



STUDY OF ANAEMIA IN HIV/AIDS PATIENTS AND ITS RELATION WITH CD4 COUNTS

Dr. Shilpa Mittal

Dr. Praveen Mangal* *Corresponding Author

ABSTRACT

Background and objectives: Anemia is an important clinical problem in patients with HIV infection and those with AIDS. It is an important cause of increased morbidity in HIV patients, and is still usually under investigated.

Very few studies have correlated the immune status with the severity of anaemia. The present study aims to analyze the type and severity of anaemia with CD4 cell counts.

Materials and Methods: This was a prospective descriptive study consisting of seventy five HIV/AIDS patients with clinically evident anaemia coming to ART centre of a tertiary health care center. Study consisted of a medical history, CD4 T-lymphocyte count, complete hemogram with red blood cell indices, and peripheral smear picture. Bone marrow examination was done after proper written consent. Anemia was classified according to the World Health Organization criteria. Data analysis was carried out using Microsoft Excel and Statistical Package for the Social Sciences software.

Results: Normocytic normochromic anaemia was the most common type of anaemia seen in 58.66% of patients. 62.16% of AIDS patients had severe anaemia whereas only 50% of Non-AIDS patients had severe anaemia. Stainable iron in bone marrow was found to be normal in most of the patients i.e. 45 (60%) out of 75 cases, out of which 22 were in AIDS group and 23 were in Non AIDS group.

Conclusion: There was increased severity of anaemia in advanced stage of disease. Variations in type and underlying mechanisms of anaemia in HIV patients warrants careful evaluation for the wellbeing of the patient.

KEYWORDS : Anemia; CD4 counts; HIV positive

INTRODUCTION

Anemia is an important clinical problem in patients with HIV infection and those with AIDS. 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 concentration¹. Despite the advent of Highly active antiretroviral therapy (HAART), HIV- related anaemia is still common, and independently associated with decreased survival.^{2,3,4} CD4 measurements are the most important indicator of mortality and wider access to affordable tests is needed in resource limited settings⁵. There are very few studies with proper statistical correlation of severity of anemia with CD4 cell counts.

The present study aims to analyze the type and severity of anaemia in HIV/AIDS patients and its relation to CD4 counts along with bone marrow stainable iron.

MATERIALS AND METHODS

A prospective descriptive clinical study of 75 HIV/AIDS patients at a tertiary health care center was done. Patients with clinically evident anemia reporting to ART center / ICTC of a tertiary health care centre and age between 18-58 years were taken. Pregnant women were excluded from the study. HIV confirmation was done by three rapid tests –SD Bioline, comb-AIDS and Retrochek on patients attending ART center / ICTC. CD-4 count was obtained from ART center done on cyflow counter and B, D facts count.

Three milliliters of peripheral venous blood sample were obtained in an ethylenediaminetetraacetic acid vial from each patient. The samples were analyzed within 4 h of collection. Hematologic parameters were obtained by sysmex KX-21 automated cell counter. Peripheral smears, stained with Leishman's stain, were examined to define the morphology of cells. Anemia based on RBC morphology was classified as normocytic normochromic, microcytic hypochromic, macrocytic. Anaemia was defined as clinically evident anaemia i.e., hemoglobin level $\leq 10\text{gm/dl}$.

Bone marrow aspiration was done after taking informed consent of the patients. The procedure was done at the posterior iliac crest with

aseptic technique after giving local anesthesia (1% lidocaine) in the skin, subcutaneous tissue, and periosteum. A sixteen-gauge bone marrow aspiration needle was used in the procedure.

Smears of the aspirated material were made on glass slides. They were allowed to dry and stained with Leishman's stain and examined by the pathologists for final opinion. Iron stores were evaluated on the aspirates by staining them with Pearl's stain. Grading of iron stores on bone marrow was done as per Gale et al⁶. Patients were divided in two groups as AIDS and Non- AIDS patients based on CD4 counts $\leq 200/\mu\text{l}$ and $>200/\mu\text{l}$.

