A Cross-Sectional Study of Anemia in Human Immunodeficiency Virus-Infected Patients in Iran

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Background: Anemia is a frequent complication of infection with human immunodeficiency virus (HIV). The causes of HIV-related anemia are multifactorial. This study was conducted to evaluate the factors associated with anemia in HIV-infected patients.

Methods: A total of 642 patients with HIV/AIDS attending the HIV Clinic at Imam Khomeini Hospital in Tehran, Iran enrolled in this study. A detailed history and physical examination was done for all the patients. Investigations included CD4+ count, hemoglobin concentration, and red blood cells morphology.

Results: Among HIV-infected patients, 87% were males. The mean duration of antiretroviral therapy was 17.9±9.2 months. The mean (±SD) hemoglobin level was 12.9 ±2.31 mg/dL. Evaluation of red blood cell morphology showed macrocytosis in 11%, normocytosis plus normochromia in 41.1%, and microcytosis plus hypochromia in 47.9% of the patients. The prevalence of anemia (defined as hemoglobin<10 mg/dL) was 10.3%. Anemia was positively associated with female sex (OR=3.01), CD4 level (CD4 count of <200) (OR= 3.49), and antituberculous drug administration (OR=4.57).

Conclusion: Female sex, stage of HIV infection, and antituberculous drug use were the most important factors associated with anemia in HIV-infected patients in our study.

Archives of Iranian Medicine, Volume 12, Number 2, 2009: 145 – 150.

Keywords: Anemia • hemoglobin • HIV • risk factors

Introduction

Anemia is a very common finding in patients with human immunodeficiency virus (HIV) infection, particularly in individuals with more advanced HIV disease. In a study of patients receiving no myelosuppressive therapies, 8% of asymptomatic HIV-seropositive patients, 20% of those with symptomatic middle-stage HIV disease, and 71% of those with Center for Disease Control (CDC)-defined AIDS were anemic.¹

Several causes of anemia have been described in HIV-positive patients, such as changes in cytokine production with subsequent effects on hematopoiesis;2–4 decreased erythropoietin concentrations;5,6 opportunistic infectious agents such as Mycobacterium avium complex⁷ and parvovirus B-19⁸ administration of chemotherapeutic agents such as zidovudine,⁹ ganciclovir,¹⁰ and trimethoprim-sulfamethoxazole (TMP-SMX);¹¹ and myelophthisis caused by cancers such as malignant lymphoma. Other mechanisms for HIV-associated anemia, although uncommon, include vitamin B12 deficiency¹² and the autoimmune destruction of red blood cells (RBCs).¹³ Direct infection of marrow precursor cells has been hypothesized, but not proven.¹⁴ HIV infection alone, without other complicating illnesses, may produce anemia in some patients.⁵

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Accepted for publication: 12 November 2008
Anemia has been associated with progression to acquired immunodeficiency syndrome (AIDS), and shorter survival times in HIV-infected patients.

Gastrointestinal bleeding should also be considered in the evaluation of HIV-infected patients with anemia. In addition to the usual causes of gastrointestinal blood loss, HIV-related infections such as cytomegalovirus colitis and malignancies such as Kaposi’s sarcoma and non-Hodgkin’s lymphoma may produce clinically significant bleeding.

Understanding the association between anemia and survival is important because different choices for treatment of anemia including recombinant human erythropoietin (r-huEPO), correction of nutritional deficiencies, blood transfusion, and in drug-induced anemia, cessation of myelosuppressive therapies, are available nowadays.

This study was conducted to evaluate the factors associated with anemia in HIV-infected patients.

**Materials and Methods**

A cross-sectional study was performed on 642 HIV-infected adults attending the HIV Clinic at Imam Khomeini Hospital in Tehran, Iran. Imam Khomeini Hospital is the biggest teaching hospital affiliated to Tehran University of Medical Sciences. Patients from all regions of the country are admitted to this hospital, representing a wide spectrum of HIV presentation in Iran.

