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Characteristics of Immunological Non-Responders in People Living with HIV at Abepura Hospital Papua

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ABSTRACT

Infection of Human Immunodeficiency Virus (HIV) lowers the body's immune system, especially $CD4^+$ cells, making it more susceptible to opportunistic infections. Approximately 10-40% of People Living with HIV/AIDS (PLHIV) fail to achieve normal levels of $CD4^+$ T cells despite continued virological suppression, a condition called Immunological Non-Responders (INR). Previous studies have shown that INR is considered a predictor of disease progression in people with HIV receiving antiretroviral (ARV)s through various mechanisms of suppression of the immune system that increases morbidity and mortality. Papua is an HIV epidemic area with a prevalence of 2.3%. This research is a cohort study conducted at Abepura Hospital from June 2019 to February 2023, which aims to identify the factors that influence the occurrence of INR in PLHIV receiving ARV therapy. There were 123 research subjects consisting of 55 people (44.7%) in the INR group and 68 people (55.3%) in the non-INR group. The results showed that the incidence of INR was higher in males than females (p=0.019), INR was significantly associated with increasing age (p=0.013), and CD4 count was low at the start of ARVs (p=0.002). There was a significant difference in CD4 counts between INR and non-INR (p<0.001). Oral candidiasis as a common opportunistic infection is more common in people with INR than in non-INR. (p=0.037). This study suggested that it is necessary to carry out a CD4 examination at the start of therapy and monitoring every 6 months to detect possible INR to prevent an increased risk of AIDS and non-AIDS, which increases mortality.

Keywords: Immunological non-responders, CD4, HIV

INTRODUCTION

Papua is an epidemic area of the Human Immunodeficiency Virus (HIV) with a prevalence rate of 2.3%. This prevalence tends to be higher in mountains and the indigenous Papuan population (2.9%) compared to lowland and urban areas where the prevalence is below 2.3%.[1]

Minister of Health Regulation (PERMENKES) number 23 of 2022 confirms that one of the indicators and targets for ending the HIV epidemic, Acquired Immune Deficiency Syndrome (AIDS) and Sexually Transmitted Infections (STI) is to find 95% of the estimated population of people with HIV/Orang Dengan HIV (ODHIV), 95% of People Living with HIV (PLHIV) receive antiretroviral (ARV) treatment and 95% of those who receive ARV treatment in which the virus has become undetectable.[1]

The goal of ARV therapy is to suppress viral load to undetectable levels and increase the number of $CD4^+$ T cells, thereby reducing morbidity and mortality in HIV-infected people. However, in some patients, optimal treatment and continued suppression of viral replication have failed to restore $CD4^+$ T cell counts. These patients are included in the Immunological Non-Responders (INR) group.[2]

The mechanism underlying the abnormal immunological response or INR is multifactorial. Broadly speaking, the factors that influence the occurrence of INR are divided into three parts, the first is due to decreased production of $CD4^+$ T cells. The mechanism of decreased $CD4^+$ production can be due to disorders of the bone marrow and hematopoietic progenitor cells, decreased size and output of the thymus, and dysregulation of cytokines (IL-7, IL-2, and IL-15). The second mechanism is immune activation resulting in increased destruction of $CD4^+$ T cells, this immune activation can occur due to microbial translocation, persistent HIV infection or HIV reservoir, coinfection, activation of Plasmacytoid Dendritic Cells (pDCs), and disturbances in the ratio of regulatory T cells (Tregs) and Th17 cells. The third mechanism is due to other factors related to immune reconstitution, such as older age, male, lower initial $CD4^+$ T cell count, and lower CD4/CD8 ratio.[3]

Immunological non-responders occur in 10-40% of HIV patients receiving ARV therapy.[4] The INR condition is considered a predictor of disease worsening in PLHIV receiving ARVs. Several studies have shown a relationship between INR and the increased risk of immune damage, immune activation, apoptosis, and immune system decline as well as AIDS and non-AIDS, which cause morbidity and mortality.[5] One of the predictors of long-term immunological recovery in advanced HIV patients is the increase in CD4 cell count during the first year of ARV treatment. Previous studies have shown that there is an increase in CD4 count and CD4:CD8 ratio after about one year of ARV treatment.[6]

This study was conducted to identify the factors that influence the occurrence of INR in HIV patients receiving ARV therapy at Abepura Hospital, Papua. Currently, there is no global agreement on the standard diagnosis of INR. The $CD4^+$ threshold in previous studies varied between <200 to <500 cells/mm^3.[3,2] This study took a $CD4^+$ threshold <350 cells/mm^3 with plasma HIV RNA <40 copies/mL after initiation of ARV therapy at least one year, which was included in the INR category.[6]

METHODS

This research is part of the InaRespond proactive multicenter research conducted in nineteen hospitals in Indonesia, including at Abepura Hospital in Papua. The research data reported is cohort data conducted on HIV sufferers at the Voluntary Counseling and Testing (VCT) Clinic of Abepura Hospital, Jayapura Papua. Data was taken from June 2019 to February 2023 and was approved by the Jakarta Health Research and Development Agency (Litbangkes) Ethics Committee in 2019 with the number IRB00003331 National Inst Hlth Rsch & Development IRB#1-Health Research.

