HIV and other predictors of serum folate, serum ferritin, and hemoglobin in pregnancy: a cross-sectional study in Zimbabwe[1–3]

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ABSTRACT

Background: Folate and iron status and hemoglobin concentrations are important to maternal and infant health.

Objective: Our goal was to identify predictors of serum folate, serum ferritin, and hemoglobin.

Design: This was a cross-sectional study of 1669 pregnant women (22–35 wk of gestation) in Harare, Zimbabwe, who were receiving prenatal care. The statistical effects of age, season, gestational age, gravidity, HIV-1 infection, malaria parasitemia, and serum α_1-antichymotrypsin (ACT) on serum folate, serum ferritin ($\log_{10}$ transformed), and hemoglobin were estimated by using multiple linear regression analyses.

Results: Serum folate ($\bar{x}$: 11.4 μmol/L) was 0.52-nmol/L (95% CI: 0.04, 1.0) lower in HIV-infected women than in uninfected women and 0.65-nmol/L (0.014, 1.28) lower in weeks 25–35 than in weeks 22–25. Serum ferritin (geometric $\bar{x}$: 11.6 μg/L) was 0.93 times (0.86, 0.99) lower in HIV-infected women and 2.25 times (1.41, 3.61) higher in women with malaria parasitemia than in uninfected women. Similarly, serum ferritin was 0.71 times (0.63, 0.79) higher in weeks 32–35 than in weeks 22–25 and 1.21 times (1.12, 1.29) higher in gravida ≥3 than in gravida 1. Elevated serum ACT was a strong predictor of serum folate, serum ferritin, and hemoglobin. HIV infection was associated with a 12.9-g/L (8.9, 16.8) lower hemoglobin concentration in women with nondepleted iron stores but low serum retinol and a 7–8-g/L lower hemoglobin concentration in women with other combinations of serum ferritin and retinol (P for interaction = 0.038). Season, age, gestational age, and gravidity were not significant predictors of hemoglobin. Low serum folate, ferritin, and retinol were associated with low hemoglobin.

Conclusions: HIV was associated with lower serum folate, serum ferritin, and hemoglobin. HIV infection was also associated with lower hemoglobin, particularly in women with stored iron and low serum retinol. Low serum folate, ferritin, and retinol were associated with low hemoglobin. *Am J Clin Nutr* 2001;73:1066–73.

KEY WORDS Hemoglobin, ferritin, folate, reproduction, pregnancy, gestational age, parity, HIV, acute phase response, malaria, Zimbabwe, women, α_1-antichymotrypsin

INTRODUCTION

Anemia is common in developing countries (1) and is closely associated with poverty. Anemia may be a cause of poor school performance, low physical activity, reduced resistance to infections, increased morbidity and mortality, and hence impaired socioeconomic development (2). Women of reproductive age are particularly at risk, and during pregnancy anemia becomes a risk factor for infant and probably maternal morbidity and mortality (3–5).

HIV infection is common in women of reproductive age in sub-Saharan Africa, where the prevalence often exceeds 25% (6). Infections with malaria parasites and hookworm, and occasionally *Schistosoma* spp., are also widespread. Infections lower hemoglobin concentrations through bone marrow suppression and hemolysis, but also impair nutritional status by reducing dietary intake and nutrient absorption and increasing nutrient utilization and losses (7).

Iron deficiency is considered the most important cause of low hemoglobin concentration (8), but vitamin A, riboflavin, vitamin B-12, folate (9, 10), and zinc are also essential to erythropoiesis. Furthermore, micronutrient deficiencies may have independent effects on other maternal, pregnancy, and infant outcomes, such as immunity (11), preeclampsia (12), maternal mortality (13), malformations (14), fetal loss, prematurity and intrauterine growth retardation (14, 15), and possibly mother-to-child HIV transmission (16, 17) and the nutritional status of infants (5, 18). However, assessing micronutrient status is difficult in the presence of infection because the micronutrient indicators are affected by the acute phase response (19). The iron storage protein ferritin, which circulates in small quantities in proportion to the amount of iron in the stores (20), is itself a "positive" acute

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phase protein because it increases during an acute phase response (21). Thus, in study populations with infections, the use of serum ferritin as a measure leads to an overestimation of iron status and may lead to spurious associations between serum ferritin and a study factor. It is therefore advisable to simultaneously measure an acute phase protein and subsequently control for the potential confounding effect of the acute phase response in the data analyses. α_1-Antichymotrypsin (ACT) and α_1-acid glycoprotein (AGP) seem to be useful (22; PI Paracha, A Jamil, K Belbase, et al, unpublished observations, 1999), whereas the time course of C-reactive protein and amyloid A may be too fast (23).

A better understanding of micronutrient status during pregnancy and the contribution of micronutrient deficiencies to low hemoglobin is essential for developing rational and targeted interventions. We conducted a cross-sectional study in 1669 pregnant women enrolled in a randomized, controlled, micronutrient supplementation trial. The role of HIV, obstetric history, and other predictors of serum concentrations of β-carotene and retinol are presented elsewhere (24). Here, we present the effects of predictors of serum folate, serum ferritin, and hemoglobin while controlling for the acute phase response by measurement of serum ACT.

