

# Prevalence, severity, and related factors of anemia in HIV/AIDS patients

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**Objective:** The prevalence of anemia in HIV infected patients has not been well characterized in Iran. This study aimed to describe the prevalence of anemia and related factors in HIV positive patients. **Materials and Methods:** In a cross-sectional study, anemia prevalence and risk factors of 212 HIV positive patients were assessed, at the behavioral disease consulting center in Isfahan. The relationship between anemia, demographic variables, and clinical histories were analyzed. Mild to moderate anemia was defined as hemoglobin 8–13 g/dL for men and 8–12 g/dL for women. Severe anemia was defined as hemoglobin, 8 g/dL. **Results:** A total of 212 HIV positive patients with a mean±SD age of 36.1 ± 9.1 years were assessed. We found that hemoglobin levels were between 4.7 and 16.5 gr/dL. In this study, the overall prevalence of anemia was 71%, with the majority of patients having mild to moderate anemia. Mild to moderate anemia and severe anemia occurred in 67% and 4% of patients, respectively. The mean absolute CD4 count was 348 ± 267.8 cells/cubic mm. Sixty one of 212 patients were at late stage of HIV infection (males=51 and female=10). Of the 212 HIV positive patients enrolled, 17 (8%) had a positive history of tuberculosis. We found a strong association between anemia and death. **Conclusion:** Normocytic anemia with decreased reticulocyte count was the most common type of anemia in overall. Prevalence of anemia in this study is relatively higher than other similar studies. Such a high prevalence of anemia needs close monitoring of patients on a zidovudine-based regimen. Better screening for anemia and infectious diseases, and modified harm reduction strategy (HRS) for injection drug users are primary needs in HIV seropositive patients.

**Key words:** AIDS, anemia, HIV positive

## INTRODUCTION

Immediately after the first reports of the acquired immunodeficiency syndrome (AIDS) cases, cytopenias of all major blood cell lines were increasingly recognized in patients with HIV infection. Anemia is the most common hematologic abnormality associated with HIV infection, affecting 60% to 80% of patients in late-stage disease. While anemia may manifest as a mere laboratory abnormality in some individuals, others may experience typical symptoms (e.g., fatigue, dyspnea, reduced exercise tolerance, and diminished functional capacity) directly related to a reduction in hemoglobin concentration.<sup>[1]</sup> The pathophysiology of HIV-associated anemia may involve three basic mechanisms: decreased RBC production, increased RBC destruction, and ineffective RBC production. Generally these include bone marrow infiltration by neoplasm or infections, a decreased production of endogenous erythropoietin, hemolytic anemia, use of myelosuppressive medications such as zidovudine (ZDV), or development as a result of the use of numerous medications. Anemia may also result from nutritional deficiencies – most commonly, deficiencies in iron, folic acid, or vitamin B12. Vitamin B12 deficiency may result from malabsorption in the

ileum or from gastric pathology caused by an array of infections or other conditions that affect the gastric mucosa in HIV-infected patients in patients with HIV disease, folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology.<sup>[2]</sup>

Owiredu *et al.* reported that highly active antiretroviral therapy-HAART may increase serum B12 levels and patients with low serum B12 in their study did not display characteristic findings of vitamin B12 deficiency, namely macrocytic anemia and neuropathy.<sup>[3]</sup> Anemia decreases survival, independently (hazard ratio 2.6, 95% CI 1.9–3.4) accelerates disease progression, and increases mortality among HIV-infected individuals. Few studies have characterized this problem in developing countries.<sup>[4,5]</sup> In a review of more than 32 000 patients infected with HIV, the yearly incidence of developing anemia increased with disease progression, affecting 3% of all patients with asymptomatic HIV infection, 12% of asymptomatic patients with CD4 cell counts <200/μL, and 37% of patients with an AIDS-related illness.<sup>[6]</sup> The association between anemia and decreased survival has been found to be independent of CD4+, T-lymphocyte count, and plasma HIV RNA

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concentration. Anemic HIV-infected people who recover from anemia have better survival rates than those who do not recover.<sup>[7-11]</sup> Risk of anemia increased by African American race, age, body mass index, history of pneumonia, oral candidacies, history of fever, ZDV use, low CD4+ cells counts (<200 cells/mL), and higher HIV-1 RNA levels in plasma.<sup>[5,12,13]</sup> ZDV has been reported as a cause of hematological disorders, especially anemia,<sup>[14,15]</sup> that most often occurs within 4 to 12 weeks of ZDV initiation.<sup>[16,17]</sup>

This study was conducted to investigate the prevalence of anemia and its related factors in patients with HIV positive in Isfahan, Iran.

