receptor concentration was considered to be greater than the normal range if it was ≥28.1 nmol/L, according to the manufacturer’s guidelines. Plasma retinol concentrations were determined by use of HPLC [15], with a CV of 4.7%.

Plasma HIV-1 load was measured by means of quantitative reverse transcriptase–PCR (Roche Amplicor Monitor, Roche), and these assays were run and validated in the AIDS Clinical Trials Group reference laboratory at Johns Hopkins Hospital (B. Jackson).

The χ² tests or exact tests were used to compare categorical variables among groups. Spearman correlation was used to examine the relationship between selected variables. Analysis of variance was used to compare means of normally distributed variables among groups. Transferrin receptor–ferritin index was defined as the ratio of transferrin receptor (milligrams per liter) to log₁₀ ferritin (micrograms per liter), as per convention [16].

### Results

Plasma HIV load was divided into quartiles, and hemoglobin, ferritin, and transferrin receptor concentrations, transferrin receptor–ferritin index, and the proportion of subjects with iron deficiency anemia, as defined by 2 different cutoffs for ferritin (12 and 30 μg/L), were compared across quartiles of plasma HIV load (table 1). There were no significant relationships among hemoglobin, ferritin, or transferrin receptor concentration or transferrin receptor–ferritin index across quartiles of plasma HIV load. CD4⁺ lymphocyte count also was divided into quartiles, and hemoglobin, ferritin, and transferrin receptor concentrations, transferrin receptor–ferritin index, and the proportion of subjects with iron deficiency anemia were compared across quartiles of CD4⁺ lymphocyte count (table 2). There were no significant relationships among hemoglobin, ferritin, or transferrin receptor concentration or transferrin receptor–ferritin index across quartiles of CD4⁺ lymphocyte count.

Because indicators of iron deficiency may be influenced by vitamin A status [17], plasma retinol was measured in all subjects. From lowest to highest quartile of plasma HIV load, mean ± SD plasma retinol concentrations were 0.75 ± 0.27, 0.69 ± 0.26, 0.73 ± 0.28, and 0.72 ± 0.29 μmol/L, respectively (p = .33). From lowest to highest quartile of CD4⁺ lymphocyte count, mean ± SD retinol concentrations were 0.67 ± 0.28,

### Table 1. Indicators of iron status in HIV-positive pregnant women in Malawi, by quartile of plasma HIV load.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma HIV load, copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;8070</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>104 ± 15 (121)</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>30.2 ± 44.3 (120)</td>
</tr>
<tr>
<td>Transferrin receptor, nmol/L</td>
<td>28.2 ± 14.3 (119)</td>
</tr>
<tr>
<td>Elevated transferrin receptor &gt;28.1 nmol/L</td>
<td>36.9 (119)</td>
</tr>
<tr>
<td>Hemoglobin &lt;110 g/L and ferritin &lt;12 μg/L</td>
<td>27.7 (119)</td>
</tr>
<tr>
<td>Hemoglobin &lt;110 g/L and ferritin &lt;30 μg/L</td>
<td>45.4 (119)</td>
</tr>
<tr>
<td>Transferrin receptor–ferritin index</td>
<td>2.66 ± 4.77 (118)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD (no.) for continuous variables and percentage with characteristic (no.) for categorical variables.
Table 2. Indicators of iron status in HIV-positive pregnant women in Malawi, by quartile of CD4+ lymphocyte count.