Statistical Analysis

Results were tabulated in Microsoft office excel worksheet and expressed as mean \pm standard deviation for continuously distributed variable and in absolute numbers and percentages for discrete variables. Appropriate standard statistical methods were utilized Chi-Square test and p-value were analyzed. p-value of less than 0.05 was considered statistically significant. Results were demonstrated in the form of tables, graphs and pie- diagrams.

OBSERVATION AND RESULTS

Total number of patients included in the study were 75, out of which 42 were males and 33 were females. (M:F= 1.27:1)

The mean age (in years) of male patients (n=42) was 38.81 and mean age of female patients (n=33) was 38.93 years.

Maximum number of patients i.e. 36 (48%) belong to age group between 31 – 40 years.

Out of 42 male patients 25 (59.52%) were in age group of 31 – 40 years, followed by 8 patients (19.04) in age group 41 – 50 years.

Similarly, out of 33 female patients, 11 patients were in age group each of 21 – 30, and 31 – 40 years. Total number of patients in AIDS group was 38 and in Non-AIDS group was 37.

Baseline haematological parameters were as mean haemoglobin (g/dl) of all patients was 7.58 ± 2.00 . Mean Total Leukocyte count (per mm^3) was 5424 ± 2889.4 and mean Platelet count (lakhs/ mm^3) was 2.11 ± 1.04 .

Distribution of type of anaemia is as given in table 1.

Table No 1: Distribution of type of Anemia in HIV patients

Type of Anemia	AIDS (n=38)		Non-AIDS (n=37)		Total	
	Count	%	Count	%	Count	%
NNA	22	57.89%	22	59.45%	44	58.66%
MHA	13	34.21%	08	21.62%	21	28.00%
MA	03	7.89%	07	18.91%	10	13.33%
	38	100%	37	100%	75	100%

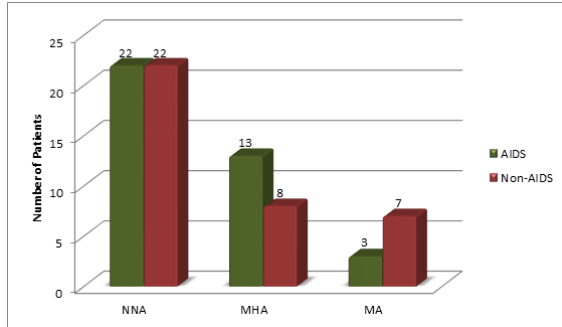


Figure 1: Distribution of type of anemia in HIV patients

Normocytic normochromic anaemia (NNA) was the commonest type of anaemia seen in 44 (58.66%) patients and accounting for 22 (59.45%) out of 37 Non AIDS and 22 (57.89%) out of 38 in AIDS patients.

Microcytic hypochromic anaemia (MHA) was second most common and was seen in 21 (28.00%) patients. Out of 21 patients 8 were Non AIDS, accounting for 21.62% of Non AIDS patients and 13 were in AIDS group, accounting for 34.21% of total AIDS patients. Macrocytic anaemia (MA) was seen in 10 (13.33%) patients. Out of 10 patients 7 were in Non AIDS group accounting for 18.91% of Non AIDS patients and 3 were in AIDS group, accounting for 7.89% of AIDS patients.(Fig. 1)

Severity of anaemia among AIDS and Non-AIDS patients is as given in table 2.

Table No 2: Distribution of severity of anaemia in HIV patients

AIDS/NON-AIDS	Hb≤8gm/dl	Hb>8gm/dl	Total
AIDS	14	23	37
NON-AIDS	19	19	38

62.16% of AIDS patients had severe anaemia whereas only 50% of Non-AIDS patients had severe anaemia.

Grading of anaemia was done according to WHO grading system i.e. moderate anaemia with haemoglobin 8-10.9 g/dl and severe anaemia with haemoglobin <8gm/dl. Out of 75 patients, 38 (50.66%) had moderate anaemia and 37 patients (49.33%) had severe anaemia.

Table No 3: Distribution of stainable iron in HIV/AIDS patients.