The study population was PLHIVs diagnosed using immunochromatography using three different methods. Viral load using EDTA plasma samples and examined with GeneXpert while CD4 T cells were examined with PIMA. The diagnosis of tuberculosis was established by using a sputum sample, which was examined by the Polymerase Chain Reaction (PCR) method using the GeneXpert instrument. Syphilis examination was carried out by the immunochromatography method, which detects antibodies qualitatively. If the results of the syphilis antibody test, were negative, they will be evaluated every six months along with viral load and CD4 tests. Hepatitis B (HBsAg) and hepatitis C (anti-HCV) tests were carried out using the ELISA method. All laboratory tests were carried out in an accredited laboratory and the results of the tests have been validated.

The inclusion criteria in this study were patients who were 18 years of age or older and had received ARVs for at least one year, with the exclusion criteria being patients who moved outside Jayapura or lost to follow-up.

The study sample was divided into the INR group if CD4 count <350 cells/mm^3 and viral load <40 copies/mL after ARV therapy for at least one year and non-INR group if CD4 count >350 cells/μL and viral load less than 40 copies/mL or if the CD4 count is any cell/μL and the viral load is more than 40 copies/mL. The collected data were analyzed univariate and bivariate using SPSS 22 for Windows. Statistical tests used 95% confidence intervals so a $p<0.05$ was considered statistically significant. Univariate analysis was performed on the characteristics of the research data indicated by frequency, mean, or median. The relationship between the two variables was carried out by bivariate analysis with the Chi-Square test and the Mann-Whitney test.

RESULTS AND DISCUSSIONS

The research sample consisted of 133 people, but 10 people were excluded because 1 person moved outside Jayapura and 9 people lost to follow-up so the research samples that were statistically analyzed were 123 people. During ARV therapy 91 people experienced viral suppression but only 55 people (44.7%) of them were included in the INR group. Out of a total of 123 people, there were 68 people (55.3%) in the non-INR group. This result is almost the same as that of Mora *et al*. in Spain, which shows an INR rate of 41.7%.[7] Other INR studies show varying figures including Oman 27% and India 21.1%.[8,9] This difference in results may be due to the absence of a global definition of INR, so several studies have used different definitions of INR on CD4 counts, definitions of viral suppression, and the length of follow-up time for ARV administration used in their studies.[2]

The results of statistical tests using the Chi-Square test showed that male gender had a significant effect on INR with $p=0.019$. The prevalence of INR in male patients was higher than in females because theoretically, males have a weaker ability to recover CD4 counts after therapy than females.[10] The Mann-Whitney test had a significant effect between age and the incidence of INR $p=0.013$. Research by Guedes *et al*. revealed that the INR group had a higher age compared to non-INR. This happens because the output of the thymus decreases due to

aging process. It is observed that during the aging process, functional thymus tissue will be bound by adipose tissue, and the organ undergoes an involution process, which results in decreased production and maturation of T cells.[11]

In this study, the initial CD4 count before receiving ARVs showed a significant difference between the INR (144.25 cells/mm^3) and non-INR (283 cells/mm^3) groups with p=0.002. A CD4 count that remains low even though the viral load has been suppressed can occur due to immune system fatigue, which causes the immune system to recover slowly. This process can occur in HIV patients who start ARV therapy with low CD4 T cell counts.[6] Several studies in several other countries have also shown a relationship between INR and low CD4 counts at the start of therapy, such as studies conducted in Oman, Canada, and the USA, low CD4 counts before giving ARVs make recovery of CD4 counts slowly despite adequate ARV therapy.[5,8,12,13]

Mycobacterium tuberculosis (MTB) and HIV infections are a major burden of communicable diseases in developing countries. In this study, it was found that almost half of the study subjects (45.1%) had MTB infection. As is known HIV will increase the risk of tuberculosis infection 16-27 times compared to HIV-negative people. *Mycobacterium tuberculosis* is the most common opportunistic infection that causes viral load exacerbations and reduces the patient's CD4 count, while decreased cellular immunity in HIV patients increases the risk of TB progression and reactivation of latent TB. This

