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Original Article

Prevalence of anemia among HIV-infected individuals and the associated factors: A single-center, retrospective review of 513 cases

Vikram K Mahajan, Niharika Dhattarwal, Karaninder Singh Mehta, Pushpinder Singh Chauhan, Anuj Sharma, Reena Kumari Sharma, Yog Raj Verma, Monika Chandel

Department of Dermatology, Venereology & Leprosy, Dr. Rajendra Prasad Government Medical College Kangra (Tanda) -176001 (Himachal Pradesh) India.

Corresponding author: Vikram K Mahajan, MD, E-mail: vkm1@rediffmail.com

ABSTRACT

Background: Although a major public health problem around the world, the prevalence of anemia and the associated factors in HIV-infected individuals remains understudied in the Indian context. **Objectives**: The objective was to assess the prevalence of anemia and the associated factors among HIV-infected individuals. **Methods**: The records of 513 HIV-affected individuals (M: F: 244:269) aged 12 to 84 years (mean ± SD: 37.5 ± 12.1) were reviewed retrospectively for the presence of anemia and the associated factors. The anemia was defined and severity was graded per the WHO guidelines. **Results**: Anemia of variable severity was present in 77.7% of the individuals. The female sex(OR: 2.09; Cl: 95%; CI: 1.41–3.10; $p < 0.05$), CD4$^+$ count ≤ 200 cells/microliter (OR: 2.36; Cl: 95%; CI: 1.59–3.52; $p < 0.0001$), WBC count < 4000 cells/mm^3 (OR: 3.29; Cl: 95%; CI: 0.97–11.14; $p < 0.04$), platelet count < 100,000 cells/dL (OR: 0.50; Cl: 95%; CI: 0.31–0.81; $p < 0.05$), before ART (OR: 3.78; Cl: 95%; CI: 2.91–4.91; $p < 0.0001$), and tuberculosis treatment (OR: 5.88; Cl: 95%; CI: 1.38–25.04; $p < 0.05$) were factors significantly associated with anemia. The mean duration of highly active antiretroviral therapy (ART) was 3.15 years, with 395 (77%) individuals being on treatment for ≤ five years. ART significantly improved hemoglobin levels ($p < 0.0001$). **Conclusion**: Anemia of variable severity remains a significant co-morbidity among HIV-infected individuals, especially females, prior to the initiation of ART, and those with a low CD4$^+$ count or thrombocytopenia and on anti-tuberculosis treatment. The fact that this was a single-center study, its small number of subjects, the retrospective design, and no information on red blood cell indices and the viral load were its important limitations.

Key words: AIDS; anemia; ART; CD4$^+$ count; HAART; HIV; India

INTRODUCTION

Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) are some of the major public health problems around the world, in particular, in the developing countries of Africa and southeastern Asia. The number of new cases has declined significantly after highly active antiretroviral therapy (ART) and intensive information, education and communication (IEC) strategies have been implemented. However, India is still estimated to have the third-largest HIV-infected population in the world, after South Africa and Nigeria, with an estimated adult prevalence of 0.22% (0.16–0.30%) in 2017 [1].

Anemia is an important cause of morbidity in HIV-infected individuals around the world, irrespective of highly active ART, which significantly improves quality of life and prevents the progression of HIV disease [2,3]. The acceleration of the disease, a decreased life

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expectancy, impaired physical function, the risk of ART-associated hepatotoxicity, psychological distress, and poor quality of life are important consequences of HIV-associated anemia [4-6].