Stainable Iron	AIDS		Non AIDS		Total	
	n=38	%	n=37	%	n=75	%
Normal	22	57.89%	23	62.16%	45	60%
Increased	03	7.89%	09	24.32%	12	16%
Decreased	13	34.21%	05	13.51	18	24%

Stainable iron in bone marrow was found to be normal in most of the patients i.e. 45 (60%) out of 75 cases, out of which 22 were in AIDS group and 23 were in Non AIDS group.

It was increased in 12 (16%) of total cases, out of which 3 were in AIDS group and 9 were in Non AIDS group. It was decreased in 18 (24%) of total cases out of which 13 were in AIDS group and 5 were in Non AIDS group.

Table No 4: Distribution of stainable iron in relation to type of Anaemia

Type of Anaemia	Stainable Iron						Total
	Normal		Increased		Decreased		
NNA	24	54.54%	11	25%	09	20.45%	44
MHA	11	52.38%	01	4.76%	09	42.85%	21
MA	10	100%	00	00%	00	00%	10

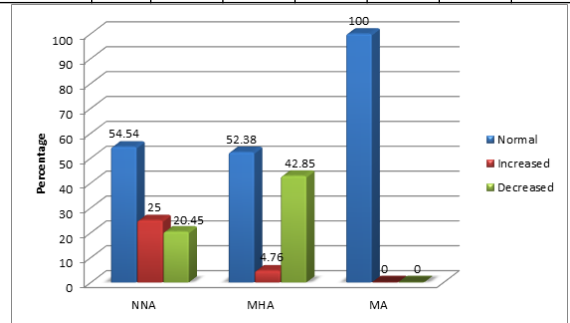


Figure 2: Distribution of stainable iron in relation to type of anaemia

Stainable iron in bone marrow in normocytic normochromic anaemia was found to be adequate in most of the cases i.e. 24 (54.54%) out of 44 cases, followed by increased stainable iron in 11 (25%) out of 44, followed by decreased 9 (20.45%) out of 44 cases.(Fig.2)

Even in microcytic hypochromic anaemia, it was found to be normal in most of cases, i.e. 11 (52.38%) out of 21 cases, decreased in 9 (42.85%) out of 21 cases, increased in 1 (4.76%) out of 21 cases. In macrocytic anaemia, it was found to be normal in 100% of cases i.e. 10 out of 10 cases.

DISCUSSION

Anaemia was graded according to the severity into moderate and severe categories according to WHO criteria⁷ in which mild anaemia is 11-12.9 g/dl, moderate is 8-10.9 g/dl, and severe is <8 g/dl.

Attili et al⁸ reported mild anaemia in 23.05%, moderate anaemia in 41.45%, and severe anaemia in 36.65%.

In present study, we observed moderate anaemia in 50.66% and severe anaemia in 49.33%.

Our results are in concordance with study done by Attili et al⁸, considering the fact that some difference in results is because patients with mild anaemia were not included in our study.

Tripathi et al⁹ studied bone marrow abnormalities in 74 HIV/AIDS patients. They reported normocytic normochromic anaemia in 88.52% of patients.

Pande et al¹⁰ studied hematological changes in HIV infection in 46 patients. Normocytic normochromic anaemia was the predominant type of anaemia in their study, seen in 63% of patients.

Khandekar et al¹¹ studied 140 bone marrow aspirates. They reported normocytic normochromic anaemia in 48.57%.

In the present study, we found normocytic normochromic anaemia in 58.66%.

Although, the incidence of normocytic normochromic anaemia in present study is lower than the study done by Tripathi et al⁹ but it is in concordance with the study done by Khandekar et al¹¹. So, the difference in incidence of normocytic normochromic anaemia with the study done by Tripathi et al⁹ can be attributable to different cohort of population.