Table 1. Basic characteristics of the research

	Group		p-value
	INR n (%)	Non-INR n (%)	
Gender			
Male	34 (61.8)	27 (39.7)	**0.019***
Female	21 (38.2)	41 (60.3)	
Age (years) (mean±SD)	37.51 ± 9.9	33.20±9.5	**0.013****
Education			
No school	3 (5.5)	2 (2.9)	
Elementary school	2 (3.6)	4 (5.9)	
Junior high school	11 (20)	5 (7.4)	0.366*
Senior high school	21 (38.2)	38 (55.9)	
College	18 (32.7)	19 (27.9)	
Work			
Doesn' t work	16 (29.1)	22 (32.4)	
Civil servant	3 (5.5)	8 (11.8)	
Private employees	17 (30.9)	16 (23.5)	
Self-employed	7 (12.7)	5 (7.4)	0.15*
Student	3 (5.5)	9 (13.2)	
Laborer	0 (0)	1 (1.5)	
Housewife	9 (16.4)	7 (10.3)	
Sexual orientation			
Heterosexual	39 (70.9)	60 (88.2)	
Homosexual	2 (3.6%)	2 (2.9)	
Bisexual	11 (20)	4 (5.9)	0.191*
Heterosexual and multi-partner	1 (1.8)	1 (1.5)	
Heterosexual and narcotics	1 (1.8)	1 (1.5)	
Heterosexual and blood-recipient	1 (1.8)	0 (0)	
Alcohol			
Yes	28 (52.8)	45 (67.2)	0.11*
No	25 (47.2)	22 (32.8)	
Length of therapy (months) (mean±SD)	42.93±28.8	37.55±26.5	**0.308****
CD4 start of therapy (mean±SD)	144.25±129.2	283±217.5	**0.002****
Current CD4 count (mean±SD)	285.9±96.5	465.8±287.4	**<0.001****

*) means p<0.05; Chi-Square test; **Mann-Whitney test; INR: Immunological Non-Responder; meanSD: normal distribution; CD4: Cluster Differentiation

Table 2. Coinfection and opportunistic infection in the INR and non-INR groups

Variables	Group		p-value
	INR n (%)	Non-INR n (%)	
TBC			
Yes	22 (40)	33 (49.3)	0.307*
No	33 (60)	34 (50.7)	
Oral candidiasis			
Yes	28 (50.9)	22 (32.4)	**0.037***
No	27 (49.1)	46 (67.6)	
Hepatitis B			
Yes	6 (10.9)	5 (7.4)	0.492*
No	49 (89.1)	63 (92.6)	
Hepatitis C			
Yes	2 (3.6)	1 (1.5)	0.439*
No	53 (96.4)	67 (98.5)	
Syphilis			
Yes	7 (12.7)	15 (22.1)	0.179*
No	48 (87.3)	53 (77.9)	

*) Chi-Square test; INR: Immunological Non-Responder; TBC: Tuberculosis

condition can cause the death of patients due to AIDS.[11] In this study the number of TB patients in the non-INR group was higher than the INR group. This could happen because in this study, did not distinguish between TB patients who had recovered (a history of TB) and TB patients who were currently being treated. Statistical test results found no significant difference between TB sufferers in the INR and non-INR groups.

Hepatitis B coinfection occurred in 11 people (0.09%), hepatitis C in 3 people (0.02%), and syphilis in 22 people (17.9%). Statistical tests conducted on hepatitis B, hepatitis C, and syphilis coinfection in ODHIV receiving ARV therapy showed non-significant differences in the incidence of INR with p=0.492; 0.439, and 0.179, respectively. The results of this study were different from that of Chan *et al*., which shows that PLHIV who have experienced suppression of the number of viruses due to the use of ARVs and have syphilis infection will decrease their CD4 count, this happens because syphilis can induce pyroptosis apoptosis of CD4 and CD8 T lymphocytes and change caspase 1 and caspase 3 causing decreased levels in the circulation. Studies have shown that HIV suppression due to ARVs becomes ineffective due to syphilis infection, and syphilitic reinfection shows a different immunological profile compared to the first syphilis infection. The difference in results is thought to be due to the syphilis examination in this study using the immunochromatography method, which detects syphilis antibodies qualitatively, making it difficult to distinguish old infections, reinfections, or first infections.[14]

HIV will significantly increase the risk of candida infection in the oral cavity and predispose to oral candidiasis. It was specifically stated that *Candida dubliniensis* was reported as the cause of oral candidiasis. In people with HIV, CD4 cell count is directly correlated with the severity of oral candidiasis. HIV patients provide significantly lower protection against the risk of antimicrobial peptides, especially histatin 5, thereby increasing oral candidiasis compared to healthy controls.[15]

Oral candidiasis, pulmonary tuberculosis, and cryptosporidiosis are the most common opportunistic infections in PLHIV, so improved hygiene, routine monitoring, and appropriate antimicrobial prophylaxis are needed to reduce morbidity and mortality caused by opportunistic infections in HIV patients.[16]

Although there were no significant differences in hepatitis B, hepatitis C, TB, and syphilis coinfection between the two groups in this study, the number of opportunistic oral candidiasis infections was found to be much higher in the INR group compared to non-INR and was statistically significant (p=0.037). These results conclude the importance of identifying the INR because the incidence of opportunistic infections will be higher in the INR group.

