Anemia in Human Immunodeficiency Virus–Infected and Uninfected Women in Rwanda

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Abstract. To determine the prevalence and risk factors of anemia among human immunodeficiency virus (HIV)–infected women in Rwanda and the influence of highly active antiretroviral therapy (HAART) on anemia, we analyzed 200 HIV-positive women and 50 HIV-negative women in a cross-sectional study. Clinical examinations and iron and vitamin B₁₂ assays were performed, and complete blood counts, serum folic acid levels, and CD4 cell count determined. The prevalence of anemia was significantly higher among HIV-positive women (29%) than among HIV-negative women (8%) ($P < 0.001$). Risk factors for anemia were lower body mass index (odds ratio [OR] = 3.4, 95% confidence interval [CI] = 2.4–4.1), zidovudine use (OR = 1.14, 95% CI = 1.01–1.29), lack of HAART (OR = 1.44, 95% CI = 1.21–1.67), oral candidiasis (OR = 1.4, 95% CI = 1.2–1.6), pulmonary tuberculosis (OR = 1.8, 95% CI = 1.7–2.2), cryptococcal meningitis (OR = 1.6, 95% CI = 1.21–1.8), *Pneumocystis jirovecii* pneumonia (OR = 1.41, 95% CI = 1.20–1.65) and CD4 lymphocyte count < 200 cells/ μ L (OR = 2.41, 95% CI = 2.01–3.07). The mean $\pm$ SD hemoglobin level of 10.9 ± 1.6 g/dL at HAART initiation significantly increased to 12.3 ± 1.5 g/dL in 8 months ($P < 0.001$). Anemia increases with HIV stage, and HAART is associated with a significant improvement in hemoglobin levels.

INTRODUCTION

Anemia is widespread in the general population of developing countries, especially among women.¹ Human immunodeficiency virus type 1 (HIV-1) infection is frequently associated with hematologic abnormalities, especially anemia. This association may have multiple causes.^{2–4} In different study settings, the prevalence of anemia has been estimated to be 30% in patients with asymptomatic HIV infection³ and 63–95% in persons with acquired immunodeficiency syndrome (AIDS).⁴ This high prevalence of anemia may be caused by a high incidence of anemia, a long duration of anemia caused by decreased mortality with highly active antiretroviral therapy (HAART) use, or a combination of both. Patients with more advanced HIV disease or a lower CD4 cell count had higher rates of anemia.^{2,5,6}

Anemia has been shown to influence the natural history of HIV disease by accelerating the rate of disease progression and increasing mortality in developed and developing country studies.^{2,5,6} Mocroft and others found that severe anemia was associated with a much faster rate of HIV disease progression and confirmed that anemia is a strong independent predictor of death.⁷ Uncorrected anemia results in multisystemic disabling symptoms and fatigue, exhaustion, increased risk of HIV dementia, poor quality of life and possibly even exacerbates poverty in communities with a high HIV prevalence.^{8–11} Conversely, survival time in HIV-infected persons may be improved after recovery from anemia.^{2,10}

The pathogenesis of anemia in HIV-infected persons is complex. Infection with HIV may lead to anemia by changes in cytokine production with subsequent effects on hematopoiesis;^{12,13} decreased erythropoietin concentrations;^{2,12,13} opportunistic infectious agents, such as *Mycobacterium avium* complex⁶ and parvovirus B-19;¹⁴ administration of chemotherapeutic agents such as zidovudine,¹² ganciclovir, dapsone, cancer chemotherapeutic medications (i.e., used for the treatment of Kaposi's sarcoma; vinca alkaloids (e.g., vincristine), bleomycin); chloramphenicol and trimethoprim/sulfamethoxazole;² and myelophthisis caused by lymphoma.