SUBJECTS AND METHODS

Study area and population

The study was conducted in the Mbare residential area of Harare, the capital of Zimbabwe. Harare is 1500 m above sea level and has a tropical climate with a mean daily maximum temperature of 25 °C and a minimum of 12 °C. There are 4 main seasons: early (June to August) and late (September to November) dry and early (December to February) and late (March to May) rainy. The common diet is maize meal porridge taken with vegetables and occasionally meat. Unemployment is very high in Mbare, especially among women, and most of those employed are general laborers. Malaria is not endemic in the study area because of the high altitude, but most women often return to their rural homes outside Harare, where malaria transmission may occur.

Information about the study was given to women registering for routine prenatal care at Edith Oppermann Maternity Hospital, and those between 22 and 36 wk of gestation were invited to participate. Women were informed about the HIV testing and were counseled before they gave their written consent to participate. Counseling was provided by a nongovernmental organization, the AIDS Counseling Trust. HIV results were made available within 2 wk of blood sampling and those willing to know their results were counseled again. A questionnaire was administered and clinical examinations and blood sampling were performed. The structured questionnaire, administered by a research nurse, was used to gather demographic data and medical and obstetric history. Gestational age was calculated from the first day of the last menstruation. When this was not possible, gestational age was estimated by fundus height.

Permission to conduct the study was obtained from the ethical and scientific committees of the Medical Research Council of Zimbabwe, the Harare City Health Department, and the Ministry of Health. The study was also approved by the Danish Central Medical Ethics Committee. The national Ministry of Health recommendations were adhered to when giving women information on breast-feeding. Women found to be sick during the clinical examination were referred for treatment to Edith Oppermann Maternity Hospital or Harare Central Hospital.

Clinical examinations and HIV testing

Clinical examinations were carried out as part of the routine prenatal care. Height, weight, triceps skinfold thickness, and arm circumference were measured and body composition and muscle-area-for-age and fat-area-for-age *z* scores were computed (25). Venous blood was collected between 0900 and 1200. Five milliliters of blood was collected in tubes containing EDTA and 5 mL in plain tubes. The samples were placed in ice-cooled insulated boxes immediately after collection and were delivered to the laboratory within 4 h. Thick and thin blood slides were made for malaria parasitemia testing. A hematology analyzer (MaxM; Coulter, Fullerton, CA) was used to measure hemoglobin.

Serum was separated and stored in liquid nitrogen at −196 °C until tested for antibodies against HIV-1/2 or was shipped to the Research Department of Human Nutrition, Denmark, for micronutrient analyses. Samples for which negative results were obtained with the Genelavia Mixt (Sanofi, France) HIV-1/2 enzyme immunoassay kit were reported as being negative. Positive and indeterminate samples were tested further with use of the Recombigen HIV-1/2 (Cambridge Biotech, Worcester, MA) enzyme immunoassay with different antigens from the Genelavia kit. Samples for which positive results were obtained with the Recombigen kit were reported as being positive.

Positive results were confirmed at the Department of Clinical Chemistry, Aalborg University Hospital, Denmark, by using a modification of a reverse transcriptase–polymerase chain reaction method developed in-house (26). HIV-1 RNA was isolated from 125 μL plasma. The following primers from the *gag* region of the virus were used: 5′-primer, AGTTGGAGGACATCAAG CAGCCATGCA AAT, and 3′-primer, TGCTATGTCAGTTCCC CTTGGTTCTCT. All samples were analyzed in 2 independent extractions and runs. Samples with discordant results were retested in duplicate. The limit of detection was 40 000 genome equivalents/L.

Measurement of serum indexes

Serum ferritin was measured by a fluoroimmunoassay kit (DELFIA Ferritin; Wallac, Turku, Finland) with the detection based on a europium-labeled monoclonal antibody against human ferritin. The detection limit of the method as given by the manufacturer was 0.5 μg/L. The interrun CV was 5%. The accuracy of the method was confirmed by participation in a national control program [Danish Institute for External Quality Assurance (DEKS), Denmark]. Serum folate was measured by a fluoroimmunoassay kit (DELFIA Folate; Wallac) in which the quantification was based on the competition between a europium-labeled pteroylglutamic acid (the stable form of folate) and sample folate for a limited number of binding sites on immobilized folate binding protein. The interrun CV was 7%. Serum retinol and ACT were measured by HPLC and automated turbidimetry, respectively, as previously described (24).