The pathogenesis of HIV associated anemia is unclear and is likely to

be multifactorial in nature. Possible causes are bleeding, insufficient dietary intake, hemolytic anaemia, changes in erythropoietin synthesis and bone marrow suppression. Myelodysplastic changes due to HIV can explain normocytic or macrocytic anaemia which is common in AIDS. Perl's Prussian blue stain was performed on all cases to evaluate iron content of the bone marrow.

According to **Khandekar et al**¹¹ stainable iron was within normal range in 50.71% cases, decreased in 29.29% of cases, while increased in 20% cases.

Pande et al¹⁰ also revealed adequate iron status in most of the cases. In their study they found adequate iron store in 82.61%, decreased in 13.04%, and increased in 4.35%. Study done by **Castella et al**¹² revealed increased iron stores in most of the cases.

In present study we reported adequate stainable iron in 60% of the cases, followed by decreased stainable iron in 24%, increased in 16%. Our findings are in tune with most other studies^{10, 11}. Lack of concordance between our study and few other studies¹² could be attributable to higher incidence of reticuloendothelial blockade in their patients which in turn can be due to advanced stage of infection at the time of presentation.

CONCLUSION

In this study we found normocytic normochromic anaemia as the most common type of anaemia. Also, the severity of anaemia is more in advanced stage of disease. Stainable iron in bone marrow is found to be adequate in most cases despite of anaemia which warrants careful evaluation for underlying causes.

REFERENCES

1. Meidani M, Rezaei F, Maracy MR, Avijgan M, Tayeri K. Prevalence, severity, and related factors of anaemia in HIV/AIDS patients. *J Res Med Sci.* 2012 Feb; 17(2):138-42.
2. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ward JW. Epidemiology of anemia in HIV infected persons: results from multistate adult and adolescent spectrum of HIV disease surveillance project. *Blood.* 1998;91:301. PMID:9414298.
3. Berhane K, Karim R, Cohen MH, Levine AM. Impact of highly active antiretroviral therapy on anemia and relationship between anemia and survival in a large cohort of HIV-infected women: Women's interagency HIV study. *J Acquir Immune Defic Syndr.* 2004;37:1245. DOI: 10.1097/01.qai.0000134759.01684.27.
4. Wenger JD, Whalen CC, Lederman MM, Spech TJ, Carey JT. Prognostic factors in acquired immunodeficiency syndrome. *J Gen Intern Med.* 1988;3:464. DOI: 10.1007/BF02595923.
5. Bhusal KR, Devkota S, Shrestha M, Khadga P. Profile of Anaemia in HIV Positive Patients. *JCMS Nepal.* 2016;12(2):70-3.
6. Gale E, Torrance J, Bothwell T. The quantitative estimation of total iron stores in human bone marrow. *J Clin Invest.* 1963;42:1076-82.
7. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and mineral nutrition information system. Geneva: World health organization; 2011 (WHO/NMH/NHD/MNM/11.1) available at <http://www.who.int/vmnis/indicators/haemoglobin.pdf>.
8. Attili SV, Singh VP, Rai M, Varma DV, Gulati AK, Sundar S. Hematological profile of HIV patients in relation to immune status- a hospital based cohort from Varanasi, North India. *Turk J Hematol* 2008;25:13-19.
9. Tripathi AK, Kalra P, Misra R, Kumar A, Gupta N. Study of bone marrow abnormalities in patients with HIV disease. *J Assoc Physicians India* 2005;53:105-10.
10. Pande A, Bhattacharya M, Pain S, Samanta A. Study of bone marrow changes in antiretroviral naive human immunodeficiency virus-infected anemic patients. *Indian J Pathol Microbiol* 2011;54(3):542-45.
11. Khandekar MM, Deshmukh SD, Holla VV, Rane SR, Kakrani AL, Sangale SA et al. Profile of bone marrow examination in HIV/AIDS patients to detect opportunistic infection, especially tuberculosis. *Indian J Pathol Microbiol* 2005;48(1):7-12.
12. Castella A, Croxon TS, Mildvan D, Witt DH, Zaluski R et al. The bone marrow in AIDS. A histologic, hematologic, and microbiologic study. *Am J Clin Pathol* 1985;84:425-32.