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# High Prevalence of Anemia among Human Immunodeficiency Virus-Infected Patients: A Cross-sectional Study in Bali-Indonesia

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**Anemia is a common condition found among Human Immunodeficiency Virus (HIV)-infected patients. T-cells that are infected with HIV virus can directly suppress the growth of progenitor cells in the bone marrow so that affecting the hemopoiesis resulted in anemia. These hematological abnormalities could potentially cause serious clinical implications. This study was conducted to determine the prevalence of anemia in HIV-infected patients in Bali. It was ahospital based cross-sectional studyconducted at two public hospital in Bali-Indonesia. A total of 243 medical record data from HIV-infected patients atWangaya Hospital Denpasar Bali and Sanjiwani Hospital Gianyar Bali between 2009 -2017 were included in analysis. Prevalence of anemia in antiretroviral (ART)-naïve patients and in patients on ART were 60.8% and 40.3%, respectively. Leucopenia and thrombocytopenia were found 8.2% and 9.4%, respectively, in ART-naïve group. While in patients with ART group leucopenia and thrombocytopenia were accounted for 6.9% and 4.3%, respectively. There was a significant difference in prevalence of anemia between ART-naïve patients and patients on ART (60.8% vs. 40.3%; $p < 0.05$). Although the prevalence of anemia was lower in patients on ART, but anemia continues to be common in a substantial portion of HIV-infected persons. Another studies are still needed to addressing the impact of anemia on HIV-infected individuals, as well as treatment strategies and future research directions.**

**Keywords:** Anemia, Antiretroviral, Bali, HIV, Indonesia.

Anemia is a common hematological abnormalities in patients with human immunodeficiency virus (HIV) infection. The prevalence of anemia among antiretroviral-naïve HIV-infected patients are around 80-90% in India[1] While in Indonesia, the prevalence is much lower which are around 45-60%[2, 3] Several studies also documented that anemia to be prevalent in up to 25% among HIV-infected patients treated with antiretroviral[4-6]

Several factors are said to account for the anemia in HIV-infected patients. Opportunistic infections and malignancies are known to be major contribution of anemia in HIV. Direct effects of HIV and its viral proteins as well as immune dysregulations during HIV infection were found

to be responsible for bone marrow suppression. In addition, the emerging role of pro-inflammatory pathways that are also thought to contribute to anemia in HIV infection[7]

Although various studies suggested HIV-related anemia complication improved with antiretroviral therapy, this group of patients still develop mild to moderate anemia. Besides, there are also quality of life impairment reported from anemia HIV-related anemia complication. Antiretroviral regimen and the duration of antiretroviral therapy were found to be the predictors of anemia in HIV-infected patients treated with antiretroviral. This might be due to Zidovudine toxicity as most patients were in Zidovudine based antiretroviral regimen[8] Baseline CD4, baseline BMI and duration of zidovudine, female gender, and TB-coinfection were found to be determinant factors for anemia in antiretroviral-treated patients[9]

Several studies reported the prevalence of anemia among HIV-infected patients in Indonesia[2, 10] However, the magnitude of anemia among HIV patients on antiretroviral are not well documented, especially in Bali. Thus, this study investigated the prevalence of anemia among HIV infected patients receiving antiretroviral in two referral hospital in Bali, Indonesia.

## MATERIAL AND METHODS

A hospital based cross-sectional study was conducted on 243 HIV-infected patients at Wangaya Hospital Denpasar Bali and Sanjiwani Hospital Gianyar Bali. Data collection sheet was used to collect the data from the patient's medical record between 2009 -2017. The following WHO definition of anemia was used to determined anemia: Hb d"12 (g/dL) for nonpregnant women, Hb d"11 (g/dL) for pregnant women, and Hb d"13 (g/dL) for men.

Cleaning was done before the analysis of the data. Descriptive statistics was used to get clear picture of variables at baseline. The prevalence of anemia was estimated in different subgroups. The group or categorical data were tested for statistical significance using Chi-square test and t-test.