Statistical analysis

The distribution of serum folate and hemoglobin conformed to normality as assessed by rankit plots, whereas serum ferritin was only normally distributed after $\log_{10}$ transformation. Two-sample *t* tests and one-way analyses of variance with Scheffe's

TABLE 1
Serum folate, serum ferritin, and hemoglobin in HIV-uninfected, pregnant women by season[1]

	Early rainy (Dec to Feb) (n = 203)	Late rainy (Mar to May) (n = 159)	Early dry (Jun to Aug) (n = 440)	Late dry (Sept to Nov) (n = 311)	P
Folate (nmol/L)[2]	11.3 (10.7, 12.0)	12.8 (12.0, 13.6)	12.3 (11.9, 12.8)	10.2 (9.8, 10.6)	<0.00001
<6.7 nmol/L (%)	15 (10, 20)	8 (3, 12)	5 (3, 7)	17 (12, 21)	<0.00001
Ferritin (μg/L)[3]	10.6 (9.6, 11.7)	10.9 (9.7, 12.1)	12.7 (11.8, 13.6)	11.3 (10.6, 12.1)	<0.008
<12 μg/L (%)	66 (60, 73)	68 (61, 75)	59 (54, 63)	64 (59, 70)	0.10
Hemoglobin (g/L)[4]	117 (115, 119)	118 (115, 120)	119 (117, 120)	117 (116, 119)	0.47
<110 g/L (%)	23 (17, 29)	23 (15, 30)	23 (19, 27)	20 (15, 25)	0.86

[1] 95% CI in parentheses.
[2] $\bar{x}$; n = 1036.
[3] Geometric $\bar{x}$; n = 1110.
[4] $\bar{x}$; n = 988.

multiple comparison post hoc tests were used to test for differences in means, whereas the chi-square test was used to test for differences in proportions between groups. Multiple linear regression analysis was used to identify and estimate statistical effects of predictors of serum folate, serum ferritin, and hemoglobin. When serum ferritin was $\log_{10}$ transformed, the regression coefficient β corresponded to a 10^{β} times change in mean serum ferritin per unit increase in the explanatory variable. The variables assessed were age, gravidity, gestational age, season, use of prenatal supplements, HIV infection, elevated ACT, and malaria parasitemia. Serum retinol was also used as an explanatory variable in the models that used hemoglobin as a dependent variable. All possible two-way interactions were tested for. When interactions were identified, interaction terms were computed to allow estimation of the statistical effect of one of the variables separately for each level of the effect-modifying variable. Dummy variables were used to assess the statistical effect of categorical variables or continuous variables when the relation was not linear. Residual analysis was performed by assessing normal plots and by plotting standardized residuals against predicted values and continuous independent variables. The level of significance used was 0.05. SPSS for WINDOWS (version 10; SPSS Inc, Chicago) was used to analyze the results.

RESULTS

Of 1669 pregnant women between 22 and 35 wk of gestation included in the study, data were available for hemoglobin from 1462 women (87.6%), for serum ferritin from 1663 women (99.6%), and for serum folate from 1539 women (92.2%). As reported elsewhere (24), most of the women were enrolled in the early (39.9%) and late (28.8%) dry seasons, compared with the early (18.5%) and late (12.7%) rainy seasons. The women's mean age was 24.4 y (range: 14–45 y) and the median number of previous pregnancies was 1 ($\bar{x}$: 1.2; range: 0–8), with 39.6% being gravida 1 ($\bar{x}$ age: 20.6 y; range: 14–40 y), 27.9% gravida 2 [23.5 (16–36) y], 17.1% gravida 3 [27.1 (18–43) y], and 14.9% gravida ≥4 [32.1 (20–45) y]. The women's mean gestational age was 29.1 wk (range: 22–35 wk). Intake of prenatal vitamin or mineral supplements before enrollment was reported by 59 women (3.7%): 28 (1.7%) had taken supplements containing iron and folic acid and 7 (0.4%) had taken iron alone. The composition of the supplements consumed by 24 women (1.4%) was unknown. Only seven (0.4%) of the 1669 women had malaria parasitemia, 526 (31.5%) were HIV-1 positive, 1113 (66.7%) were HIV-1 negative, and 30 (1.8%) had an indeterminate HIV status. The mean serum ACT concentration was not significantly different between HIV-infected and uninfected women, but was higher in the dry (0.34 g/L) than in the rainy (0.32 g/L) seasons (24).

Serum folate

The mean serum folate concentration of the HIV-uninfected women was 11.6 nmol/L (95% CI: 11.4, 11.9), with 65% having marginal (6.7–13.5 nmol/L) and 10% having low (<6.7 nmol/L) values. There were significant differences in serum folate by season (**Table 1**): the peak concentration was in the late rainy (12.8 nmol/L) and the nadir was in the late dry (10.2 nmol/L) season. However, no significant differences were found by age, gestational age, or gravidity. HIV-infected women had a lower mean serum folate concentration and a higher prevalence of concentrations <6.7 nmol/L than did HIV-uninfected women (**Table 2**).