## MATERIALS AND METHODS

This single-center population-based study was conducted in year 2010. Prevalence of anemia and correlated factors in total registered patients of 212 HIV positive were assessed, at the counseling center for behavioral diseases in Isfahan, Iran. The enrolled patients with HIV infection were subjects who tested with the enzyme-linked immuno-absorbent assays (ELISA) that were confirmed using the Western blot test. The study was approved by the medical university's ethics committee Isfahan University of Medical Sciences.

Laboratory and demographic data collected in the counseling center for behavioral disease such as hemoglobin, mean corpuscular volume-MCV, CD4 count, age, sex, marital status, education level, drug history (antiretroviral or nonantiretroviral and prophylactic), past medical history (treatment or documented infection with hepatitis B virus- HBV, hepatitis C virus-HCV, and mycobacterium tuberculosis), stage of the infection HIV, and AIDS based on statement of WHO stage IV HIV disease<sup>[18]</sup> and high-risk behaviors for HIV acquisition. Additional biochemical tests of serum ferritin, reticulocyte count, and serum level of B12 and folic acid were carried out for 50 available patients with anemia (41 men and 9 women).

Mild to moderate anemia was defined as hemoglobin 8–13 and 8–12 g/dL in men and women patients, respectively. Severe anemia was defined as hemoglobin  $\leq$  8 g/dL. Univariate comparisons between patients with anemia and patients without anemia were performed using Fisher's exact, Mann–Whitney, and logistic regression analysis tests. All tests of significance were two sided, and a *P*-value of less than 0.05 was considered significant using the statistical software SPSS version 18 for Windows.

## RESULTS

We enrolled a total of 212 HIV positive cases, (males=179

(84.8%)) with a mean $\pm$ SD age of 36.2 $\pm$ 9.1 years (males=36.8 $\pm$ 8.5 years; females=32.9 $\pm$ 11.2 years). In these cases 126 (77.8%) were illiterate or had elementary education, 36 (22.2%) had high-school or university education. Demographic and laboratory data are summarized in [Table 1]. The rates (%) of injection drug users (IDU) and having homosexual and/or heterosexual contact were 123/151 (81.5%) and 88/106 (83%), respectively. However, there were 61 and 106 patients missing information about the mentioned risk factors, respectively. From 98 patients, who answered the questions regarding the two risk factors, 61 (62.2%) responded positive to both of them.

It was difficult to define precisely the timing of HIV infection acquisition but according to the time of diagnosis, Mean  $\pm$  SD of duration of HIV diagnosis in anemic patients was 3.91 $\pm$ 2.8 years and in nonanemic patients was 3.92 $\pm$ 2.1 years, but this difference was not significant (*P*-value=0.74).

The overall prevalence of anemia was 71%, with the majority of patients having mild to moderate anemia. Mild to moderate anemia and severe anemia occurred in 67% and 4% patients, respectively.

Hemoglobin levels of the patients were between 4.7 and 16.5 g/dL. Mean  $\pm$  SD of hemoglobin in anemic and nonanemic patients were 11.2  $\pm$  1.3 and 14.22  $\pm$  1.1 g/dL, respectively. However, the difference was not statistically significant (*P*-value = 0.41). Microcytosis was more common in women than men (19% vs. 15%; *P*-value=0.418). In this study, 88 (72.7%) anemic patients were normocytic with decreased reticulocyte (men = 74.3%, women = 62%). Twenty (16.5%) and 13 (10.7%) have microcytic anemia and macrocytic anemia, respectively.

The mean $\pm$ SD absolute CD4 count was 348  $\pm$  267.8 cells/cubic mm (range 8–1594). Generally 54 patients had used ZDV (600 mg daily). In ZDV users 43(79.6%) were anemic (*P*-value=0.38).

Out of 212 patients, 75 patients missed to provide the CBC test. Of all anemic patients 40% had iron (ferritin<30  $\mu$ g/L) and 23% had vitamin B<sub>12</sub> (<200 pg/mL) deficiencies. There was no patient with folic acid deficiency (<6 ng/mL). Mean  $\pm$  SD of folic acid, vitamin B12 and ferritin in anemic patient were 16.9  $\pm$  7.1 ng/mL, 347.2  $\pm$  227.5 pg/mL and 74.6  $\pm$  66.6  $\mu$ g/L, respectively.

