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Predictors of anemia among a cohort of people living with HIV on anti-retroviral therapy in a southern state of India

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Abstract

Introduction: Anemia is the most prevalent hematological complication among people living with HIV (PLHIV), and its presence can affect the disease progression and mortality. This study aims to ascertain the prevalence of anemia and the factors predicting it in PLHIV on anti-retroviral therapy (ART).

Material and methods: In this study, 228 PLHIV who were enrolled for ART in the study period of 3 years from 2012 (February) to 2015 (February) were selected and the data from their records were used for analysis. Factors predicting anemia in PLHIV were assessed using univariate and multivariate logistic regression.

Results: The prevalence of anemia in our study was found to be 62.2% (n = 142). The mean hemoglobin among PLHIV was 11.8 g/dl. Age below 45 years, female gender, low body mass index (BMI), smoking, consumption of alcohol, stage 3 or 4 HIV, presence of opportunistic infections, low CD4 count and low total leukocyte count were the factors found to be associated with anemia among PLHIV. On multivariate analysis, low BMI, low CD4 count and low total leukocyte count were the factors significantly predicting anemia among PLHIV.

Conclussions: Anemia is a common complication seen among PLHIV. Low CD4 count, low total leukocyte count and undernutrition significantly predict anemia among PLHIV.

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Key words: anemia, PLHIV, cohort, predictors, South India.

Introduction

Human immunodeficiency virus (HIV) causes opportunistic infections and acquired immunodeficiency syndrome (AIDS) related neoplasms as the virus affects CD4 cells, leading to a poor immune response to various infective organisms [1].

With prevalence of 0.26% among the adult population, HIV in India has reached epidemic proportions [2]. Karnataka, a southern state in India, has prevalence of HIV of 0.36% among the general population, which is much higher compared to national statistics [2, 3].

Anemia is a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs. Anemia is defined as low hemoglobin levels or low hematocrit. According to the World Health Organization (WHO), hemo-

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globin (Hb) levels to diagnose anemia are < 13 g/dl in males and < 12 g/dl in females [4].

HIV is associated with hematological disorders and anemia is the most prevalent hematological complication [5, 6]. Anemia in people living with HIV (PLHIV) may be due to numerous pathophysiological changes taking place during the course of illness and it is very difficult to pinpoint the exact reason. The effect on hematopoiesis due to the viral effect on cytokine production, decrease in the level of erythropoietin, presence of numerous opportunistic infections and use of anti-retroviral drugs such as zidovudine are some of the reasons [6]. These along with underlying anemia especially in HIV patients from low income countries tend to further complicate the illness and affect the survival of patients [6, 7].

Many studies have been conducted to detect the prevalence of anemia among PLHIV, and the results have been variable, ranging from 19% to 69% [8-11]. Decreased quality of life, accelerated disease progression, and increased mortality in HIV-infected individuals are independently associated with anemia, irrespective of other indicators of poor prognosis, i.e. low CD4 cell count, high HIV viral load or the manifestation of AIDS-defining conditions [6, 12, 13].

There are a few socio-demographic, immunological and clinical factors that have been associated with anemia in PLHIV. Some of these factors are female gender [6, 8, 9, 14], older age [12, 15], high HIV viral load [15-18], low CD4 cell count [8, 9, 11, 12, 14-18], presence of opportunistic infections [14, 16, 17] and low body mass index (BMI) [9, 11, 12]. Leukopenia and thrombocytopenia have also been associated with anemia in PLHIV [6].

Data regarding prevalence of anemia in PLHIV and its impact on the disease from India are lacking. Most of the studies determining the factors associated with anemia are from developed countries and from developing nations of Africa, with very limited studies from India. Results obtained from our study could differ from other countries due to factors such as malnutrition, the diverse spectrum of opportunistic infections, etc., present in our study area. Hence, this study aims to ascertain the prevalence and factors predicting anemia in PLHIV on anti-retroviral therapy in a southern state of India.

Material and methods

This study was conducted in an Anti-Retroviral Therapy (ART) Centre attached to Kasturba Medical College Hospital in Mangaluru, India. A total of 228 PLHIV who were started on ART in the study period of 3 years from 2012 (February) to 2015 (February) were included in this study. The criteria to initiate ART on PLHIV were CD4 count < 350 cells/mm^3 or WHO stage 3 or 4 [6, 8]. Exclusion criteria included PLHIV who were started on ART before February 2012.

PLHIV were considered anemic if their hemoglobin levels were < 13 g/dl for males and < 12 g/dl for females. The anemic PLHIV were then classified into mildly anemic (> 11 g/dl), moderately anemic (8-11 g/dl) and severely anemic (< 8 g/dl) [4].