In multiple linear regression analyses, season, gestational age, use of prenatal supplements, HIV infection, and elevated serum ACT were independent predictors of serum folate (**Table 3**). Serum folate was 2.04-nmol/L lower in the late dry season and 1.56-nmol/L lower in the early rainy season than in the late rainy season. Women between 25 and 35 wk of gestation had significantly lower serum folate than did women between 22 and 25 wk of gestation, whereas those reporting use of micronutrient supplements in their current pregnancy had a 2.41-nmol/L higher serum folate concentration than did women not taking supplements. HIV infection was associated with a 0.52-nmol/L lower value, whereas serum ACT concentrations >0.4 g/L were associated with ≈1-nmol/L lower serum folate. Exclusion of elevated

TABLE 2
Serum folate, serum ferritin, and hemoglobin in pregnant women by HIV status[1]

	HIV negative (n = 1113)	HIV positive (n = 526)	P
Folate (nmol/L)[2]	11.6 (11.4, 11.9)	11.0 (10.6, 11.4)	0.009
<6.7 nmol/L (%)	10 (8, 12)	16 (13, 20)	0.001
Ferritin (μg/L)[3]	11.6 (11.2, 12.1)	11.4 (10.7, 12.0)	0.57
<12 μg/L (%)	63 (60, 66)	64 (59, 68)	0.82
Hemoglobin (g/L)[4]	118 (117, 119)	109 (108, 110)	<0.00001
<110 g/L (%)	22 (19, 25)	54 (49, 58)	<0.00001

[1] 95% CI in parentheses. HIV status was indeterminate in 30 women.
[2] $\bar{x}$; n = 1539.
[3] Geometric $\bar{x}$; n = 1663.
[4] $\bar{x}$; n = 1462.

TABLE 3
Regression coefficients (β) and 95% CIs for predictors of serum folate (nmol/L) in pregnant women[1]

Variable	β (95% CI)	*P*
Season[2]		
Early dry	−0.39 (−1.12, 0.33)	0.29
Late dry	−2.04 (−2.81, −1.28)	<0.00001
Early rainy	−1.56 (−2.40, −0.72)	0.0002
Gestational age (wk)[3]		
25–35	−0.65 (−1.28, −0.014)	0.049
Use of prenatal supplements[4]	2.41 (1.28, 3.53)	0.00003
HIV infection[4]	−0.52 (−1.00, −0.04)	0.035
Serum ACT (g/L)[5]		
0.3–0.4	−0.24 (−0.72, 0.23)	0.32
0.4–0.5	−1.19 (−2.04, −0.34)	0.006
>0.5	−0.98 (−2.28, 0.31)	0.14

[1] n = 1428. Adjusted R^2 = 0.05.
[2] Defined as early (June to August) and late (September to November) dry and early rainy (December to February), with use of late rainy (March to May) as the reference category.
[3] Gestational age of 22–25 wk was used as the reference category.
[4] Coded as 0 for no and 1 for yes.
[5] Serum α_1-antichymotrypsin (ACT) ≤0.3 g/L was used as the reference category.

serum ACT from the model led to a slight underestimation of the association between gestational age and serum folate. The constant in the model gives the mean at the intercept, that is, the 0 value of all the explanatory variables. For the model presented in Table 3, the constant was 13.3, which means that HIV-negative women with serum ACT concentrations <0.3 g/L, between 22 and 25 wk of gestation, and enrolled in the late rainy season had a mean serum folate concentration of 13.3 compared with the overall mean of 11.4 nmol/L. No interactions were found between any of the variables assessed.

Serum ferritin

The geometric mean serum ferritin concentration in HIV-uninfected women was 11.6 μg/L (95% CI: 11.2, 12.1), with 63% having concentrations below the cutoff of 12 μg/L used to define depleted iron stores. Serum ferritin differed by season (Table 1), with the lowest values in the early rainy season (10.6 μg/L) and the highest in the early dry season (12.7 μg/L). There were no significant differences between HIV-infected and uninfected women in either the geometric mean serum ferritin concentration or the proportion of women with depleted iron stores (Table 2). In contrast, the geometric mean serum ferritin concentration differed significantly by age (P = 0.03), gravidity (P = 0.001), and gestational age (P < 0.00001).

In multiple linear regression analyses, season, gravidity, gestational age, use of prenatal supplements, HIV infection, malaria parasitemia, and elevated serum ACT were predictors of serum ferritin (**Table 4**). With serum ferritin $\log_{10}$ transformed, the regression coefficients of the early and late rainy seasons were −0.04, corresponding to a $10^{-0.04}$ or 0.91 times lower mean serum ferritin concentration in those seasons than in the reference season (the early dry season, when serum ferritin peaked). Interestingly, mean serum ferritin in gravida ≥3 was 1.21 times higher than in gravida 1, whereas serum ferritin concentrations in gravida 2 and gravida 1 were not significantly different. Serum ferritin decreased with gestational age such that women with gestational ages of 25–28, 28–32, and 32–35 wk had a 0.84, 0.76, and 0.71 times lower mean serum ferritin concentration, respectively, than did women enrolled in week 22–25 of gestation. Despite the similar geometric mean serum ferritin concentration in HIV-infected and uninfected women in the bivariate analyses, HIV was found to be slightly but significantly inversely associated with serum ferritin when serum ACT was controlled for in the multivariate analyses. In contrast, the 7 women with malaria parasitemia had a 2.25 times higher mean serum ferritin concentration than did the women without parasitemia. Mild elevations in serum ACT were not a predictor of serum ferritin, whereas serum ACT concentrations of 0.4–0.5 and >0.5 g/L were associated with a 1.26 and 3.16 times higher serum ferritin. In addition to being an independent predictor of serum ferritin, inclusion of serum ACT in the model served to control for otherwise confounding effects of the acute phase response. For example, the role of season and malaria would have been overestimated and the effect of HIV would have vanished if the acute phase response had not been controlled for. No interactions were found between any of the variables assessed.