Treated patients received triplet therapy included ZDV, Plus lamivudine, and Efavirenz except for 4% of treated patients that ZDV was replaced by stavudine. All patients with CD4<200 cells/ $\mu$ L received Co-trimoxazole in order to Toxoplasmosis prophylaxis. None of patient received anti-HCV therapy.

**Table 1: Comparison of demographic and laboratory data between anemic and nonanemic HIV/AIDS patients**

Characteristics	Anemic	Non-anemic	P-value
Sex			
Male	77 (68.8)	35 (31.2)	0.75
Female	18 (72)	7 (28)	
Marital status			
Single	28 (63.6)	16 (36.4)	0.74
Married/ divorce	37 (63.8)	21 (36.2)	
Education			
Illiterate/primary	50 (63.3)	29 (36.7)	0.66
High school/university	14 (58.3)	10 (41.7)	
MCV			
Normal	56 (63.6)	32 (36.4)	0.06
Decreased	6 (46.2)	7 (53.8)	
Increased	17 (85)	3 (15)	
CD4 count			
<100	13 (76.5)	4 (23.5)	0.008
100-249	29 (82.9)	6 (17.1)	
>250	36 (53.7)	31 (46.3)	
AIDS			
Yes	41 (78.8)	11 (21.2)	0.059
No	54 (63.5)	31 (36.5)	
Age Mean±SD year	36.8 ± 9.7	35.6 ± 7.2	0.18
Hemoglobin Mean±SD g/dL	11.2 ± 1.3	14.2 ± 1.1	0.41
Reticulocyte count Mean±SD	0.91 ± 0.31	0.99 ± 0.17	0.12
Ferritin Mean±SD µg/L	74.6 ± 66.6	—	—
B12 Mean±SD pg/ml	347.2 ± 227.5	—	—
Folic acid: Mean±SD ng/ml	16.9 ± 7.1	—	—
Length of diagnosis Mean±SD Year	3.91 ± 2.8	3.93 ± 2.1	0.974
Death			
No	72 (64.3)	40 (35.7)	0.007
Yes	23 (92)	2 (8)	

Values in parentheses are row percentage; MCV: Mean corpuscular volume

In addition, 57/212 (26.9%) of the cohort received ZDV (600 mg daily), 37/212 (17.5%) co-trimoxazole (SD tablet per 12 h), and 7/212 (3.3%) capsule fluconazole (100–200 mg daily). 41/57 (71.9%) and 27/36 (75.0%) of patients who took, respectively, ZDV and co-trimoxazole were anemic.

From 212 HIV positive patients enrolled, 61 (28.8%) were at a late HIV stage (AIDS), (males = 51 and female = 10). Out of 61 cases who were diagnosed as AIDS, 16 (26.2%) had CD4>200 cells/µL. However, because of oral thrush or recurrent pneumonia allocated to the AIDS stage IV of HIV group. Anemia was more common in AIDS patients (41/52 (78.8%)) than others, this difference was marginally significant ( $P$ -value=0.055).

We found a strong association between anemia and death ( $P$ -value=0.007). In addition, the logistic regression was conducted to check the related factors with anemia. The variables that had the  $P$ -value more than 0.3 in the initial analysis were selected to enter the model. Goodness of

fitting logistic regression was checked using the Hosmer and Lemeshow test. The chi-square test (8 degrees of freedom) was  $X^2(8)=6.37$  ( $P$ -value=0.606). The risk of anemia in the increased and decreased MCV were about two and six times more than the normal MCV, respectively, but not statistically significant (OR=2.04 and 6.08, respectively). More information has been shown in [Tables 1 and 2].

## DISCUSSION

Anemia is a frequent complication of infection with the human immunodeficiency virus type 1 (HIV-1), and could be clinically important. Multifactorial origin of anemia complicates determining its original cause and/or its proper treatment.<sup>[19]</sup> The prevalence of anemia in this study was 71% that was significantly higher than prevalence of anemia reported in similar studies not only in Iran but also in other countries.<sup>[20,21]</sup> In Iran, Ramezani *et al.* (2008) reported that mild anemia occurred in 46% of subjects while severe anemia was not observed.<sup>[22]</sup> In a study on 642 Iranian HIV