The prevalence of HIV-associated anemia ranges from 10% in asymptomatic HIV disease to 92% among individuals with advanced AIDS and is 24% and 58% in individuals taking ART and up to 35% in those ART-naïve [4,7-10]. However, it may occur at any stage of HIV disease, with a severity that may correlate with the progression of the disease with a higher prevalence among ART-naïve individuals when compared to those taking ART [11,12]. A multitude of factors, such as sex, low nutritional status, anti-tuberculosis treatment (ATT), opportunistic infections, an advanced stage of the HIV disease, a $CD4^+$ count below 200 cells/microliter, a white blood cell (WBC) count below 4000 cells/microliter, and a platelet count below 200,000 cells/microliter, as well as zidovudine-containing ART regimens, have been identified to be associated with anemia among HIV-affected individuals [8,4,13-16]. Pure red cell aplasia, characterized by a normal leukocyte and platelet count, a corrected reticulocyte count below 1%, less than 5% of erythrocyte precursors in the bone marrow, and no hemolysis is an uncommon cause of refractory anemia in HIV/AIDS and occurs consequent to an autoimmune response selectively affecting erythroid cell lines or ART-induced myelosuppression [16-18]. However, the magnitude of these factors varies across regions depending upon socioeconomic and health conditions. Although the prevalence of anemia among the HIV-affected and the associated factors remain understudied in the Indian context, it was 23% to 61.5% in some studies, irrespective of their ART status [6,18-20]. Since the management of anemia in these individuals improves survival and the overall quality of life, we performed this study with the aim to assess the prevalence of anemia and the associated factors in HIVinfected individuals under follow-up in this part of the country having a low prevalence of HIV/AIDS and for an overall paucity of relevant data.

MATERIALS AND METHODS

The medical records of 518 patients on regular ART registered in the institute-affiliated ART Center and under follow-up between January 2015 and December 2019 were analyzed retrospectively for this hospital-based, descriptive, observational study after obtaining approval from the Institutional Ethics Committee. Sociodemographic details, the $CD4^+$ count, previous illnesses and their treatment (if any), hemoglobin (Hb) levels, and the results of complete blood counts recorded before the initiation of ART together with the most recent one were noted from their ART records or booklets maintained in the center. Hemoglobin levels of at least 12 g/dL were considered normal and anemia was defined per the WHO guidelines as hemoglobin values below 13 g/dL in adult males, below 12 g/dL in adult females, below 11 g/dL in children below five years of age, and below 11.5 g/dL in children between five to eleven years of age [21]. The severity of anemia was graded as mild with Hb = 11.0−11.9 g/dL, as moderate with Hb = 8.0−10.9 g/dL, and as severe with Hb < 8.0 g/dL.

Statistical Analysis

Five patients had incomplete records and were excluded from the final analysis. Continuous data was presented as means and standard deviations and categorical variables as frequencies and percentages. Categorical and parametric data was analyzed by χ^2 test and Student's *t*-test. The non-parametric Mann–Whitney test was employed for variables not distributed normally. Odds ratios (ORs) were calculated with a 95% confidence interval. A *p* value below 0.05 (5%) with a 95% confidence interval was considered statistically significant.

RESULTS

Table 1 shows the baseline characteristics of the 513 study subjects, comprising 244 (47.6%) males and 269 (52.4%) females (M: F: 0.9:1) aged between 12 and 84 years (mean ± SD: 37.5 ± 12.1 years). Among the subjects, 380 (74.1%) were aged 16–45 years. The $CD4^+$ cell count varied from 5 to 1824 (mean ± SD = 240.12 ± 187.94) cells/microliter and were ≤ 200 cells/microliter in 252 (49.1%) individuals. All had been on highly active ART with good treatment adherence for two weeks to nine years and four months (mean: 3.15 years), and 395 (77%) individuals had been undergoing treatment for not longer than five years. The major ART regimens were tenofovir + lamivudine + efavirenz (TLE) in 282 (55%), zidovudine + lamivudine + nevirapine (ZLN) in 177 (34.5%), and zidovudine + lamivudine + efavirenz (ZLE) in 22 (4.29%) individuals, respectively. Overall, 199 (38.8%) had been taking zidovudine-containing ART regimens. Six (1.2%) developed nevirapine hypersensitivity syndrome and the initial