Hemoglobin

The mean hemoglobin concentration of the HIV-uninfected women was 118 g/L, with 22% being anemic. HIV-infected women had a lower mean hemoglobin concentration and a considerably higher prevalence of anemia than did uninfected women (Table 2). Surprisingly, hemoglobin did not differ significantly by gestational age, gravidity, age, or season.

HIV infection and elevated serum ACT were negative predictors of hemoglobin in the multiple linear regression analysis.

TABLE 4
Regression coefficients (β) and 95% CIs for predictors of serum ferritin (μg/L; $\log_{10}$ transformed) in pregnant women[1]

Variable	10^{β} (95% CI)	*P*
Season[2]		
Late dry	0.96 (0.89, 1.04)	0.35
Early rainy	0.91 (0.83, 0.995)	0.03
Late rainy	0.91 (0.82, 1.005)	0.06
Gravidity[3]		
2	1.04 (0.96, 1.13)	0.33
≥3	1.21 (1.12, 1.29)	<0.00001
Gestational age (wk)[4]		
25–28	0.84 (0.75, 0.93)	0.001
28–32	0.76 (0.69, 0.84)	<0.00001
32–35	0.71 (0.63, 0.79)	<0.00001
Use of prenatal supplements[5]	1.30 (1.10, 1.53)	0.002
HIV infection[6]	0.93 (0.86, 0.99)	0.03
Malaria parasitemia[6]	2.25 (1.41, 3.61)	0.001
Serum ACT (g/L)[7]		
0.3–0.4	0.98 (0.91, 1.04)	0.47
0.4–0.5	1.26 (1.12, 1.41)	0.0001
>0.5	3.16 (2.63, 3.72)	<0.00001

[1] n = 1519. Adjusted R^2 = 0.16.
[2] Defined as late dry (September to November) and early (December to February) and late (March to May) rainy, with use of early dry (June to August) as the reference category.
[3] Gravida 1 was used as the reference category, assessed at age 24 y.
[4] Gestational age <25 wk was used as the reference category.
[5] Previous use of micronutrient supplements in the current pregnancy.
[6] Coded as 0 for absent and 1 for present.
[7] Serum α_1-antichymotrypsin (ACT) ≤0.3 g/L was used as the reference category.

TABLE 5
Regression coefficients (β) and 95% CIs for predictors of hemoglobin concentration (g/L) in pregnant women[1]

Variable	β (95% CI)	*P*
Serum ACT (g/L)[2]		
0.3–0.4	−2.0 (−3.4, −0.6)	0.006
0.4–0.5	−5.4 (−7.9, −2.8)	0.00004
>0.5	−7.1 (−11.2, −3.0)	0.001
HIV infection[3]		
Low serum ferritin, normal serum retinol	−7.5 (−9.5, −5.4)	<0.00001
Low serum ferritin, low serum retinol	−7.6 (−10.6, −4.4)	<0.00001
Normal serum ferritin, normal serum retinol	−7.7 (−10.5, −4.9)	<0.00001
Normal serum ferritin, low serum retinol	−12.9 (−16.8, −8.9)	<0.00001
Micronutrient status[4]		
Low serum folate (<10.8 nmol/L)	−3.6 (−5.6, −1.6)	0.00001
Low serum ferritin (<12 μg/L)	−6.5 (−8.2, −4.8)	<0.00001
Low serum retinol (<0.70 μmol/L)	−1.7 (−3.6, 0.2)	0.09

[1] n = 1294. Adjusted R^2 = 0.16.
[2] Serum α_1-antichymotrypsin (ACT) ≤0.3 g/L was used as the reference category.
[3] Coded as 0 for absent and 1 for present and assessed for different combinations of serum ferritin (< or >12 μg/L) and retinol (< or >0.70 μmol/L) concentrations. P for interaction = 0.038.
[4] Reference categories are serum folate >10.8 nmol/L, serum ferritin >12 μg/L, and serum retinol >0.70 μmol/L.