**Table 2: Summarized results of fitting logistic regression to 99 HIV/AIDS patients**

Characteristics	OR (95%CI)	P-value
MCV		
Normal	1	—
Increase	2.04 (0.51–8.2)	0.314
Decrease	6.08 (0.98–37.8)	0.053
CD4		
>=250	1	—
100-249	0.98 (0.19–4.86)	0.978
<100	1.65 (0.47–5.84)	0.435
AIDS		
No	1	—
Yes	2.27 (0.72–7.2)	0.165
Age		
Year	1.03 (0.98–1.1)	0.292

\*:95% confidence interval; MCV: Mean corpuscular volume

positive patients, Jabbari *et al.* reported that the prevalence of anemia (defined as hemoglobin<10 mg/dL) was 10.3%.<sup>[23]</sup> Chakravarty *et al.* studied on 1256 patients ZDV; 203 (16.2%) (143 females) developed anemia (<8 g/dL). Severe anemia (<6.5 g/dL) was documented in 100 (7.9%) patients.<sup>[24]</sup>

A peripheral smear should be examined as it provides a great deal of information on cell morphology and can give clues to the cause of the alteration in hematopoiesis. According to our study normocytic, anemia with decreased reticulocyte count is the most common form of anemia in HIV positive patients in Isfahan. This form of anemia may be caused by medication toxicity, viral suppression, marrow infiltration by malignancy, mixed nutritional deficiencies, and chronic infections. Mata-Marin *et al.* reported normocytic normochromic anemia as the most common form of anemia (85%) in HIV positive patients, and in their study there was no case of macrocytic anemia.<sup>[25]</sup> In Iran, Jabbari *et al.* reported macrocytosis in 11%, normocytosis plus normochromia in 41.1%, and microcytosis plus hypochromia in 47.9% of the patients.<sup>[21]</sup> Chakravarty *et al.* reported a normocytic, normochromic picture in 42% of the patients and others showed macrocytic changes.<sup>[23]</sup>

We found anemia is slightly more common in women than men. Wills *et al.* and Diallo *et al.* showed that anemia was more frequent in women than in men.<sup>[26,27]</sup> Jabbari *et al.* reported borderline relationship between female sex and anemia and CD4 level (CD4 count of <200).<sup>[23]</sup> Mildvan *et al.* studied prevalence of anemia in 9690 HIV infected patients, and showed 39.5% (1721) of patient receiving no antiretroviral therapy and 35.5% (7252) of patients receiving HAART were anemic. They found anemia was more prevalent among men and patients with CD4<200 cells/mm<sup>3</sup>.<sup>[28]</sup> Mata-Marin *et al.* in a cross-sectional study in the Mexican population (54 men and 9 women) with no

antiretroviral therapy, found anemia, and severe anemia in 20.3% and 1.58% of the patients, respectively.<sup>[25]</sup>

The proportion of anemia produced by iron deficiency may be underestimated in our study because the diagnosis of iron deficiency was based only on ferritin concentration. Ferritin is an acute phase protein, which is elevated in cases of infections.

Moore *et al.* analyzed data from 905 patients, and found HAART is an effective treatment of the anemia of HIV infection.<sup>[29]</sup>

Kiragaa and colleges in a study on 5494 patients suggest that baseline severe anemia (Hb ≤8 g/dL) should not be used as a criterion for avoiding the use of ZDV in initiating HAART in resource-limited settings.<sup>[20]</sup> They found early severe anemia occurred in 4% of patients during the first 6 months on ART, and baseline severe anemia OR = 5.27 (95% CI 3.00–9.26) associated with early severe anemia. Injection drug use was an independent risk factor for iron deficiency anemia among HIV positive cases.<sup>[30]</sup> We found anemia was more common in patients with advanced stages of disease (AIDS) rather than in HIV infection itself. Anemia is a predictor of death in HIV positive patients.<sup>[31,32]</sup> In our study, there was a strong association between anemia and death.

The main limitations of our study include lack of measurement in erythropoietin levels, viral load and parvovirus IgM titers and also relatively moderate to high amount of missing data of the cohort.

Prevalence of anemia in this study is relatively higher than other similar studies. Such a high prevalence of anemia needs close monitoring of patients on a ZDV-based regimen especially in women, IDUs and patients receiving drugs with bone marrow suppressant effect such as cotrimoxazole. Normocytosis with decreased reticulocyte is the most common form of anemia in Isfahan and may be due to medical toxicity, chronic infections, or mixed nutritional deficiency. Better screening for anemia and infectious diseases, and modified harm reduction strategy (HRS) for IDUs (especially in women) are the primary needs in HIV seropositive patients in Isfahan.

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