Table 1: Baseline characteristics of the patients

Baseline Characteristics		No. of Patients (%) *n*=513
Sex	Male	244 (47.56)
	Female	269 (52.44)
	Females: males	1:1.10
Age range (mean±SD) 12–84 (37.45±12.11) years	< 15 yrs.	34 (6.63)
	16–30 yrs.	80 (15.59)
	31–45 yrs.	300 (58.48)
	46–60 yrs.	86 (16.76)
	> 60 yrs.	13 (2.53)
CD4 cell count (at presentation) range (mean±SD) 5–1824 (240.12±187.94) cells/microliter	> 500 cells/microliter	38 (7.41)
	> 350–500 cells/microliter	52 (10.14)
	> 200–350 cells/microliter	171 (33.33)
	> 100–200 cells/microliter	135 (26.31)
	< 100 cells/microliter	117 (22.81)
Duration of ART range (mean) 2 wk. – 9 yrs. 4 months (3.15 yrs.)	< 1 yrs.	112 (21.83)
	> 1–2 yrs.	92 (17.93)
	> 2–3 yrs.	59 (11.50)
	> 3–4 yrs.	63 (12.28)
	> 4–5 yrs.	69 (13.45)
	> 5–6 yrs.	52 (10.14)
	> 6–7 yrs.	38 (7.41)
	> 7 yrs.	28 (5.46)
ART Drug Regimen	TDF+3TC+EFV (TLE)	282 (54.97)
	ZDV+3TC+NVP (ZLN)	177 (34.50)
	ZDV+3TC+EFV (ZLE)	22 (4.29)
	Abc+NVP	13 (2.53)
	TDF+3TC+NVP (TLN)	11 (2.14)
	Abc+EFV	5 (0.97)
	d4T+3TC+NVP (SLN)	2 (0.39)
	d4T+3TC+EFV (SLE)	1 (0.19)
Hemoglobin Levels Mean±SD (range) (g/dL)	Before ART	10.8±2.02 (4.4–16.5)
	After ART	12.3±1.7 (6–17)
	p value	**< 0.0001**
Anemia	Before ART	368 (77.7)
	After ART	206 (40%)
	P value	**<0.0001**
Grade of Anemia Before ART	Mild anemia (Hb=11–11.9 g/dl)	72 (13.9)
	Moderate anemia (Hb=8–10.9 g/dl)	257 (50.2)
	Severe anemia (Hb<8 g/dl)	39 (7.6)
	No anemia (Hb ≥ 13 g/dL for men, ≥ 12 g/dL for women) adults and adolescents	145 (28.3)

ART: antiretroviral therapy; Abc: abacavir; d4T: stavudine; EFV: efavirenz; 3TC: lamivudine; NVP: nevirapine; TDF: tenofovir disoproxil fumarate; ZDV: zidovudine; Hb: hemoglobin; SD: standard deviation; SLE: stavudine+lamivudine+efavirenz; SLN: stavudine+lamivudine+nevirapine; TLE: tenofovir+lamivudine+efavirenz; TLN: tenofovir+lamivudine+nevirapine; ZLE: zidovudine+lamivudine+efavirenz; ZLN: zidovudine+lamivudine+nevirapine. A *p* value<0.05 at a 95% confidence interval was considered statistically significant and is shown in bold

nevirapine-based ART regimens were changed to a tenofovir + lamivudine + efavirenz (TLE) regimen. Thirty (5.8%) received anti-tuberculosis treatment (ATT) for pulmonary tuberculosis in a recommended dose and schedule before ART was initiated. The only individual with an HBV co-infection had not received any drug(s) other than ART. Laboratory reports for the leukocyte and platelet count were available only for 492 and 451 cases, respectively. Information for body weight/BMI, iron supplements, alcohol abuse, and the presence of concurrent or opportunistic infections or their treatment and prophylaxis, particularly for *Pneumocystis jiroveci* pneumonia, was not available in the records.