HIV-infected women had an 8.6-g/L (95% CI: 7.2, 10.0) lower hemoglobin concentration than did uninfected women. Women with serum ACT concentrations of 0.3–0.4, 0.4–0.5, and >0.5 g/L had 2.0- (95% CI: 0.6, 3.4), 5.4- (95% CI: 2.8, 7.9), and 7.1- (95% CI: 3.0, 11.2) g/L lower hemoglobin concentrations, respectively, than did women with a serum ACT concentration ≤0.3 g/L. Malaria parasitemia was not a predictor, nor was gestational age, neither as continuous variables nor as dummy variables. Similarly, neither gravida 2 nor gravida ≥3 had hemoglobin concentrations significantly different from those of gravida 1.

On the basis of this model, the role of serum folate and ferritin (and of serum retinol, as presented in reference 24) on hemoglobin was assessed. Interestingly, a 3-way interaction was found between HIV status, serum ferritin, and serum retinol (P = 0.038); none of the 3-way interactions with serum folate were significant. The statistical effect of HIV infection was therefore estimated separately for the 4 possible combinations of the binary serum ferritin (cutoff of 12 μg/L) and retinol (cutoff of 0.70 μmol/L) variables. As shown in **Table 5**, HIV-infected women had significantly lower hemoglobin concentrations than did uninfected women for all combinations of serum ferritin and retinol. However, the interaction was obviously due to HIV being associated with a much lower (12.9 g/L; 95% CI: 8.9, 16.8) hemoglobin concentration in women with normal serum ferritin and low serum retinol than in women with any other combination of serum ferritin and retinol.

There were no significant differences in body weight between HIV-infected and uninfected women (24) and inclusion of body mass index or muscle-area-for-age and fat-area-for-age z scores did not affect the interaction or the estimated effects of HIV. With HIV infection thus accounted for, low serum ferritin, low serum folate, and low serum retinol were still predictors of hemoglobin (Table 5).

The 3-way interaction between HIV, serum ferritin, and serum retinol is illustrated in **Figure 1**. In the figure, the difference between the thick and thin solid lines at the intercept represents the statistical effect of depleted iron stores on hemoglobin in HIV-uninfected women with serum retinol concentrations >0.70 μmol/L and corresponds to the adjusted regression coefficient of low serum ferritin (β = −6.5) presented in Table 5. Similarly, the difference between the solid and broken thick lines at the intercept represents the statistical effect of low serum retinol on hemoglobin in HIV-uninfected women with nondepleted iron stores and corresponds to the adjusted regression coefficient of low serum retinol (β = −1.7). As shown, although the combination of nondepleted iron stores and low serum retinol was associated with a relatively high mean hemoglobin concentration in HIV-uninfected women, it was associated with a relatively low hemoglobin concentration in HIV-infected women. Inclusion of the micronutrient status indicators in the model increased the adjusted R^2 from 0.09 to 0.16. In this model, in which all of the explanatory variables were binary, with 0 representing the normal state, the constant of 126 g/L represented the mean of HIV-uninfected women with low serum ACT, nondepleted iron stores, and normal folate and retinol concentrations. In contrast, the overall mean hemoglobin concentration was 115 g/L.

To reevaluate the conventional cutoffs for serum folate, ferritin, and retinol, dummy variables were computed and their effects on hemoglobin assessed in HIV-uninfected women. As shown in **Table 6**, serum ferritin between 12–15 and 15–24 μg/L had no significant effects on hemoglobin when compared with values >24 μg/L. In contrast, the serum ferritin categories <12 μg/L were associated with lower hemoglobin concentrations, although values between 9 and 12 μg/L were not significant. Interestingly, the hemoglobin deficit increased with decreasing serum ferritin even below the cutoff used to indicate depleted stores, in that serum ferritin values of 9–12 and 6–9 μg/L were associated with hemoglobin deficits of 2.3 and 4.4 g/L, respectively, and serum ferritin values <6 μg/L were associated

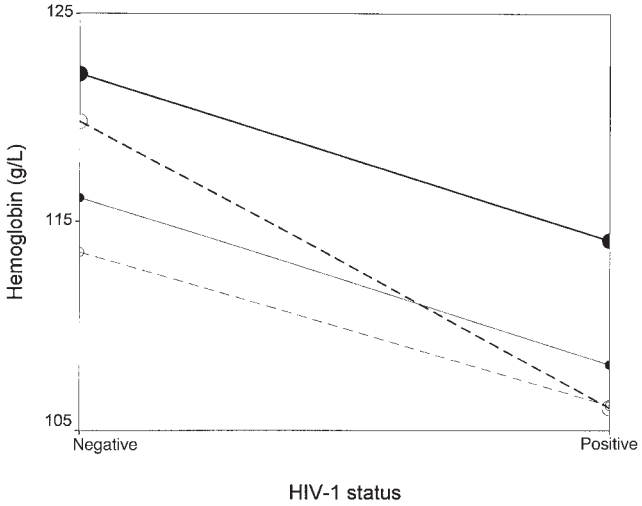

FIGURE 1. Mean hemoglobin concentration in 1669 pregnant women with different combinations of low (<12 μg/L) or normal (≥12 μg/L) serum ferritin and low (<0.70 μmol/L) or normal (≥0.70 μmol/L) serum retinol according to HIV-1 status. The thick and thin lines represent women with normal and low serum ferritin, respectively, and the solid and broken lines represent women with normal and low serum retinol, respectively.