This study has several limitations, namely screening for syphilis using rapid antibodies (immunochromatography) causing antibodies to still be detectable despite latent conditions or chronic infection and there was no data on testing for cryptococcus, toxoplasmosis, and other opportunistic infections.

This study shows that the incidence of INR is significantly associated with low initial CD4 counts, male patients, increasing age, and opportunistic infections of oral candidiasis. Since it is important to know the condition of the INR, recommended that people with HIV check their CD4 count before starting therapy and carry out a monitoring examination every 6 months if the CD4 count is low to detect the possibility of developing INR, which can increase the risk of developing AIDS and non-AIDS, which increases the incidence of death.

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REFERENCES

1. Peraturan Menteri Kesehatan no 23, 2022. Penanggulangan human immunodeficiency virus, acquired immune deficiency syndrome dan infeksi menular seksual. Jakarta, 2022; 23: 001-118.
2. Yang X, Su B, Zhang T, Zhang X, Liu Y, Wu H. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responderss. J Leukoc Biol, 2020; 107: 597–612.
3. Bono V, Augello M, Tincati C, Marchetti G. Failure of $CD4^+$ T-cell recovery upon virally-effective cART: An enduring gap in the understanding of HIV+ immunological non-responders. New Microbiol, 2022; 45(3): 155–72.
4. Hailu, Araya W. Immunological and virological discordance among people living with HIV on highly active antiretroviral therapy in Tigray, Northern Ethiopia. BMC Infectious Diseases, 2021; 21: 561.
5. Adi N, Allahna E, Xun W, Emmanuel B, Yakubu A, *et al*. Clinical factors and outcomes associated with immune non-response among virally suppressed adults with HIV from Africa and the United States. Scientific Reports, 2022; 12: 1196.
6. Aiwei Z, Fernando R, Jaja Z, Se G, Pierre de T, *et al*. HIV sheltering platelets from immunological non-responders induce a dysfunctional glycolytic CD4 T cell profile. Frontiers in Immunology, 2022; 2: 78192.
7. Mora EG, Massanella M, Garcia E, Giles D, Bernado M, *et al*. Elevated humoral response to cytomegalovirus in HIV-infected individuals with poor $CD4^+$ T cell immune recovery. PLos One, 2017; 1-17.
8. Ali ZG, Boulassel MR. Factors associated with immune discordant responses in treated HIV-infected Omani patients. Open AIDS J, 2019; 13(1): 25–30.
9. Anusuya GS, Sikhamani R, Somani J, Gurusamy M, Nadol P, *et al*. Virological discordance in patients on first line antiretroviral therapy with Immunological failure in Tambaram, India. Natl J Res Comm Med, 2017; 6(1): 030–7.
10. Boatman J, Baker J, Emery S, Furrer H, Mushatt D, *et al*. Risk factors for low $CD4^+$ count recovery despite viral suppression among participants initiating antiretroviral treatment with $CD4^+$ counts > 500 cells/mm3: Findings from the Strategic Timing of AntiRetroviral Treatment (START) Trial. J Acquir. Immune Defic Syndr, 2019; 81(1): 10–17
11. Guedes M, Carvalho SW, Andrade SJ, Maria BC, Souto F, Guimaraes R. Thymic exhaustion and increased immune activation are the main mechanisms involved in impaired immunological recovery of HIV-positive patients under ART. Viruses, 2023; 15(2): 440.
12. Darraj M, Shafer LA, Chan S, Kasper K, Keynan Y. Rapid CD4 decline prior to antiretroviral therapy predicts subsequent failure to reconstitute despite HIV viral suppression. J Infect Public Health, 2018; 11(2): 265–9.
13. Adi N, Allahna E, Xun W, Emmanuel B, Yakubu A, *et al*. Clinical factors and outcomes associated with immune non-response among virally suppressed adults with HIV from Africa and the United States. Scientific Reports, 2022; 12: 1196.
14. Chan P, Tang T, Kwong R, Chan L, Chan H, *et al*. Effects of syphilis infection among HIV-1-positive individuals on suppressive antiretroviral therapy. AIDS Research and Therapy, 2022; 19: 69.
15. Vila T, Sultan A, Jauregui D, Rizk MA. Oral candidiasis: A disease of opportunity. J Fungi, 2020; 6: 15.
16. Rozhana S, Bagui M N. Clinico-microbiological study on 100 HIV seropositive patients from Bangladesh. Kuwait Medical Journal 2019; 51(1): 66-71.