Other mechanisms for HIV-associated anemia, although uncommon, include vitamin B₁₂ deficiency and the autoimmune destruction of erythrocytes.³ Direct infection of marrow precursor cells¹² has been hypothesized, but not proven. However, studies from developed countries suggest that use of HAART reduces the risk of anemia in patients with HIV infection and improves hemoglobin values in many patients who are already anemic at the time of HAART initiation.^{6,15–17}

A better understanding of the causes of anemia is important because there are different approaches for treating anemia, such as transfusion, correction of nutritional deficiencies, recombinant human erythropoietin,¹⁸ cessation of myelosuppressive therapies,¹⁹ and prevention or treatment of the opportunistic infections.

This study was conducted to evaluate the prevalence of anemia, the relationship of anemia with nutritional deficiencies, and the association of anemia with the stage of HIV infection. We evaluated the effect of HAART on anemia in this study population. To our knowledge, this is the first study of this kind to be carried out in Rwanda.

MATERIALS AND METHODS

A cross-sectional study was performed on 200 HIV-infected and 50 uninfected adult women attending the HIV clinic and hospitalization rooms at the Department of Internal Medicine at the Butare University Teaching Hospital, Butare, Rwanda. This study was reviewed and approved by the Rwanda National Ethics Committee. A written informed consent was obtained from each woman. A detailed history was obtained and complete physical examination was performed for all women by using a standard questionnaire that was completed by the attending physician. Clinical information included age, sex, medical history, date of HIV diagnosis, use of antiretroviral drugs, duration of antiretroviral therapy, and use of antituberculosis therapy and other drugs.

The World Health Organization HIV disease stage classification was assessed for every HIV-positive woman on the basis of her medical history and physical examination. Laboratory analyses performed included complete blood count; measurement of serum iron, serum folic acid, serum vitamin B₁₂ levels; and CD4 cell counts. Anemia was defined as a hemoglobin

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level < 11.5 mg/dL by using reference ranges based upon local control measurements in our laboratory, which is located at a moderate altitude of 1,768 meters. This limit was also shown by Beutler and Waalen.²⁰ Serum iron, vitamin B₁₂, and folic acid levels were measured in the clinical laboratory of Ghent University Hospital. However, erythropoietin assays and the screening for malaria and chronic helminthes were not systematically conducted.

The first-line HAART regimen at the time of the study consisted of stavudine or zidovudine, plus lamivudine, plus nevirapine or efavirenz; more patients used stavudine and nevirapine than zidovudine and efavirenz. For all the patients who initiated HAART at the time of entry into the study, they were all given a stavudine-based regimen. No patients were taking other antiretroviral medications such as tenofovir, abacavir, or protease inhibitors. Women receiving zidovudine for prevention of mother-to-child transmission of HIV were not included in the study population.

Most patients were given cotrimoxazole prophylaxis with a dose of 1 tablet of 960 mg daily when CD4 cell counts were less than 350 cells/ μ L when possible. However, because many patients initially only sought care when they have reached advanced stages of immunosuppression, some patients were given prophylaxis at lower CD4 cell counts. No persons were offered ferrous sulfate and/or multivitamins for the treatment of anemia.

Statistical analysis. All statistical analyses were performed with SPSS software windows version 16.0 (SPSS Inc., Chicago, IL). Continuous variables with normal distribution were summarized by the using mean and SD. Frequencies and percentages were calculated for all the categorical variables. Student's *t*-test was used to compare the mean hemoglobin of the various CD4 cell count strata and for comparisons of hemoglobin before and after antiretroviral treatment.

Values were tested for statistical significance by using chi-square or Fisher's exact tests in the appropriate situation. A *P* value ≤ 0.05 was considered significant. Univariate logistic regression was performed to assess the relationship between each risk factor and the risk of anemia. Variables that were statistically significant (*P* < 0.05) in the univariate analysis were included in the multivariate analysis by using a logistic regression model adjusting for the possible confounding variables. The results were reported as unadjusted and adjusted odds ratios and 95% confidence intervals.