TABLE 6
Effects of different concentrations of serum folate, ferritin, and retinol on hemoglobin in pregnant, HIV-negative women expressed as regression coefficients (β) with 95% CIs[1]

	Adjusted for ACT[2]		Not adjusted for ACT[3]	
Variable	β (95% CI)	*P*	β (95% CI)	*P*
Serum folate (nmol/L)[4]				
10.8–13.5	−1.3 (−3.4, 0.8)	0.24	−1.5 (−3.6, 0.7)	0.18
6.7–10.8	−2.9 (−4.7, −1.1)	0.002	−3.2 (−5.1, −1.4)	0.001
≤6.7	−5.0 (−7.7, −2.4)	0.0002	−5.3 (−8.0, −2.6)	0.0001
Serum ferritin (μg/L)[5]				
15–24	−0.3 (−3.3, 2.7)	0.86	0.4 (−2.6, 3.3)	0.80
12–15	1.0 (−2.3, 4.3)	0.55	1.7 (−1.6, 4.9)	0.31
9–12	−2.3 (−5.1, 0.4)	0.09	−1.1 (−3.8, 1.5)	0.40
6–9	−4.4 (−7.1, −1.7)	0.001	−3.1 (−5.7, −0.5)	0.02
≤6	−13.4 (−16.3, −10.4)	<0.00001	−12.1 (−14.9, −9.2)	<0.00001
Serum retinol (μmol/L)[6]				
0.70–1.05	−1.4 (−3.2, −0.3)	0.11	−1.7 (−3.5, 0.03)	0.054
<0.70	−2.2 (−4.2, −0.1)	0.04	−3.0 (−5.0, −0.9)	0.004

[1] n = 889.
[2] Adjusted R^2 = 0.174.
[3] Adjusted R^2 = 0.156.
[4] Serum folate >13.5 nmol/L was used as the reference category.
[5] Serum ferritin >24 μg/L was used as the reference category.
[6] Serum retinol >1.05 μmol/L was used as the reference category.

with a deficit of 13.4 g/L. Similarly, with use of serum folate values >13.5 nmol/L as the reference, values <10.8 nmol/L had negative effects on hemoglobin, whereas the regression coefficient of serum folate values between 10.8 and 13.5 nmol/L was negative but not significant. Serum retinol values <0.70 μmol/L were associated with a 2.2-g/L lower hemoglobin value than were values >1.05 μmol/L, whereas values between 0.70 and 1.05 μmol/L were not significantly associated with hemoglobin. Neither fetal sex nor plurality was associated with serum folate, serum ferritin, or hemoglobin.

DISCUSSION

Iron and folate deficiencies are of concern in reproductive women, and it is recommended that all women take daily prenatal iron and folate supplements (27).

Folate

Folate deficiency is a cause of macrocytic anemia (28), which was first described in pregnant Indian women (29, 30). Furthermore, folate deficiency leads to neural tube defects (31) and possibly intrauterine growth retardation and premature delivery (14). In our study, serum folate differed considerably by season and declined with gestational age. The mean serum folate concentration in the present study was only one-half of that found in pregnant Nepali women, although, as pointed out by those authors, their data may not have been representative (32). In addition to a possibly lower dietary intake of folate, the higher mean gestational age in our study than in the Nepali study (29.1 compared with 18.5 wk) may also have contributed to the lower serum folate because gestation is associated with enhanced catabolism of folate (33). Reported intake of prenatal supplements was associated with higher serum folate, but this may have been due to confounding by socioeconomic status (24). HIV was a negative predictor of serum folate, probably because of reduced intake and absorption and increased catabolism.

Iron

Two-thirds of the women had depleted iron stores. In Zimbabwe, as in other developing countries (34), the typical diet is maize porridge with few vegetables and fruit and occasionally flesh foods. The iron content of such a diet is low and is mainly poorly bioavailable nonheme iron. Hence, the seasonal variation in storage iron was probably the result of seasonal variation in dietary intake of enhancers (meat, fish, seafood, and organic acids) and inhibitors (phytates, phenoles, and calcium) of nonheme-iron absorption, rather than in iron intake per se. We found that iron stores were 30% lower in weeks 32–35 of gestation than in weeks 22–25. We did not find a decline in iron stores with increasing gravidity. In fact, iron stores were 20% greater in gravida ≥3 than in gravida 1, probably attributable to good breast-feeding and confinement practices. However, there was no effect of gravidity on hemoglobin.