The ethical approval was obtained from Medical Faculty of Udayana University/Sanglah Hospital Research and Ethics Committee before commencement of study. A formal letter of permission was submitted to Wangaya Hospital VCT Clinic and Sanjiwani Hospital VCT Clinic. Approved was obtained in order to conduct the research in the study sites. Confidentiality was kept by recoding the data with codes and unauthorized person didn't have access to the data.

## RESULTS

A total of 243 HIV-infected patients were enrolled in this study. At baseline, 171 patients (70%) were HIV-infected patients without antiretroviral therapy (naive) and 72 patients (30%) were HIV-infected patients with antiretroviral therapy. Table 1 gives an overview of patient's baseline characteristics, including sex and age.

Age of patients in this study was in the range of 18-64 years old, with median of age 35 years old. Most of them (68.7%) were in reproductive age group (< 40 years old), both in group of naive treatment (71.3%) and in group with antiretroviral therapy (62.5%). Patients were predominantly male (62.6%), with proportion of 64.9% and 56.9% in naive-treatment group and antiretroviral-treatment group, respectively. Only a small proportion of patients (37.4%) were female and only 76 patients (31.3%) were above 40 years old.

Data from complete blood count examination were collected. Hematologic parameters such as white blood cell, red blood cell, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, dan platelet were evaluated. Mean or median of each parameter, both in naive-treatment group and antiretroviral-treatment group, can be seen in Table 2.

Red blood cell count, hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were significantly different between naive-treatment group and antiretroviral-treatment group ($p < 0.05$; Mann Whitney U Test). White blood cell and platelet count in naive-treatment group were lower than antiretroviral-treatment group, but the difference was not statistically significant ($p > 0.05$).

Based on hematologic parameter described in Table 2, it can be identified some hematologic abnormalities that is anemia, leucopenia, and

thrombocytopenia (Table 3). Anemia is the most common hematologic abnormalities that occurred, both in naive-treatment group (60.8%) and in antiretroviral-treatment group (40.3%). Leucopenia and thrombocytopenia were occurred in smaller proportion, which is 8.2% and 9.4%, respectively, in naive-treatment group. While in antiretroviral-treatment group the proportion of leucopenia and thrombocytopenia was 6.9% and 4.3%, respectively. The prevalence of anemia in naive-treatment group was higher than those in antiretroviral-treatment group (60.8% versus 40.3%, $p < 0.05$). Likewise, the prevalence of leucopenia and thrombocytopenia were higher in naive-treatment group rather than those in antiretroviral-treatment group, but this differences were not statistically significant.

## DISCUSSION

Human immunodeficiency virus infection still a burden in developing countries, including Indonesia. The prevalence of this infection remaining high and still increasing in the past few years. Human immunodeficiency virus type-1 (HIV-1) is the major causative agent of acquired immune deficiency syndrome (AIDS). In Indonesia, especially in Bali, CRF01_AE subtype was the predominant HIV-1 subtype found[11]

Hematological abnormalities were common among HIV-infected patients. Among them, anemia was the most common complication observed. In this study, prevalence of anemia in naive-treatment group was 60.8%, higher than in antiretroviral-treatment group that was 40.3%. Previous studies revealed that prevalence of anemia in naïve-treatment patients ranged from 40%-70%, while in antiretroviral-treatment group the prevalence ranged from 15%-45%[2,3,6,12,13]

There are several factors that contribute to anemia in HIV-infected patients. Some studies indicate that pathophysiology of anemia in HIV-

**Table 1.** Baseline characteristics of HIV-infected patients with and without antireroviral treatment

| Characteristics | HIV-infected patients N (%) | Patients without antiretroviral therapy N (%) | Patients with antiretroviral therapy N (%) |
|---|---|---|---|
| Sex | | | |
| • Male | 152 (62.6) | 111 (64.9) | 41 (56.9) |
| • Female | 91 (37.4) | 60 (35.9) | 31 (43.1) |
| Age | | | |
| • <40 years old | 167 (68.7) | 122 (71.3) | 45 (62.5) |
| • ≥40 years old | 76 (31.3) | 49 (28.7) | 27 (37.5) |