A detailed history and physical examination was obtained from all HIV-infected patients using a standard questionnaire accomplished by the attending physician. Clinical and laboratory information included age, sex, medical history, antiretroviral drugs consumption, duration of antiretroviral therapy, antituberculous (anti-TB) and antitoxoplasmosis drugs consumption, route of HIV transmission, type of anemia, and CD4 counts were determined for all of the HIV-infected patients. RBC morphology was assessed by light microscopy, on Giemsa-stained samples. Anemia was defined as a hemoglobin (Hb) <10 mg/dL.

A written informed consent was obtained from each patient. The study protocol was reviewed and approved by Institutional Review Board of Tehran University of Medical Sciences.

Statistical analysis was performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA). Values were tested for statistical significance using Chi-square test in appropriate situation. A \( P \) value of 0.05 or less was considered significant. Multiple logistic regression was performed to describe the association of demographic variables (sex, age, and route of HIV exposure), stage of the disease (CD4 T-lymphocyte count <200 cells/µL), and concurrent illnesses and chemotherapeutic agents with the occurrence of anemia. The results were reported as unadjusted and adjusted odds ratios (OR), with 95% confidence intervals (CI).

**Results**

The study included a total of 642 patients with HIV/AIDS. Table 1 shows the demographic characteristics of the patients. The mean age of the patients was 36.3±9.2 years (range: three to 75 years). Of the patients, 87% (557 patients) were males. The mean duration of antiretroviral therapy was 17.9±9.2 months (range: one to 120 months). The mean Hb was 12.9 mg/dL±2.31 (SD). Injection drug use was the highest transmission route of HIV (52.8%). TMP-SMX, isoniazid (INH) plus vitamin B6, acyclovir, fluconazole, and anti-
lipid drugs were used by 154 (24.2%), 152 (23.9%), 14 (2.2%), 61 (9.6%), and three (0.5%) patients, respectively. Frequencies of antiretroviral drugs used by the patients are as follows: 127 (19.9%) zidovudine (AZT)+lamivudine (3TC)+nelfinavir (NFV), 30 (4.7%) patients AZT+3TC+efavirenz (EFV), 25 (3.9%) patients AZT+3TC+nevirapine (NVP), 24 (3.7%) patients stavudine (d4T)+3TC+NFV, nine (1.4%) patients d4T+3TC+EFV, 16 (2.5%) patients d4T+3TC+NVP, and six (0.9%) patients other drugs.

RBC morphology showed macrocytosis in 11%, normocytosis plus normochromia in 41.1%, and microcytosis plus hypochromia in 47.9% of the patients.

Table 2 indicates the factors associated with anemia in HIV-infected patients. There was no association between the type of anemia and underlying disease (P>0.271), and prescription of acyclovir (P=0.117) and fluconazole (P=0.368). Also, no relationship between the type of anemia and the antiretroviral regimens including AZT+3TC+NVP (P=0.433), d4T+3TC+EFV (P=0.166), and d4T+3TC+NVP (P=0.316) was detected. Anemia was positively associated with drug history, use of TMP-SMX, antituberculosis drugs, antiretroviral regimens [AZT+3TC+NFV (P=0.005), AZT+3TC+EFV (P=0.007), d4T+3TC+NFV (P<0.0001)], and also opportunistic infections (P=0.001) and stage of HIV infection (P<0.001).

Anemia was positively associated with female sex, clinical AIDS (a CD4 count of <200), and administration of anti-TB drugs. It was negatively associated with heterosexual route of transmission and INH plus pyridoxine administration (Table 3).

**Discussion**

A variety of hematologic abnormalities associated with HIV infection has been described in different studies. Although primarily characterized by a specific deficit in CD4 T-lymphocytes, depletion of other cell lines including neutrophils, thrombocytes, and RBCs have been observed in HIV-infected individuals. While some investigators have suggested that anemia occurs particularly in the later stages of HIV infection, others have reported it as an early sign of HIV infection.