We previously reported that gestational age and gravidity have significant, albeit slight, effects on serum ACT concentrations, suggesting that pregnancy itself leads to a mild acute phase response. With use of dummy variables, 0.4 g/L was suggested as a cutoff for ACT in pregnant women, although values between 0.3 and 0.4 g/L have slight effects on serum retinol (24). In contrast, there was no effect of mild ACT elevations (0.3–0.4 g/L) on serum ferritin, whereas moderate (0.4–0.5 g/L) and high (>0.5 g/L) elevations were associated with a 1.3 and 3.2 times higher serum ferritin concentration.

HIV and malaria

Malaria parasitemia was associated with a doubling of storage iron. This is well known to occur in both clinical and asymptomatic malaria (35) and probably mirrors the reduction in hemoglobin resulting from either bone marrow suppression or hemolysis, whereby iron is confined to the stores or translocated from the circulation to the stores, respectively. The increased erythropoiesis after hemolysis may also increase iron absorption (36).

In contrast, HIV-infected women had slightly lower iron stores in our study of generally asymptomatic women, whereas increased stores, due to an accumulation of iron in macrophages in liver, bone marrow, and other tissues, were reported in patients with more advanced HIV disease (37). Nevertheless, we found that HIV was associated with a deficit in hemoglobin, and expected this to be reflected in an increase in storage iron. The negative effect of HIV on storage iron was likely due to reduced intake and absorption of iron outbalancing the shift in iron from circulating erythrocytes to the stores. HIV-induced enteropathy (38) and iron malabsorption were described by others (39).

Iron may prove to play a complex role in HIV (40) because it is important for immune function (11) but is also required by enzymes involved in HIV replication and, by virtue of its prooxidant properties, may even stimulate replication (40, 41). Epidemiologic studies suggest that supplementary iron (42, 43), inadequate chelation of iron overload (44), and the size of the iron stores (45) are associated with HIV progression.

Hemoglobin

In a recent trial in western Kenya, iron considerably reduced the helminth reinfection rate in adults with low hemoglobin (46). But iron supplementation reduced hemoglobin (A Olsen, unpublished observations, 1999), probably because of an increased HIV progression. Accordingly, we expected a stronger negative statistical effect of HIV on hemoglobin in women with undepleted compared with depleted iron stores. However, HIV had its strongest statistical effect on hemoglobin in women with high serum ferritin and low serum retinol. This may have been because more advanced HIV led to reductions in hemoglobin with translocation of iron from erythrocytes to the stores, while at the same time impairing vitamin A status. However, this is not likely because we found less storage iron in HIV-infected women. Additionally, the interaction remained after we controlled for body composition, a measure of disease progression. Currently, administration of 60–120 mg Fe is recommended to pregnant women to reduce anemia (27), a risk factor for infant and pregnancy-related maternal mortality (3). If iron proves harmful when given to individuals with HIV, then a major public health dilemma is imminent. Studies to clarify the effects of iron supplementation in HIV infection are urgently needed.

In healthy women, hemoglobin concentrations have a U-shaped pattern during pregnancy, with the nadir at weeks 20–24 (47). The absence of an expected increase in hemoglobin in weeks 22–35 may have been the result of nutritional deficiencies impairing hemopoiesis, although deficiencies of iron, folate, and retinol were not responsible. Low serum folate, ferritin, and retinol were all independent negative predictors of hemoglobin. This is in accordance with a recent study in anemic, pregnant Malawian women (48) in which iron status was determined on the basis of bone marrow aspirates, and serum retinol, folate, and vitamin B-12 were measured. In that study, only 23% of the women had isolated iron depletion, 26% had iron in their stores but were vitamin deficient, and 32% had both iron and vitamin deficiencies.

Assuming that hemopoiesis ranks high in the hierarchy of biological functions requiring iron, folate, and vitamin A, we evaluated various cutoffs with use of dummy variables (Table 5). Although cutoffs from 10 to 30 μg/L were previously used (2, 49, 50), our data confirmed that 12 μg/L is an appropriate cutoff for serum ferritin. Contrary to the widespread notion that serum ferritin values below the cutoff all indicate depleted stores, we found that the statistical effect on hemoglobin increased with decreasing serum ferritin even <12 μg/L. Keeping in mind that cutoffs for serum folate are method specific and that hypersegmentation of neutrophils may be a more sensitive index (51), our data suggest that 10.8 or even 13.5 nmol/L is a more appropriate cutoff for serum folate than is 6.7 or 4.5 nmol/L (32). Serum retinol <0.70 μmol/L was associated with lower hemoglobin, and values between 0.70 and 1.05 μmol/L marginally significantly so. If ACT was not adjusted for, the effect of low concentrations of serum ferritin and serum retinol on hemoglobin would have been under- and overestimated, respectively, reflecting that ferritin is a positive and retinol a negative acute phase reactant.

Conclusion

We found that HIV, malaria, elevated ACT, and obstetric history are associated with serum folate and ferritin. Furthermore, the statistical effect of HIV on hemoglobin was larger in women with iron in their stores and low serum retinol. This finding is in accord with, but does not confirm, the notion that iron may be detrimental in HIV-infected individuals. Clarifying the role of iron in HIV infection should be a research priority.

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