**Table 2.** Hematologic pamaremeterof patients

| Hematologic parameter | Patients without antiretroviral therapy | Patients with antiretroviral therapy | *p*-value |
|---|---|---|---|
| | (n=171) | (n=72) | |
| White blood cell (x$10^3$ /µL) | 6.0 ± 2.8 | 6.4 ± 2.9 | 0.384 |
| Red blood cell (x$10^3$ /µL) | 4.3 ± 0.6 | 4.0 ± 0.8 | 0.013* |
| Hemoglobin (g/dL) | 12.2 ± 1.8 | 12.4 ± 2.4 | 0.039* |
| Hematocrit (%) | 36.4 ± 5.3 | 37.1 ± 7.1 | 0.052 |
| Mean corpuscular volume (L) | 84.8 ± 9.1 | 95.2 ± 11.4 | 0.000** |
| Mean corpuscular hemoglobin (pg) | 28.6 ± 2.3 | 32.1 ± 4.3 | 0.000** |
| Mean corpuscular hemoglobin concentration (%) | 33.5 ± 1.7 | 33.7 ± 1.6 | 0.27 |
| Platelet (x$10^3$ uL) | 249.5 ± 100.2 | 261.4 ± 87.7 | 0.148 |

*P<0.05; **P<0.01

**Table 3.** Hematologic abnormalities among HIV-infected patients with and without antireroviral treatment

| Hematologic abnormalities | Patients without antiretroviral therapy (n=171) | Patients with antiretroviral therapy (n=72) | *p*-value |
|---|---|---|---|
| Anemia | 104 (60.8) | 29 (40.3) | 0.003* |
| -Mild | 88 (51.5) | 22 (30.6) | |
| -Moderate | 12 (7.0) | 4 (5.6) | |
| -Severe | 4 (2.3) | 3 (4.2) | |
| Leucopenia | 14 (8.2) | 5 (6.9) | 0.742 |
| -Mild | 6 (3.5) | 4 (5.6) | |
| -Moderate | 6 (3.5) | 0 | |
| -Severe | 1 (0.6) | 1 (1.4) | |
| Trombositopenia | 16 (9.4) | 3 (4.3) | 0.185 |
| -Mild | 11 (6.4) | 1 (1.4) | |
| -Moderate | 2 (1.2) | 0 | |
| -Severe | 3 (1.8) | 2 (2.8) | |

*P<0.05

infected patients may a result of direct effect of virus itself. HIV infection results changes in cytokine production with subsequent effects on hematopoiesis. The virus inhibit hematopoietic progenitor cell survival and proliferation, thus also result in erythrocyte differentiation impairment. In addition, HIV infection also effect on stromal cell with cytokine dysregulation and development of autoantibodies to erythropoietin and erythrocyte[14, 15] In other circumstances, HIV itself also influences the mtDNA contents and cause toxicity as a result of chronic HIV inflammation[16]

The other factor that contribute to anemia in HIV-infected individual was CD4 count. Previous studies indicated that the frequency and severity of anemia increased in line with decline in CD4 count[4, 17, 18] CD4 is a cell receptor carried by T-helper lymphocytes and monocytes commonly present in the bone marrow. This receptor is found on the cell surface that is the target of HIV. HIV infection on monocyte cell in the bone marrow alter the release of cytokines, which indirectly suppress the capacity of hemopoietic progenitor cells to respond adequately to anemia. This mechanism explain why the severity of anemia increased in line with decline in CD4 count[7, 14, 19] Other studies exploring risk factors of anemia in HIV-infected patients revealed that nutritional status and opportunistic infections also have contribution to anemia in this group of patients[15]

