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Duration of Zidovudine Consumption Correlates with Anemia in People Living with HIV

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Article Info

ABSTRACT

Anemia is the most common hematological disorder associated with Human Immunodeficiency Virus (HIV). WHO recommends zidovudine (AZT) as one of the first-line drugs for this infection. It is important to note that the drug has the adverse effect of causing anemia. Therefore, this study correlates AZT consumption duration and the incidence of anemia in HIV-infected individuals. This cross-sectional study was conducted at health service facilities using 213 participants. The data were obtained from medical records, and the assessed parameter was hemoglobin (Hb) level after less or more than a year of zidovudine administration. Furthermore, they were analyzed using a *Pearson's coefficient* contingency test ($p<0.05$) to evaluate the correlation between groups with and without anemia at each period of drug administration. The results showed a significant correlation ($p<0.05$) between sex and age with anemia incidence in HIV patients administered AZT. The duration of this drug intake in HIV-infected individuals was correlated with anemia ($p= 0.024$; OR=0.369). This indicates the use of AZT for at least one year may be a protective factor for the incidence of anemia in patients with HIV compared to the group consuming this drug for less than one year.

INTRODUCTION

Anemia is the most common hematological disorder associated with Human Immunodeficiency Virus (HIV) (Bhardwaj *et al.*, 2020). The prevalence of this complication in HIV infection ranges from 11.7% to 92% (Geletaw, et. al 2017) with a multifactorial cause (Assefa *et al.* 2015). The pathophysiology of anemia includes four basic mechanisms, namely a) blood loss due to medical conditions, b) inadequate erythrocyte production due to viral infiltration into the bone marrow, myelosuppressive HIV therapy, inadequate production of endogenous erythropoietin (EPO), or ineffective EPO response, c) increased destruction of erythrocytes due to hemolysis, and d) nutrient deficiencies such as iron, folic acid, and vitamin B12 (Adediran *et al.* 2016; Chandiwana *et al.* 2021; Geletaw, *et al.* 2017; Meidani *et al.* 2012, Opie 2012) The pathogenesis of this complication in HIV is associated with decreased erythropoietin concentration, opportunistic infections, and zidovudine therapy (Redig *et al.,* 2013). Furthermore, the pro-inflammatory process is the cause of anemia in antiretroviral therapy, similar to the occurrence of the disorder in other chronic diseases. (Goyal *et al.* 2016; Redig and Berliner 2013). HIV infects and grows on CD4 T-lymphocytes (Abbas, *et. al* 2017), causing the cells to secrete cytokines. Meanwhile,

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an increased cytokine response could affect the process of hematopoiesis, including erythropoiesis (Nyambura Mugwe, et. al 2016). Some studies suggested that the incidence of the disease is increased in patients living with HIV/AIDS (PLWHA) receiving zidovudine treatment. The drug could increase intracellular reactive oxygen species (ROS) and damage mitochondrial DNA (mtDNA) (Assefa *et al*., 2015; Dash *et al*., 2015; Ikunaiye *et al*., 2018; Kuwalairat and Winit-Watjana, 2014; Singh *et al*., 2016; Weil, 2015).

Zidovudine is the most common HIV drug that causes low hemoglobin (Hb) levels (Goyal *et al*. 2016). Its consumption by the patient with this virus is known to correlate with the incidence of anemia closely and requires routine monitoring of the drug's toxic effects on Hb levels (Barik, 2016; Goyal *et al*., 2016; Kallianpur *et al*., 2016; Melese *et al*., 2017; Phe *et al*., 2013). The complication could occur in the first 2 - 48 weeks after zidovudine was administered, specifically in the first 24 weeks (Kuwalairat *et al.,* 2014).

Azido thymidine (AZT) or zidovudine is a first-time nucleoside reverse transcriptase inhibitor (NRTIs) and has been recommended by WHO since 1990 to treat AIDS. Furthermore, it is commonly used when tenofovir is contraindicated (Parkes-Ratanshi *et al*., 2015). Minister of Health Regulation No. 87 of 2014 on Antiretroviral Treatment Guidelines states that AZT is a first-line alternative therapy (Kementrian Kesehatan RI, 2015). Therefore, this drug is widely used in Indonesia as a first-line regimen in HIV therapy. Bobat (2020), showed that AZT causes side effects, such as anemia/neutropenia and lipoatrophy, while 3TC (Lamivudine) is relatively well-tolerated. This drug has been associated with more frequent and severe mitochondrial toxicity than 3TC.

Study concerning the effects of AZT on the risk of anemia in PLWHA remains limited, and none was found as it relates to Indonesia, specifically in Semarang. Therefore, this study examines the correlation of AZT consumption to the incidence of anemia in PLWHA. The results are expected to be a primary prevention consideration and a breakthrough in treating anemia in PLWHA receiving AZT treatment. Local governments consider serial hemoglobin monitoring to be a primary prevention method.