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;252</th>
<th>252–390</th>
<th>391–566</th>
<th>&gt;566</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>99 ± 16 (110)</td>
<td>101 ± 17 (113)</td>
<td>103 ± 12 (116)</td>
<td>101 ± 13 (118)</td>
<td>.15</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>28.5 ± 34.1 (113)</td>
<td>35.5 ± 56.9 (119)</td>
<td>30.1 ± 40.6 (117)</td>
<td>26.8 ± 32.3 (114)</td>
<td>.45</td>
</tr>
<tr>
<td>Transferrin receptor, nmol/L</td>
<td>29.1 ± 15.0 (107)</td>
<td>27.6 ± 12.9 (114)</td>
<td>27.1 ± 11.6 (112)</td>
<td>30.1 ± 16.6 (114)</td>
<td>.37</td>
</tr>
<tr>
<td>Elevated transferrin receptor &gt;28.1 nmol/L</td>
<td>46.7 (107)</td>
<td>35.1 (114)</td>
<td>35.7 (112)</td>
<td>40.3 (114)</td>
<td>.39</td>
</tr>
<tr>
<td>Ferritin &lt;110 g/L and ferritin &lt;12 µg/L</td>
<td>27.7 (108)</td>
<td>23.0 (113)</td>
<td>26.1 (115)</td>
<td>26.3 (114)</td>
<td>.95</td>
</tr>
<tr>
<td>Hemoglobin &lt;110 g/L and ferritin &lt;30 µg/L</td>
<td>54.6 (108)</td>
<td>45.1 (113)</td>
<td>46.9 (115)</td>
<td>50.0 (114)</td>
<td>.58</td>
</tr>
<tr>
<td>Transferrin receptor–ferritin index</td>
<td>2.56 ± 7.19 (106)</td>
<td>1.72 ± 3.46 (113)</td>
<td>2.37 ± 3.65 (111)</td>
<td>2.56 ± 3.52 (112)</td>
<td>.48</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD (no.) for continuous variables and percentage with characteristic (no.) for categorical variables.

0.74 ± 0.30, 0.75 ± 0.25, and 0.73 ± 0.27 µmol/L, respectively (P = .17).

Spearman’s correlations were used to characterize the relationships among hemoglobin, ferritin, and transferrin receptor concentrations, plasma HIV load, CD4+ lymphocyte count, and retinol concentration (table 3). There were no significant correlations between indicators of iron status and indicators of HIV disease severity (plasma HIV load and CD4+ lymphocyte count), except for a significant negative correlation between plasma HIV load and hemoglobin level. Retinol concentrations were significantly correlated with hemoglobin level but were not correlated with indicators of iron status and indicators of HIV disease severity.

**Discussion.** The present study suggests that iron status is not related to markers of HIV disease severity (plasma HIV load and CD4+ lymphocyte count) in HIV-infected pregnant women in Malawi. These findings do not corroborate previous studies that have suggested that iron overload is more severe in adults with more advanced HIV disease [7, 8]. The present study does not show that plasma ferritin concentrations are elevated in women with more advanced HIV disease, contrary to a previous finding of high serum ferritin concentrations in subjects with more rapid HIV disease progression [4]. The present study cannot shed insight on the relationship between iron overload in HIV-positive adults with thalassemia major [3], because the study population did not include such subjects. Studies suggesting that alterations in iron metabolism are associated with increased progression of HIV disease and mortality have not been conducted in populations that have high risk for iron deficiency [3–5, 7, 8]. It is unclear whether altered iron metabolism, as suggested by the increased deposition of iron observed in bone marrow, is involved in the etiology of opportunistic infections and high mortality rates in patients with HIV infection [5]. Another study from Malawi shows that...
iron status was not significantly different between HIV-negative and HIV-positive pregnant women, as determined by means of biopsy of bone marrow specimens [14]. Elevated ferritin concentrations were associated with increased HIV disease progression [4], but ferritin is a positive acute-phase reactant. The association of elevated serum ferritin concentrations with increased HIV disease severity might be due to underlying infection and inflammation, rather than to alterations in iron metabolism.

Anemia is extremely common during pregnancy, occurring among an estimated 35%–75% of pregnant women in developing countries [17, 18]. In many countries in sub-Saharan Africa, 15%–35% of pregnant women who present for antenatal care may be HIV positive, and they are given iron and folate supplements as a standard part of care. These data suggest that iron supplementation to achieve normal iron status is probably safe in HIV-positive pregnant women in developing countries. Severe anemia in pregnant women is associated with increased maternal mortality rates [19], and anemia has been associated with decreased likelihood of survival in many different studies [20]. The available evidence linking iron status with HIV disease severity and mortality is based on epidemiological observation, and this association may possibly represent an epiphenomenon. More definitive evidence is needed regarding the effect of iron supplementation on HIV infection from randomized, double-masked, controlled clinical trials.

Acknowledgments

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References