In the present study, the prevalence of anemia in antiretroviral-treatment group was 40.3%. This result was similar with study conducted by Akilimali *et al* that reported up to 43% patients remain anemic after 12 month of antiretroviral therapy[13] Another study in Ethiopia shown the lower prevalence of anemia after 12 month of antiretroviral therapy, that is around 14%[6] Antiretroviral therapy contributes to anemia resolution in many studies[4, 5, 12, 20] However in some cases HIV infected patients cannot always be resolved with durable efficient antiretroviral therapy. It is possibly due to residual inflammation or immune activation rather than unbalanced iron homeostasis[21] Study conducted by Lipshultz *et al* reported that having hemoglobin <14 g/dL for men and <13 g/dL for women was independently associated with monocyte activation in HIV-infected adults on stable antiretroviral therapy. It has been shown that even after suppressing HIV with antiretroviral, persistent systemic inflammation and activation of the immune system occur[22]

The prevalence of having mild anemia in this study was 30.6%. This results are congruent with another studies that reported at least 33% of

patients with antiretroviral therapy experienced mild anemia[22] Although this is slightly higher than in a recent cross-sectional study of HIV-infected adults on ART, the SILCAAT study, the discrepancy is likely due to the different definition used for anemia[23]

## CONCLUSION

The magnitude of anemia among HIV-infected patients was variable based on the study settings and the definition of anemia. Treatment with antiretroviral contributes to anemia resolution due to the positive effect of antiretroviral on the differentiation and survival of erythrocytes. In spite of this, the overall burden of anemia in ART treated patients remains high. Although the prevalence of anemia was lower in patients receiving antiretroviral, but anemia continues to be common in a substantial portion of HIV-infected persons. Another studies are still needed to addressing the impact of anemia on HIV-infected individuals, as well as treatment strategies and future research directions.

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**Conflict of interest**

We have no conflicts of interest to disclose.

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## REFERENCES

1. Panwar A, Sharma S, Kumar S, Sharma A. A study of anemia in human immunodeficiency virus patients: Estimating the prevalence, analyzing the causative effect of nutritional deficiencies, and correlating the degree of severity with CD4 cell counts. *Med. J. DY Patil Univ;* **9**(3):312-8 (2016).
2. Wisaksana R, Sumantri R, Indrati A. R, Zwitser A, Jusuf H, de Mast Q, *et al*. Anemia and iron homeostasis in a cohort of HIV-infected patients in Indonesia. *BMC Infect. Dis.,* **11**(1):213 (2011).
3. Aryastuti S. A, Dewi S. R, Cahyawati P. N, Suryana K, Masyeni S. Hematological and immunological profile of ART-Naïve HIV-infected patients in Bali-Indonesia: a descriptive study. *IOP Conf. Ser. Mater. Sci. Eng.,* **434**:012150 (2018).
4. Woldeamanuel G. G, Wondimu D. H. J. B. H. Prevalence of anemia before and after initiation of antiretroviral therapy among HIV infected patients at Black Lion Specialized Hospital, Addis Ababa, Ethiopia: a cross sectional study. *BMC Hematol.,* **18**(1):7 (2018).
5. Huibers M. H. W,Bates I,McKew S,Allain T. J,Coupland S. E,Phiri C, *et al*. Severe anaemia complicating HIV in Malawi; Multiple co-existing aetiologies are associated with high mortality. *PLoS ONE,* **15**(2): e0218695 (2020).
6. Redig A. J, Berliner N. Pathogenesis and clinical implications of HIV-related anemia in 2013. *Hematology;* **1**: 377-81 (2013).
7. Gedefaw L, Yemane T, Sahlemariam Z, Yilma D. Anemia and Risk Factors in HAART Naïve and HAART Experienced HIV Positive Persons in South West Ethiopia: A Comparative Study. *PloS ONE,* **8**(8):e72202 (2013).
8. Kuwalairat P, Winit-Watjana W. Chumphon Community Hospital Antiretroviral Clinic Group. Determinants for zidovudine-induced anemia in HIV adult patients: A Thai multicenter study. *Arch. Pharm. Pract.,* **5**(1):6-13 (2014).
9. Suryana K, Suharsono H, Budiasa G, Antara J, Astuti P, Indah I. A, *et al*. Correlations between Cluster Differentiation 4 Counts, Human Immunodeficiency Virus Clinical Stages, and Hemoglobin Level among Human Immunodeficiency Virus Patients with Anemia in Merpati Clinic, Wangaya Hospital, Denpasar, Bali, Indonesia: A Cross-Sectional Study. *Asian J. Pharm. Clin. Res.,* **12**(3) (2019).
10. Khairunisa S, Masyeni S, Witaningrum A. M, Yunifiar M. Q, Indriati D, Kotaki T, *et al*. Genotypic characterization of human immunodeficiency virus type 1 isolated in Bali, Indonesia. *HIV AIDS Rev.,* **17**(2):81-90 (2018).
11. Gebremedhin K. B, Haye T. B. Factors Associated with Anemia among People Living with HIV/AIDS Taking ART in Ethiopia. *Adv. Hematol.,* **8** (2019).
12. Akilimali P. Z, Kashala-Abotnes E, Musumari PM, Kayembe P. K, Tylleskar T, Mapatano M. A. Predictors of Persistent Anaemia in the First Year of Antiretroviral Therapy: A Retrospective Cohort Study from Goma, the Democratic Republic of Congo. *PloS One*, **10**(10):e0140240 (2015).