SUBJECTS AND METHODS
The subjects enrolled in this study were 213 adults diagnosed with HIV/AIDS from health facilities in the Central Java Province, Indonesia. The participants volunteered after obtaining informed consent. Furthermore, the exclusion criteria are subjects with opportunistic infection and malignancy. The patients received Duviral®, which contains twice daily, 300 mg of zidovudine (AZT) and 150 mg of lamivudine (3TC). The duration of the drug consumption was categorized into less and over one year. Finally, there are two classifications, namely group A with and without anemia which consisted of 52 and 161 participants, respectively.

This study uses a cross-sectional design, where demographic and clinical data were obtained from medical records. This was approved by the Ethics Committee of Medical and Health Research, Faculty of Medicine, Diponegoro University (No.123/EC/FK-RSDK/III/2018) before the investigation started. The assessed parameter was Hb level after less and more than a year of zidovudine administration. Furthermore, approximately five ccs of venous blood were obtained to examine Hb levels. This examination was performed using an automatic hematology analyzer Sysmex Type XN-1000, and Hb levels were categorized as anemia when <12 g/dL in women and <13 g/dL in men. The values are expressed as mean ± standard deviation (SD), and data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA. Additionally, a Pearson's coefficient contingency test (p<0.05) was performed to assess the correlation between two groups (anemia and without anemia) at each period of zidovudine administration.

RESULTS AND DISCUSSION
The univariate descriptive statistical analysis of the participants' socio-demographic characteristics showed that the majority of them were male (64.8%), within the age categories of 30-39 years old (43.7%). Furthermore, the most common risk factor is heterosexual (56.3%). Previous studies also stated that HIV is transmitted in 3 ways, but the most contagious is sexual contact with heterosexual and homosexual partners (Abbas *et al*., 2017). The characteristics of risk factors and body mass index of the study subjects did not play a role in the incidence of anemia with p = 0.060 and p = 0.062 (> 0.05). Table 1 shows the descriptive socio-demographic analysis.

This study showed a significant association (p=0.001) between sex and the risk of anemia in HIV patients. This is in line with the result that women living with HIV/AIDS are more prone to anemia than men (Nnamani *et al*., 2021). A significant correlation (p=0.002) was also discovered between age and the incidence of

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anemia in patients administered AZT. A previous study stated that the prevalence of anemia increases with age (p=0.026). Furthermore, aging is associated with increased proinflammatory cytokines that contribute to EPO resistance (Assefa *et al*. 2015). In terms of hematological function, bone marrow cellularity and red blood cell longevity decrease with age, as does the sensitivity of erythroid progenitor cells to erythropoietin. The thymus organ, which produces various growth factors needed for erythropoiesis, will involute. Therefore, the growth factors produced are also reduced, affecting erythropoiesis (Kaushansky *et al*., 2016).

The analysis results using the Chi-square difference test obtained a p-value of 0.024 (p <0.05), indicating there was a statistically significant relationship between the duration of AZT consumption and the incidence of anemia. This is corroborated by the value of the Odds Ratio (95% Confidence Interval) 0.369 (0.151-0.901). Table 2 shows that patients consuming this drug for more than or equal to one year have a 0.369 chance of developing anemia. Therefore, the consumption of AZT for more or equal to one

year is a protective factor in the incidence of anemia in patients with HIV.

AZT has an active C atom which increases ROS (reactive oxygen species) and damages mitochondrial DNA (mtDNA) due to the accumulation of low-frequency random mutations known as mutational loads (Weil 2015). This drug has the adverse effect of decreasing the replication of human mitochondrial DNA by inhibiting the polymerase enzyme, which interferes with DNA synthesis (Barik 2016; Ghodkea *et al*. 2012; Ukoha *et al*. 2015). Furthermore, AZT inhibits thymidine kinase activity causing the depletion of thymidine triphosphate needed for mtDNA replication. This implies the toxicity effect of this drug begins with a decrease in ATP concentration and glutathione depletion before mtDNA damage is detected. The decreased glutathione levels cause an increase in ROS that could interfere with DNA, protein, and lipids (Lin *et al*., 2018; Salem *et al*., 2016; Vaneet *et al*., 2015). The component of AZT that causes ROS release is azido, because thymidine does not increase ROS/RNS release (Deavall *et al*., 2012).