Multifactorial origin of anemia complicates determining its original cause and/or its proper treatment. Diallo et al. showed that anemia was more frequent in women than in men (P=0.00003). We also found a borderline relationship between female sex and anemia (P=0.05). According to Fangman and Scadden’s study, women, blacks, injection drug users, and people with advanced stages of HIV infection have a higher risk of anemia. Therefore, factors that affect the course of HIV-infected patients should be considered in the treatment of anemia. The frequency of anemia in our study is higher than previous reports. This may be due to the high prevalence of antiretroviral regimens including protease inhibitors, which are associated with anemia. The risk of anemia in patients receiving antiretroviral regimens including protease inhibitors was compared with those receiving regimens without these drugs. The results showed a significantly higher risk of anemia in patients receiving protease inhibitors (P<0.001). This result is consistent with previous studies. The influence of antiretroviral regimens on anemia could be due to depletion of RBCs, interference with heme biosynthesis, and induction of proinflammatory cytokines.

**Table 2.** Factors associated with anemia in HIV-infected patients.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Type of anemia</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macrocytosis</td>
<td>Normocytosis plus normochromia</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>105</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>120</td>
</tr>
<tr>
<td>Exposure route</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug history</td>
<td>24</td>
<td>65</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>13</td>
<td>36</td>
</tr>
<tr>
<td>Antituberculosis drugs</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Opportunistic infections</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Stage of HIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infected</td>
<td>3</td>
<td>63</td>
</tr>
<tr>
<td>AIDS</td>
<td>29</td>
<td>57</td>
</tr>
</tbody>
</table>

TMP-SMX= trimethoprim-sulfamethoxazole; INH=isoniazid.
Table 3. Logistic regression models showing the associations of incident anemia in HIV-infected patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Presence of anemia (Hb&lt;10 mg/dL) (n=642)</th>
<th>Unadjusted OR (CI 95%)</th>
<th>Adjusted OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, Female</td>
<td></td>
<td>0.935 (0.429–2.038)</td>
<td>4.156 (1.291–13.378)</td>
</tr>
<tr>
<td>Age≥45 years old</td>
<td></td>
<td>1.075 (0.565–2.047)</td>
<td>0.925 (0.424–2.020)</td>
</tr>
<tr>
<td>HIV exposure route</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal-fetal transmission</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Injecting drug use</td>
<td>2.111 (1.019–4.374)</td>
<td>6.632 (0.930–47.277)</td>
<td></td>
</tr>
<tr>
<td>Heterosexual sex</td>
<td>0.443 (0.236–0.832)</td>
<td>0.366 (0.145–0.923)</td>
<td></td>
</tr>
<tr>
<td>Hemophilia/transfusion recipient</td>
<td>1.239 (0.468–3.281)</td>
<td>3.748 (0.322–43.590)</td>
<td></td>
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<tr>
<td>Other</td>
<td>0.852 (0.107–6.765)</td>
<td>4.426 (0.240–81.705)</td>
<td></td>
</tr>
<tr>
<td>Stage of disease</td>
<td>1.414 (0.847–2.360)</td>
<td>1.357 (0.298–6.176)</td>
<td></td>
</tr>
<tr>
<td>CD4 count &lt; 200 cells/µL</td>
<td>3.078 (1.803–5.257)</td>
<td>2.396 (0.946–6.067)</td>
<td></td>
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<tr>
<td>Concurrent illness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV infection</td>
<td>0.985 (0.573–1.695)</td>
<td>0.917 (0.461–1.822)</td>
<td></td>
</tr>
<tr>
<td>HBV infection</td>
<td>1.475 (0.496–4.393)</td>
<td>1.268 (0.357–4.504)</td>
<td></td>
</tr>
<tr>
<td>Hemophilia</td>
<td>0.821 (0.188–3.583)</td>
<td>0.674 (0.050–9.044)</td>
<td></td>
</tr>
<tr>
<td>Opportunistic infections</td>
<td>1.685 (0.984–2.885)</td>
<td>0.879 (0.377–2.047)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapeutic agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zidovudine</td>
<td>1.693 (0.898–3.191)</td>
<td>4.358 (1.401–13.552)</td>
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</tr>
<tr>
<td>Lamivudine</td>
<td>0.911 (0.537–1.545)</td>
<td>3.464 (0.290–41.347)</td>
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<tr>
<td>Nelfinavir</td>
<td>0.620 (0.356–1.081)</td>
<td>0.170 (0.018–1.602)</td>
<td></td>
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<tr>
<td>Efavirenz</td>
<td>2.207 (0.520–9.379)</td>
<td>0.454 (0.033–6.312)</td>
<td></td>
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<tr>
<td>Nevirapine</td>
<td>2.336 (0.551–9.095)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Stavudine</td>
<td>0.310 (0.152–0.629)</td>
<td>—</td>
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<tr>
<td>Acyclovir</td>
<td>0.661 (0.085–5.137)</td>
<td>0.785 (0.077–7.961)</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>1.351 (0.612–2.982)</td>
<td>0.929 (0.306–2.826)</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>1.687 (0.974–2.923)</td>
<td>1.445 (0.572–3.648)</td>
<td></td>
</tr>
<tr>
<td>Antituberculous drugs</td>
<td>4.015 (1.990–8.103)</td>
<td>4.326 (1.788–10.470)</td>
<td></td>
</tr>
<tr>
<td>Isoniazid plus vitamin B6</td>
<td>0.476 (0.230–.988)</td>
<td>0.351 (0.146–0.842)</td>
<td></td>
</tr>
</tbody>
</table>