13. Gibellini D, Clò A, Morini S, Miserocchi A, Ponti C, Re MC. Effects of human immunodeficiency virus on the erythrocyte and megakaryocyte lineages. *World J. Virol.,* **2**(2):91-101 (2013).
14. Masaisa F, Gahutu J. B, Mukiibi J, Delanghe J, Philippé J. Anemia in human immunodeficiency virus-infected and uninfected women in Rwanda. *Am. J. Trop. Med. Hyg.,* **84**(3):456-60 (2011).
15. Masyeni S, Sintya E, Megawati D, Sukmawati N. M. H, Budiyasa D. G, Aryastuti S. A, *et al*. Evaluation of antiretroviral effect on mitochondrial DNA depletion among HIV-infected patients in Bali. HIV/AIDS (Auckland, NZ), **10**:145-50 (2018).
16. Ahumareze R. E, Rankin J, David A, Wapmuk A, Disu E, Balogun Y, *et al*. Prevalence of anaemia and the relationship between haemoglobin concentration and CD4 count in HIV positive children on highly active antiretroviral therapy (HAART) in Lagos. *Nigeria Curr. Pediatr. Res.,* 29-36 (2016).
17. Lai J. L, Chen Y. H, Liu Y. M, Yuan J. J, Lin J, Huang A. Q, *et al*. Prevalence and risk factors of anaemia in hospitalised HIV-infected patients in southeast China: a retrospective study. *Epidemiol. Infect.,* **147**: e81 (2019).
18. Wande I. N, Fuadi M. R, Hadi S. The Correlation between total lymphocyte count, hemoglobin levels, lymphocyte/leukocyte ratio (LLR), and lymphocyte/neutrophil ratio (LNR) to CD4 levels in patients with Human Immunodeficiency Virus infection at Sanglah Hospital. *Bali Med. J.,* **8**(2) (2019).
19. Ezeamama E. A, Sikorskii A, Bajwa K. R, Tuke R, Kyeyune B. R, Fenton I. J, *et al*. Evolution of Anemia Types During Antiretroviral Therapy—Implications for Treatment Outcomes and Quality of Life Among HIV-Infected Adults. *Nutrients,* **11**(4) (2019).
20. Quiros-Roldan E, Castelli F, Lanza P, Pezzoli C, Vezzoli M. The impact of antiretroviral therapy on iron homeostasis and inflammation markers in HIV-infected patients with mild anemia. *J. Transl. Med.,* **15**(1):256 (2017).
21. Lipshultz H. M, Hileman C. O, Ahuja S, Funderburg N. T, McComsey G. A. Anaemia is associated with monocyte activation in HIV-infected adults on antiretroviral therapy. *Antivir. Ther.,* **20**(5):521-7 (2015).
22. Borges A. H, Weitz J. I, Collins G, Baker J. V, Levy Y, Davey R. T, *et al*. Markers of inflammation and activation of coagulation are associated with anaemia in antiretroviral-treated HIV disease. *AIDS* (London, England),; **28**(12):1791-6 (2014).