Hemoglobin levels varied from 4.4 to 16.5 g/dL (mean ± SD: 10.8 ± 2.02 g/dL). Three hundred and

sixty-eight (77.7%) individuals had anemia before the initiation of ART, which was mild in 72 (13.9%), moderate in 257 (50.2%), and severe in 39 (7.6%) cases, respectively. The female sex (OR: 2.09; Cl: 95%; CI: 1.41–3.10; $p < 0.05$), a CD4$^+$ count ≤ 200 cells/microliter (OR: 2.36; Cl: 95%; CI: 1.59–3.52; $p < 0.0001$), a WBC count < 4000 cells/mm^3 (OR: 3.29; Cl: 95%; CI: 0.97–11.14; $p < 0.04$), a platelet count < 100,000 cells/mm^3 (OR: 0.50; Cl: 95%; CI: 0.31–0.81; $p < 0.05$), before the initiation of ART (OR: 3.78; Cl: 95%; CI: 2.91–4.91; $p < 0.0001$), ATT intake (OR: 5.88; Cl: 95%; CI: 1.38–25.04; $p < 0.05$), and nevirapine hypersensitivity (OR: 0.38; Cl: 95%; CI: 0.07–1.95; $p < 0.04$) were significantly associated with anemia (Table2). However, zidovudine-containing ART regimens were not found to be significantly associated with anemia (OR: 0.71; Cl: 95%; CI: 0.49–1.02; $p = 0.067$). Meanwhile, ART significantly improved hemoglobin levels and corrected anemia, as only 206 (40%) individuals had anemia after ART, as opposed to 368 (77.7%) with anemia before the initiation of ART ($p < 0.0001$).

DISCUSSION

The demographic profile of HIV-affected individuals in this study was similar to what has been described in the past [6,22]. The prevalence of anemia ranged between 10% to 28% in asymptomatic HIV-infected individuals in the pre-AIDS stage and 71–92% in those with AIDS across countries [6,9-11,13]. In general, the prevalence of anemia was higher in those ART-naïve than those on ART, and severity varied from mild to severe [4,9,11-13]. In a recent study, it was mild in 13%, moderate in 14%, and severe in 7.4% of cases, respectively [11]. In our study, the prevalence of anemia was 77.7% before ART was initiated, which was moderate in almost half of the cases, as opposed to mild in 13.9% and severe in 7.6% of the individuals. The prevalence decreased to 28.3% after ART, conforming to the above epidemiological trends. Apart from anemia of a chronic disease, HIV-associated anemia may be due to nutritional deficiencies, blood loss, medication-induced hemolysis, or bone marrow suppression (trimethoprim–sulfamethoxazole, zidovudine, amphotericin) [18]. The female sex, a CD4$^+$ count below 200 cells/microliter, and ATT intake were other significant factors associated with anemia in this study. These observations suggest that HIV infection by itself is an important cause of anemia in the majority of the HIV-affected. HIV infection is believed to affect bone marrow functioning directly or from opportunistic infections especially during the early phase of uncontrolled HIV multiplication, or because of drug toxicity over a period of time [20]. Anemia in individuals before the initiation of ART, a WBC count below 4000 cells/mm^3, and thrombocytopenia (platelet count < 100,000/mm^3) in this study, as well

Table 2: Factors associated with anemia

Factors		Total No. of Patients	Anemia		OR (CI: 95%)	Confidence Interval	*p* value
			No. of Patients				
			Yes	No			
Sex	Female	269	212	57	2.09	1.41–3.10	**0.0002**
	Male	244	156	88	-	-	-
CD4$^+$cell count	≤ 200 cells/microliter	252	201	51	2.36	1.59–3.52	**< 0.0001**
	> 200 cells/microliter	261	163	98	-	-	-
WBC* count	< 4000/mm^3	26	23	3	3.29	0.97–11.14	**0.04**
	> 4000/mm^3	466	326	140	-	-	
Platelet** count	< 100,000/mm^3	306	208	98	0.50	0.31–0.81	**0.005**
	> 100,000/mm^3	145	117	28	-	-	-
ART Status	Before ART	513	368	145	3.78	2.91–4.91	**< 0.0001**
	After ART	513	206	307	-	-	-
ART Regimen	With ZDV	199	70	129	0.71	0.49–1.02	0.067
	Without ZDV	314	136	178	-	-	-
ATT***	Yes	30	28	2	5.88	1.38–25.04	**0.016**
	No	483	340	143	-	-	-
NVR DHS	Yes	6	3	3	0.38	0.07–1.95	**0.04**
	No	507	365	142	-	-	-