Approval from the Institutional Ethics Committee of Kasturba Medical College, Mangaluru (Manipal Academy of Higher Education) was obtained. Using a data extraction sheet, the data were collected from records of the ART centre. The sheet was divided into 3 sections containing general data, clinical/laboratory data and therapeutic data, which included information on BMI (Asia-Pacific classification), WHO staging of HIV, opportunistic infections (OIs), CD4 count, laboratory values, etc.

Data analysis

Statistical Package for Social Sciences version 11.5 (SPSS, Inc., Chicago, IL, USA) was used to analyze the compiled data. Descriptive statistics such as mean (standard deviation) and median (IQR) were used to express the data. The independent *t*-test and Mann-Whitney test were used to assess the difference between quantitative variables among outcome groups (anemia/no anemia). Statistical significance was implied if the p value < 0.05. To assess the various factors associated with anemia among PLHIV on ART, univariate and multivariate analysis was used and $p < 0.05$ was considered as an indication of a statistically significant association between predictive and outcome variables. Multivariate analyses were conducted only on variables found to be significant in univariate analysis. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were reported.

Results

A total of 228 PLHIV were started on ART during 2012 to February 2015 (99 in 2012, 41 in 2013 and 88 in 2014). Prevalence of anemia in our study was found to be 62.2% (n = 142), 46.4% (n = 66) were mildly anemic, 47.8% (n = 68) were moderately anemic and 5.6% (n = 8) were severely anemic. Mean hemoglobin among PLHIV was 11.8 g/dl (SD = 2.3).

Demographic details of the study population are described elsewhere [19]. About 31.6% (n = 72) of PLHIV had a low BMI (< 18.5 kg/m^2). WHO stage 3 and 4 diseases was present in 45.6% (n = 104) of PLHIV. OIs were present in 50% (n = 114), the most prevalent being tuberculosis (n = 60, 52.6%).

For statistical evaluation, we combined mild, moderate and severe anemia as presence of anemia, to compare it with PLHIV without anemia. Mean baseline characteristics among the PLHIV with and without anemia were compared. BMI and total leukocyte levels were observed to be significantly lower among the anemic PLHIV in contrast to PLHIV who did not have anemia ($p < 0.05$), as depicted in Table 1.

Comparison of laboratory parameters among PLHIV with and without anemia is shown in Table 2. It was noted that the anemic PLHIV had a lower CD4 count, higher erythrocyte sedimentation rate (ESR) and alanine transam-

Table 1. Comparison of baseline characteristics among people living with HIV (*N* = 228)

Variable	Anemia status		*t* value	Mean difference (CI)	*p* value
	Anemia (*n* = 142), mean (SD)	No anemia (*n* = 86), mean (SD)			
Age (years)	42.84 (9.4)	44.3 (8.6)	1.17	1.46 (–0.98-3.91)	0.241
BMI (kg/m^2)	19.08 (3.01)	21.68 (3.52)	5.17	0.612 (0.38-0.84)	0.000
TLC (cells/mm^3)	5781 (2171)	6400 (1976)	2.08	618.60 (32.00-1205.2)	0.039

**Independent t-test was done*

Table 2. Comparison of baseline characteristics among people living with HIV (*N* = 228)

Variable	Anemia status		Mann-Whitney *U* (Z)	*p* value
	Anemia (*n* = 142), median (IQR)	No anemia (*n* = 86), median (IQR)		
CD4 count (cells/mm^3)	217 (131-339)	369 (251-486)	3581 (–5.17)	0.000
Platelet (million cells/dl)	0.202 (0.154-0.265)	0.212 (0.157-0.253)	2076 (–0.07)	0.938
ESR (mm/hour)	70 (45-95)	24 (8-37)	1530 (–7.42)	0.000
Urea (mg/dl)	19 (16-25)	19 (15.5-23)	2920 (–0.75)	0.451
Creatinine (mg/dl)	0.8 (0.7-1.0)	0.8 (0.7-1.0)	4208 (–1.00)	0.313
Bilirubin (mg/dl)	0.3 (0.2-0.6)	0.4 (0.3-0.60	643 (–1.07)	0.282
AST (IU/l)	30 (23-47)	30 (23-41)	3509 (–0.44)	0.661
ALT (IU/l)	23 (17-41)	29 (23-41)	3104 (–2.81)	0.005

**Mann-Whitney test was done*

inase (ALT) values in comparison to PLHIV who were not anemic ($p < 0.05$) and this difference was found to be statistically significant ($p < 0.05$).