RESULTS

A total of 250 women (200 HIV-positive women and 50 HIV-negative) were included in the study. The anthropometric and biological characteristics of HIV-positive and HIV-negative women at enrollment are shown in Table 1. All women were greater than 18 years of age, and age did not differ significantly between the HIV-positive and HIV-negative women. Mean body mass index was significantly lower among HIV-positive than among HIV-negative women. Fifty (25%) HIV-positive women were receiving HAART. The mean hemoglobin concentration was significantly lower in HIV-positive women than in HIV negative women (12.5 ± 2.9 g/dL and 13.8 ± 1.4 g/dL, respectively; *P* < 0.001), and the prevalence of anemia was significantly higher among HIV-positive than among HIV-negative women (29% and 8%, respectively; *P* < 0.0001). However, hemoglobin levels did not differ

TABLE 1
Anthropometric and biological characteristics of HIV-positive and HIV-negative women at study enrollment, Rwanda*

Characteristics	HIV positive (n = 200)	HIV negative (n = 50)	<i>P</i> value
Age, years	36.9 $\pm$ 9.1	37.2 $\pm$ 14.4	0.83
Body mass index	24.7 $\pm$ 5	25.9 $\pm$ 6.9	0.001
Mean hemoglobin concentration (g/dL)	12.5 $\pm$ 2.9	13.8 $\pm$ 1.4	0.001
CD4 lymphocyte count, cells/ μ L	503 $\pm$ 21	—	—
Serum vitamin B12, ng/L	341 $\pm$ 15	457 $\pm$ 52	0.003
Serum iron, μ g/dL	76 $\pm$ 34	67 $\pm$ 41	0.09
Serum folic acid, ng/mL	10.2 $\pm$ 6.2	11.4 $\pm$ 3.3	0.056

*Values are mean $\pm$ SD. HIV = human immunodeficiency virus. Body mass index is weight (kg) divided by the square of height (m²).

between HIV-negative women and women with asymptomatic HIV infection (*P* = 0.85). Conversely, significant differences were observed between the different HIV stages. The prevalence of anemia among HIV-positive women by clinical HIV stage (I, II, III, and IV) was 9%, 26%, 40%, and 88%, respectively (*P* < 0.001).

Women with CD4 cell counts greater than 500 cells/ μ L had a mean $\pm$ SD hemoglobin level of 13.2 ± 2.1 g/dL, those with cell counts of 200–500 cells/ μ L had a mean $\pm$ SD hemoglobin level of 12.1 ± 2.2 g/dL, and those with CD4 cell counts less than 200 cells/ μ L had a mean $\pm$ SD hemoglobin level of 10.2 ± 2.5 g/dL. The decrease in mean hemoglobin values with decreased CD4 cell count strata was statistically significant (*P* < 0.001).

The mean vitamin B₁₂ concentration was significantly lower among HIV-positive than among HIV-negative women. The mean concentrations of serum iron and folic acid did not differ significantly between HIV-positive women and HIV-negative women.

The mean $\pm$ SD serum folic acid concentration was significantly lower among anemic women (8.8 ± 3.8 ng/mL) than among non-anemic women (11.2 ± 6.2 ng/mL) (*P* = 0.003), and the mean $\pm$ SD serum iron concentration was significantly lower among anemic women (62.9 ± 38.6 μ g/dL) than among non-anemic women (77.9 ± 33.7 μ g/dL) (*P* = 0.005). The mean $\pm$ SD serum vitamin B₁₂ concentration did not differ significantly between anemic women (325 ± 260 ng/L) and non-anemic women (385 ± 253 ng/L) (*P* = 0.09).

Risk factors associated with anemia. In HIV-positive women, univariate analysis showed that multiple factors were associated with anemia (Table 2). Lower body mass index, zidovudine use, lack of HAART, CD4 cell counts less than 200 cells/ μ L, and opportunistic diseases such as pulmonary TB, cryptococcal meningitis, oral candidiasis, and *Pneumocystis carinii* pneumonia were associated with increased risk for anemia. Herpes zoster infection and lower serum concentration of folic acid and iron were not associated with increased risk of anemia.

In the multivariate model, lower body mass index, zidovudine use, lack of HAART, CD4 cell counts less than 200 cells/ μ L, and pulmonary TB were associated with increased risk of anemia.