HCV=hepatitis C virus; HBV=hepatitis B virus.

disease suffer more from anemia and should be screened.29 In our study, anemia was more frequent in patients with advanced disease (AIDS) rather than in HIV infection itself. Dancheck et al. suggested that injection drug use was an independent risk factor for iron-deficiency anemia among HIV-seropositive women.30 We also found such risk factors for anemia among HIV-seropositive patients (OR=3.645).

In our study, 315 out of the 642 patients had abnormal RBC morphology, in which microcytosis plus hypochromia was the most frequent finding, while in Eley et al.’s study, anisocytosis was the most frequent observation.31 It was usually correlated with an increased RBC distribution width in many children.32

Administration of TMP-SMX can cause drug-associated aplastic anemia or immune-mediated destruction of specific populations of blood cells.11 We could not find any association between the administration of TMP-SMX and anemia in the studied patients (P=0.253).

In general, the likelihood of anemia increases with progressive immunologic deterioration and with the advancement of HIV-related disease.24 A CD4+ T-lymphocyte count less than 200 cells/µL is independently associated with the development of anemia. Our data also showed such a relationship, and the patients with AIDS were more likely to develop anemia compared with HIV-infected patients.

It is claimed that both AZT and d4T induce a metabolic defect in developing RBC precursor.33 However, AZT, but not d4T, has broader myelosuppressive effects both in vitro and in vivo. Its mechanism of induction of anemia possibly relates to the reduction of globin mRNA synthesis.34 According to Moyle et al.’s study, AZT-based highly active antiretroviral therapy (HAART) had a greater negative impact on hematologic parameters compared with the d4T-based regimens. The AZT recipients are more likely to experience anemia and neutropenia events of any grade than the d4T recipients.35
indicated that anemia was influenced by antiretroviral regimens of AZT+3TC with NFV, AZT+3TC with EFV, and d4T+3TC+NVF. However, Moore and Forney, and Semba et al.’s studies are in contrast to these results. They found that HAART was an effective treatment of 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.

The main limitation of our study was that the data which allow the classification of the causes of anemia such as reticulocyte counts, erythropoietin levels, and parvovirus IgM titers were not measured.

Anemia in HIV-infected patients, if persistent, is associated with substantially decreased survival. Consideration should be given to evaluate the effects of treating anemia in a prospective study design. If recovery from anemia is shown to directly increase survival, screening for anemia should be aggressive and the patients with anemia should be treated.

Acknowledgment

We would like to thank Dr. Gholamreza E. Javid for performance of statistical analysis. The study support was provided by the Department of Medicine, Tehran University of Medical Sciences, Tehran.

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