In order to ascertain the factors associated with anemia, univariate analysis was conducted. Out of the demographic factors, PLHIV aged < 45 years, female gender, smokers and PLHIV who consumed alcohol had higher proportions of anemia. However, none of these demographic factors were found to be significantly associated with anemia ($p > 0.05$). During analysis of the clinical parameters, PLHIV who were underweight (BMI < 18.5 kg/m^2), those with WHO stage 3 or 4 disease, presence of OIs and presence of tuberculosis were factors found to be associated with anemia and this observation was found to be statistically significant ($p < 0.05$). Obesity was statistically associated with negative prediction of anemia. When laboratory parameters were compared, CD4 count < 350 cells/mm^3, low total leukocyte count (TLC < 4,000 cells/mm^3) and low platelet count were found to be associated with anemia, among which low CD4 count and low TLC were found to be statistically significant in univariate analysis.

Factors found significantly associated with anemia in univariate analysis were considered for multivariate analysis. Out of the factors found significant in univariate analysis, only low CD4 count and low BMI were the factors found to be significantly predicting anemia in multivariate analysis. Univariate and multivariate analysis of the factors associated with anemia is depicted in Table 3.

Discussion

HIV infection is known to cause hematological complications such as anemia, leukopenia and thrombocytopenia, out of which anemia is the commonest complication [20]. Anemia in PLHIV is usually due to decreased red blood corpuscle (RBC) production due to various causes, followed by raised RBC breakdown, nutritional deficiencies or a combination of these. Etiology of anemia in PLHIV is multifactorial [5, 6]. HIV virus may infect the bone marrow stromal cells or can increase secretion of cytokines such as tumor necrosis factor which impede hematopoiesis, resulting in reduced production of RBCs. Co-infection of parvovirus B19 with HIV may hinder RBC production. Other aspects of HIV infection which might indirectly give rise to anemia are opportunistic infections, neoplasms, nutritional deficiencies due to anorexia and malabsorption or adverse reaction to antiretroviral drugs, especially zidovudine, which is known for its myelosuppressive effects. In a study conducted by Sullivan *et al.*, 22% of the anemia in their study was drug related [6], highlighting the effect of the drugs on anemia in PLHIV. Other uncommon causes of anemia in PLHIV may include hemolysis (thrombotic thrombocytopenic purpura)

Table 3. Multivariate analysis of the factors predicting anemia (*N* = 228)

Factor	*N* (%)	Anemia *n* = 142 (62.3%)	Unadjusted OR (CI)	*p* value	Adjusted OR (CL)	*p* value
Age						
< 45 years	146 (64.0)	97 (66.4)	1.62 (0.93-2.83)	0.085	1.41 (0.66-3.03)	0.367
> 45 years	82 (36.0)	45 (54.8)				
Gender						
Female	95 (41.6)	63 (66.3)	1.346 (0.77-2.32)	0.289	1.24 (0.68-2.25)	0.471
Male	133 (58.4)	79 (40.7)				
BMI for Asians* (kg/m^2) (*n* = 224)						
Low (< 18.5 kg/m^2)	87 (38.9)	71 (81.6)	3.39 (1.71-6.71)	0.000	4.14 (1.35-12.65)	0.013
Normal (18.5-22.9 kg/m^2)	90 (40.2)	51 (56.6)				
Overweight (23-24.9 kg/m^2)	27 (12.0)	12 (44.4)	0.61 (0.26-1.45)	0.266	0.70 (0.15-3.32)	0.661
Obese (> 25 kg/m^2)	20 (08.9)	6 (30.0)	0.32 (0.12-0.93)	0.036	0.34 (0.24-5.45)	0.304
Smoking						
Yes	36 (16.1)	20 (55.5)	1.38 (0.67-2.83)	0.380	1.16 (0.53-2.53)	0.699
No	192 (84.2)	122 (63.5)				
Alcohol consumption						
Yes	76 (33.3)	42 (55.3)	1.54 (0.87-2.7)	0.134	1.53 (0.60-3.87)	0.370
No	152 (66.7)	100 (65.8)				
Stage of HIV						
Stage 3 and 4	104 (45.6)	82 (78.8)	4.04 (2.24-7.28)	0.000	1.95 (0.66-5.73)	0.224
Stage 1 and 2	124 (54.4)	60 (48.3)				
Opportunistic infections						
Present	114 (50.0)	86 (75.4)	3.18 (1.18-5.58)	0.000	1.34 (0.46-3.83)	0.589
Absent	114 (50.0)	56 (49.1)				
CD4 count						
< 350	147 (64.5)	107 (72.7)	3.61 (2.04-6.41)	0.000	2.83 (1.41-5.67)	0.003
> 350	81 (35.5)	35 (43.2)				
Total leukocyte count (*n* = 206)						
Low	30 (14.6)	26 (86.6)	4.71 (1.57-14.08)	0.005	6.8 (1.17-40.00)	0.033
Normal	176 (85.4)	102 (58)				
Platelet count (*n* = 137)						
Low	111 (81.0)	72 (64.8)	1.47 (0.56-3.8)	0.427	1.13 (0.43-3.07)	0.799
Normal	26 (19.0)	19 (73.0)				

**Asia Pacific classification*

and gastrointestinal bleeding. Anemia of a chronic disease like picture is seen sometimes in PLHIV with anemia due to decreased RBC production and a reduced reticulocyte response due to chronic HIV infection.