Analysis of the effect of HAART on anemia. A comparison between hemoglobin levels on entry in the study and 8 months later was performed. Among 50 women receiving HAART, 30 started HAART at the time of entry into the study, and 20 were already receiving HAART (mean $\pm$ SD treatment

TABLE 2
Risk factors associated with anemia in HIV-positive women, Rwanda*

Risk factors	Anemic (n = 58), no. (%)†	Non-anemic (n = 142), no. (%)†	Total (n = 200), no. (%)	Univariate analysis unadjusted OR (95% CI)	Multivariate analysis adjusted OR (95% CI)	P value
Underweight (BMI < 18.5)	17 (29.3)	15 (10.6)	32 (16)	5.3 (3.98–6.99)	3.4 (2.4–4.1)	0.02
Not receiving HAART	49 (84.4)	101 (71.1)	150 (75)	1.61 (1.39–1.84)	1.44 (1.21–1.67)	0.01
Zidovudine use	10 (17.2)	5 (3.5)	15 (7.5)	1.16 (1.05–1.32)	1.14 (1.01–1.29)	0.03
Pulmonary tuberculosis	15 (25.8)	16 (11.26)	31 (15.5)	2.4 (2.1–2.6)	1.81 (1.7–2.2)	0.01
Oral candidiasis	10 (17.2)	23 (16.2)	33 (16.5)	2.2 (1.8–2.5)	1.4 (1.2–1.6)	0.01
Cryptococcal meningitis	6 (10.3)	2 (1.4)	8 (4)	2.0 (1.5–2.6)	1.6 (1.21–1.8)	0.001
Herpes zoster	7 (12)	0 (0)	7 (3.5)	0.96 (0.84–1.1)	–	0.8
<i>Pneumocystis jiroveci</i> pneumonia	6 (10.3)	2 (1.4)	8 (4)	2.1 (1.7–2.5)	1.41 (1.20–1.65)	0.003
CD4 cell count < 200 cells/μL	20 (34.5)	5 (3.5)	25 (12.5)	3.05 (1.70–5.31)	2.4 (2.01–3.07)	0.001

* HIV = human immunodeficiency virus; OR = odds ratio; CI = confidence interval; BMI = body mass index; HAART = highly active antiretroviral therapy.

† Percentages are relative to the total number of anemic or non-anemic women.

period = 6 ± 3 years). For women who initiated HAART at the time of entry into the study, the mean $\pm$ SD hemoglobin level of 10.9 ± 1.6 g/dL significantly increased to 12.3 ± 1.5 g/dL during the 8 months of follow-up ($P < 0.001$). For the groups of women who never received HAART, the mean $\pm$ SD increase in hemoglobin from 11.8 ± 1.6 g/dL to 12.4 ± 1.5 g/dL did not reach statistical significance during the 8 months of follow-up ($P = 0.07$). Among the women who initiated HAART at the time of entry into the study, no one received anemia treatment (iron, folic acid, transfusion) during the study period.

DISCUSSION

Using the definition of anemia (hemoglobin level < 11.5 g/dL), our study showed that the overall prevalence of anemia was 24.8% (29% in HIV-positive women and 8% in HIV-negative women), which is similar to the prevalence reported in New York by Semba and others⁶ (28% in HIV-positive women). In a study by Mugisha and others in Uganda, the prevalence of anemia was 18.3% in female participants. The HIV-infected women were particularly more likely than HIV-negative women to be anemic (23.6% and 12.8%, respectively; $P = 0.031$).²¹

The lower percentage of anemia among HIV-negative women in our study may be related to the fact that the women coming to outpatient department of Butare University Teaching Hospital come from the urban area and have a better nutritional status than the general population.