ART: anti-retroviral therapy; ATT: anti-tuberculosis treatment; CI: confidence interval DHS: drug hypersensitivity syndrome; g/dL: grams/deciliter; Hb: hemoglobin; NVR: nevirapine; OR: odds ratio; WBC: white blood cells; ZDV: zidovudine. A *P* value<0.05 at a 95% confidence interval was considered statistically significant and is shown in bold.*Reports were available for 492 cases only.
**Reports were available for 451 cases only.
***ART was initiated only after the completion of ATT comprising rifampicin (10 mg/kg), isoniazid (5 mg/kg), ethambutol (15 mg/kg), pyrazinamide (25 mg/kg) for two months in the intensive phase followed by rifampicin and isoniazid for four months in the continuation phase

as the other significant factors ($p < 0.5$) associated with anemia, also reflect some amount of HIV-induced myelosuppression. However, none of our patients were pregnant, had chronic renal failure, or received a blood transfusion for severe anemia or blood loss. Individuals with a low $CD4^+$ cell count have a high viral load and are at an increased risk for opportunistic infections, which in turn may lead to an increased prevalence of anemia. Concurrent opportunistic infections (parvovirus B19, *Pneumocystis jiroveci*, tuberculosis, *Mycobacterium avium complex*) and their therapies, for instance, tuberculosis, due to chronicity, bone marrow suppression, malnutrition, and hemoptysis, and any severe drug toxicity (including from nevirapine) possibly enhance the risk of anemia in these individuals, as was observed in this study [11,15,18]. An improvement in anemia after ART, as in this study, is due to the suppression of the viral replication/load and improved immunity against opportunistic infections and the overall gain in health reinforcing the foregoing [13]. Although zidovudine-containing ART regimens, especially the during the early phase of ART initiation, have been identified to be associated with anemia, especially among individuals with low baseline hemoglobin and pure red cell aplasia in particular, we made no such observations, as individuals with low Hb were not put on zidovudine-containing regimens [13,16,23]. However, only a small number of individuals were on zidovudine-containing ART for a meaningful conclusion and may not reflect the real effect of zidovudine. In our study, details on other risk factors of anemia, such as *Pneumocystis jiroveci* prophylaxis and the nutritional status of the HIV-affected, could not have been ascertained from the records

Limitations

This study was limited by a small number of subjects, its single-center stratification, and its retrospective study design. Data on red blood cell indices, types of anemia, and other potential risk factors, such as alcohol abuse, dietary habits and nutritional statuses, opportunistic infections and their treatment or prophylaxis, which might have influenced the results, at least in some of the cases, was not recorded. The analysis of anemia on a yearly basis was impossible due to retrospective analysis. As ART is regimen-based, the identification of an individual drug responsible for anemia was not possible. Viral-load studies were not part of the study.

CONCLUSION

Anemia may be a significant co-morbidity among HIV-infected individuals, especially in females, before the initiation of ART, and those with a $CD4^+$ count ≤ 200 cells/microliter, a WBC count < 4000 cells/mm^3, thrombocytopenia, a history of nevirapine adverse reactions, or ATT intake. While 50% of affected individuals may have moderate anemia, the spectrum varies from mild to severe anemia, necessitating screening for the overall health of the affected individual. ART significantly improves pre-existing HIV-associated anemia in the majority. The low potential of zidovudine-containing regimens for ART-associated anemia perhaps does not reflect the real effect of zidovudine because of the small number of these cases. Well-designed and prospective studies addressing the limitations of this study are highly desirable to further our understanding of factors associated with anemia and its effect on the overall health of the HIV-affected and the interventions needed.

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Statement of Ethics

The study was approved by the Institutional Ethics Committee (registration number: ECR/490/Inst/HP/2013/RR-16) vide letter no. HFW-H-DRPGMD/Ethics/17/2018-91, dated 19-05-2018. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975 as revised in 2013.

Statement of Human and Animal Rights

All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 2008 revision of the Declaration of Helsinki of 1975.

Statement of Informed Consent

Informed consent for participation in this study was obtained from all patients.

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