Table 1. The Characteristics of Subject Receiving Zidovudine

Subject characteristics	Total subject, N (%), (mean ± SD)	Anemia N (%), (mean ± SD)	Without anemia N (%), (mean ± SD)	p-value
Sex				0.001*
Male	138 (64.8)	24 (17.4)	114 (82.6)	
Female	75 (35.2)	28 (37.3)	47 (62.7)	
Age (years)	36.9 ± 8.6	40.12 ± 8.38	35.98 ± 8.49	0.002**
20-29	35 (16.4)	5 (14.3)	30 (85.7)	
30-39	93 (43.7)	19 (20.4)	74 (79.6)	
40-49	60 (28.2)	18 (30)	42 (70)	
50-59	25 (11.7)	10 (40)	15 (60)	
Risk factor				0.060**
Homosexual	72 (33.8)	10 (13.9)	62 (86.1)	
Heterosexual	120 (56.3)	38 (31.7)	82 (68.3)	
Bisexual	9 (4.2)	2 (22.2)	7 (77.8)	
Parenteral transmission	11 (5.2)	2 (18.2)	9 (81.8)	
Other	1 (0.5)	-	1 (100)	
Body mass index (kg/m²)	22.3 ± 3.7	21.47 ± 3.53	22.58 ± 3.76	0.062**
Hemoglobin level (gr%)	13.8 ± 1.9	11.41 ± 1.00	14.53 ± 1.38	0.000**

* Uji Chi-square; **Uji Mann- Whitney U

Table 2. Duration of AZT Consumption with the Incidence of Anemia

The Duration of AZT	Anemia	Without anemia	p-value	OR; CI 95%
≥ 1 year	42 (22.1)	148 (77.9)	0.024*	0.369;0.151-0.901
< 1 year	10 (43.5)	13 (56.5)		Ref

* Uji Chi-square

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The participants in this study were HIV patients with long-time administration of AZT therapy. A study showed that anemia often occurs after 4-12 weeks of initial consumption of this drug (Kiragga *et al.*, 2010). However, some studies stated that the disorder arises after six months of AZT consumption (Assefa *et al.*, 2015; Dash *et al.*, 2015; Ikunaiye *et al.*, 2018; Kuwalairat *et al.*, 2014; Singh *et al.*, 2016; Weil, 2015). Hb levels of patients receiving this drug decreased progressively within 6 months after usage and increased gradually. Several studies stated that after using ARVs, there was a significant improvement in the hematological profile, namely a decrease in anemia or a significant increase in Hb levels (Geletaw *et al.*, 2017). Enawgaw *et al.* (2014), stated that HIV-infected patients receiving this therapy increased their erythrocyte count within six months of starting treatment. Hb levels in most patients would be recovered one month after the termination of AZT consumption. However, the myelotoxicity effects of this drug could last up to three months, resulting in a more extended period for Hb levels to normalize (Dash *et al.*, 2015). According to the theory, anemia in HIV patients is affected by sex, CD4 level, and history of tuberculosis infection (Alamdo *et al.*, 2015).

Assefa *et al.* (Assefa *et al.*, 2015) discovered that moderate and severe anemia was higher in women than in men both before and after antiretroviral therapy (ART) administration. This is because women are at risk of routine blood loss through menstruation and childbirth. The complication can be discovered in approximately 63% - 95% of people infected with HIV due to various etiologic factors leading to decreased red blood cell (RBC) production, increased RBC destruction, or ineffective RBC production (Saini *et al.*, 2019). The prevalence of anemia was increased among patients with a CD4 cell count <200 cells/mm^3. In addition, the likelihood of this complication also increases due to the progressive immunological decline in HIV patients (Alamdo *et al.*, 2015). The AZT therapy in these patients is also closely correlated with the incidence of anemia and requires regular monitoring of its toxic effects on Hb levels (Barik, 2016; Goyal *et al.*, 2016; Kallianpur *et al.*, 2016; Melese *et al.*, 2017; Phe *et al.*, 2013).

Assefa *et al.* (2015), stated that the prevalence of anemia decreased by 67% within 6 months after administering ARVs. The administration of this therapy can reduce the incidence of opportunistic infections, which reduces proinflammatory cytokines such as TNF that suppress erythropoiesis. This indirectly shows the effectiveness of ARVs in reducing HIV-associated anemia by decreasing the incidence of opportunistic infections and improving patients' nutritional status (Assefa *et al.*, 2015; Gedefaw *et al.*, 2013). A similar study stated that hemoglobin levels increased significantly in HIV-infected patients after receiving ARVs for the first 12 months (Johannessen *et al.*, 2011). Another plausible explanation is that these drugs can suppress HIV, directly affecting the bone marrow and preventing anemia (Gedefaw *et al.*, 2013).

This study provides information on Hb monitoring in patients receiving AZT, but there is still limitation related to demographic variance. Therefore, a case-control study should be conducted to discover the relationship between AZT consumption duration and the incidence of anemia.

CONCLUSION

The duration of AZT consumption is significantly related to the incidence of Anemia in HIV patients. Therefore, the intake of this drug for more than or equal to one year is a protective factor in the incidence of anemia in patients with HIV compared to the group having consumption of less than one year.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest concerning this study.

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