In our study, anemia was more frequent in patients with advanced disease (AIDS) rather than in persons infected with HIV. The prevalence of anemia was highest among women who had a CD4 lymphocyte count < 200 cells/μL and lowest among women with a CD4 lymphocyte count > 500 cells/μL, which showed that risk of anemia is higher with more advanced HIV infection. Concerning the World Health Organization AIDS clinical stages, our data also showed such a relationship; the patients with AIDS (stage IV) were more likely to develop anemia than HIV-infected asymptomatic patients. The increasing anemia with HIV disease progression supports data from prior studies.^{6,15,22,23} We surprisingly found that anemia is not more prevalent in asymptomatic HIV-positive patients than in HIV-negative women. This finding may be caused by the effect of secondary events, especially the opportunistic infections, which are associated with anemia. It is also possible that the duration of HIV infection plays a role. Concomitant medications may be involved in the development of anemia. However, no traditional drug use was mentioned among the study participants.

We found that zidovudine use was associated with anemia; this finding was also shown in other studies.^{6,24} Zidovudine has broad myelosuppressive effects *in vitro* and *in vivo*. Its mechanism of induction of anemia is possibly related to the reduction of globin mRNA synthesis.²⁵ According to the study of Moyle and others, zidovudine-based HAART had a greater negative effect on hematologic parameters than stavudine-based regimens. Recipients of zidovudine are more likely to experience anemia and neutropenia events of any grade than recipients of stavudine.¹⁷

Our study showed decreased anemia with HAART use, which supports data from prior studies.^{6,13,24,26,27} Studies by Moore and Forney²⁶ and by Semba and others²⁷ found that HAART was an effective treatment for anemia of HIV infection, and the potential mechanisms that might be involved included a reduction in opportunistic infections and the anemia of chronic disease, and an improvement in nutritional status.

Our study showed that pulmonary tuberculosis was associated with a higher risk of anemia among HIV-positive women. This supports data from other studies.^{23,28} There are likely multiple factors for the etiology of anemia in tuberculosis; it is partially derived from anemia of chronic disease (associated with increased levels of interleukin-6) and partly from deficiencies of micronutrients such as iron, vitamin A, and selenium.²⁸ Also, in some cases, severe anemia may be the only clue to diagnosing occult tuberculosis infection of the bone marrow.²⁹ In addition to HAART, accurate diagnosis and good treatment of tuberculosis is necessary for the management of anemia in HIV-infected patients.

Other opportunistic infections such as cryptococcal meningitis, oral candidiasis, and *Pneumocystis jiroveci* pneumonia were associated with a higher risk of anemia. Semba and others found that oral candidiasis and bacterial pneumonia were associated with a high risk of anemia.⁶

Lower body mass index was also associated with a high risk of anemia. This association may be caused by deficiencies of many micronutrients, including iron, folate, B12, and vitamin A, which contribute directly to anemia. We found that the mean concentration of serum iron did not differ significantly between HIV-positive women and HIV-negative women and normocytic normochromic anemia was found in 85% cases of anemia, which excludes iron deficiency anemia. It was shown that HIV-positive women have a similar risk of iron-deficiency anemia as HIV-negative women; Semba and others have demonstrated that iron-deficiency anemia accounts for approximately half of anemia among HIV-positive and HIV-negative female injection drug users.^{6,10}

It is our concern that adequate treatment of anemia is not always considered in developing countries because most attention is paid to HIV infection and the frequent complications such as opportunistic infections. Previous studies suggest that recovery from anemia is associated with improved survival among persons with HIV infection.^{2,30} If recovery from anemia is shown to directly increase survival, screening for anemia should receive more attention and the patients with anemia should be managed properly. Diagnosis of anemia is made by measurement of hemoglobin, which is one of the simple techniques in the laboratory.

In conclusion, our findings showed that anemia is a frequent complication of HIV/AIDS infection in female patients. The severity of anemia depends on the clinical and immunologic stage of the disease, worsening in presence of most opportunistic infections or low CD4 cell count, and is especially highly prevalent in patients with low body mass index. HAART produces an improvement of hemoglobin levels, except for the well-known myelosuppressive potential of zidovudine. Treating physicians should pay attention to the screening of anemia, especially in HIV-infected persons. The treatment of patients with anemia should target its cause, but it is important to know that early start of HAART may prevent anemia or reduce its severity. Our study had a small sample size. A wider study that includes several HAART regimens and a long-term follow-up is needed.

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