Previous studies have shown that anemia decreases the survival time of PLHIV and predicts mortality [6, 10, 13, 16, 18]. Anemia affects the natural history of HIV and increases the rate of progression of the disease, resulting in increased mortality in both developed and developing nations. In a study conducted in Europe, a decrease of 1 g/dl of hemoglobin increased the mortality by 57%, illustrating the effect of anemia on mortality [10]. There is also evidence that improvement of hemoglobin in PLHIV increases the survival time [6]. Anemia might not necessarily have a causal relationship with mortality, but it might be an indicator of a compendium of comorbid systemic states [16, 18]. Even though treatment with ART increases the hemoglobin concentration, reversal of anemia is usually incomplete, indicating a complex mechanism for the development of cytopenias [11]. Anemia can only be partially treated with use of ART. Anemia is so frequent among PLHIV that in our study we found the prevalence of anemia to be as high as

62.2%, with the majority coming under category of moderate anemia (Hb of 8-11 g/dl). The prevalence of anemia in PLHIV in our study setting is similar to the results obtained in a study in south India [9]. With the importance of anemia in predicting mortality, such high prevalence of anemia in the setup needs to be studied further to evaluate the predictors of anemia in PLHIV and indirectly reduce mortality.

In our study, anemic patients also had a significantly low BMI, low total leukocyte count, low CD4 count and high erythrocyte sedimentation rate. Female gender, age < 45 years, low BMI (< 18.5 kg/m^2), alcohol consumption, smoking, WHO stage 3 and 4 disease, presence of opportunistic infections, low CD4 count (< 350 cells/mm^3), low total leukocyte count (< 4000 cells/mm^3) and low platelet count (< 0.15 million cells/dl) were the factors associated with anemia in our study. However, only low BMI, low CD4 count and low total leukocyte count were the factors significantly associated with anemia.

Nutritional status was measured using body mass index in our study. Undernutrition, i.e. BMI < 18.5 kg/m^2, had an independent significant association with anemia, and it is consistent with findings from studies conducted in south India [9], Rwanda [10] and the Netherlands [14]. PLHIV who had anemia also had significantly low mean BMI compared to the non-anemics. Undernutrition could result in anemia due to nutritional deficiencies of iron and vitamins necessary for hematopoiesis. Both undernutrition and anemia are associated with increased mortality. In our study, overweight (> 23 kg/m^2) and obese individuals (> 25 kg/m^2) showed a protective effect against anemia, though the association was not statistically significant, consistent with the findings from a study conducted in Puerto Rico [18].

Low CD4 count was independently associated with anemia. Median CD4 count was also significantly lower in the anemic population of the study. The results were similar to studies conducted in African nations, Europe and North America [8, 9, 11, 14, 15, 17, 18, 20]. Low CD4 count indicates the late stage of the disease and anemia is expected as a common hematological complication.

Leukopenia (< 4000 cells/mm^3) was also significantly associated with anemia and mean total leukocyte count was significantly lower in the PLHIV who had anemia. Studies conducted in the United States of America [6] and Puerto Rico [18] also showed similar findings. HIV infection affects bone marrow production of hematopoietic cells, and in our study the effect was seen in red blood cells and white blood cells, but not with megakaryocytes. From our study we conclude that anemia is a common complication seen among PLHIV with high prevalence. Low CD4 count and undernutrition significantly predict anemia among PLHIV. A limitation of the study is the lack of longitudinal data to determine the temporal relationship of anemia with the factors associated with anemia. There was a lack of data regarding the laboratory parameters which would help in determining the probable etiology of anemia in PLHIV. Data obtained for the study were from records which were not primarily collected for the purpose of research and hence lacked some data. Most of the consideration in PLHIV is given in treating HIV with ART and treatment complications such as opportunistic infection, and little attention is given to evaluation and management of anemia. Studies have shown that improvement in hemoglobin level increases survival time, and hence screening for anemia should be advised on a regular basis and management of anemia should be provided after necessary evaluation. Emphasis should also be given to serial weight checking and nutritional supplements as good nutrition has protective effect against anemia.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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