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Differences peripheral of blood in HIV patients with and without cognitive disorders at "Prof. Dr. IGNG Ngoerah" Hospital

Ketut Widyastuti[1], I Made Susila Utama[2], Anak Agung Ayu Putri Laksmidewi[1]

[1]Department of Neurology, Faculty of Medicine, Udayana University, Bali, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine Udayana University, Bali, Indonesia

ABSTRACT

Background and objective. The prevalence of mild neurocognitive Disorder (MND) has increased after using antiretroviral therapy (ARV), which is around 51.5%. Inflammation plays an essential role in the neurodegenerative state of HIV patients. Peripheral blood images can assess increased macrophage activation by evaluating the number of leukocytes, neutrophils, total lymphocytes, hemoglobin, platelets, platelets, and neutrophil-lymphocyte ratio (NLR) to determine the condition of systemic inflammation in HIV patients with cognitive impairment. This study to know differences in the appearance of peripheral blood in HIV patients with and without cognitive impairment.

Method. Observational analytic study with a cross-sectional design on 44 HIV patients who were outpatients at the polyclinic Voluntary Counseling and Testing (VCT) at Prof Dr. IGNG Ngoerah Denpasar Hospital. Subjects were divided into 2 groups based on cognitive status assessed using the MoCA-INA questionnaire. The peripheral blood images considered were the average levels of leukocytes, neutrophils, lymphocytes, hemoglobin, hematocrit, platelets, and the ratio of neutrophils to lymphocytes.

Results. 31-40 years old, male, with >9 years of education, has a CD4 count >200, and has received ARV therapy >1 year. The Shapiro-Wilk normality test performed on both groups showed that the data were normally distributed with $p>0.05$. Bivariate analysis using an independent t-test with a 95% confidence level ($\alpha=0.05$) found significant differences in mean leukocyte, neutrophil, lymphocyte, and neutrophil-lymphocyte ratios ($p<0.05$) between groups of HIV patients with cognitive impairment and no cognitive impairment.

Conclusion. Inflammatory markers from the peripheral blood picture are increased in HIV-infected persons.

Keywords: peripheral blood, neutrophils, lymphocytes, cognitive, HIV, MoCA-INA

List of abbreviations

ARV - Antiretroviral Therapy
HAND - HIV-Associated Neurocognitive Disorder
HIV - Human Immunodeficiency Virus
INA - Indonesia
MND - Mild Neurocognitive Disorder
MoCA - Montreal Cognitive Assessment
NLR - Neutrophil-Lymphocyte Ratio
VCT - Voluntary Counseling and Testing
SPSS - Statistical Package for Social Sciences

INTRODUCTION

People living with HIV often experience neurological complications, including cognitive impairment HIV-associated neurocognitive Disorder (HAND). The development of HIV therapy with combination antiretrovirals (ARVs) has reduced the incidence of cognitive impairment compared to the pre-ARV era. The administration of ARV therapy influences the occurrence of cognitive impairment complications, opportunistic infections, age, smoking, and level of education [1]. Studies on HIV patients at Prof. Dr. IGNG

Corresponding author:
Ketut Widyastuti
E-mail: kt_widyastuti@unud.ac.id

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Ngoerah Denpasar Hospital showed risk factors for cognitive impairment in people with HIV, including low CD4 nadir, low CD4 current, low education level (< 9 years), and HIV diagnosis of more than one year [2,3].

Cognitive impairment is one of the early symptoms of functional decline that reflects damage to brain neurons, so it is essential to know cognitive function in patients with HIV. Neurocognitive Disorder screening using the Montreal Cognitive Assessment (MoCA) found a HAND prevalence of 75% in HIV patients receiving antiretroviral therapy (ARV) (Hasbun et al., 2012). The prevalence of HAND in Indonesia was found to be 51%, with the most affected domain being memory (63%) [4].

HIV infection in the brain occurs in macrophage cells, including monocyte-derived perivascular macrophages and microglia. HIV attacks the brain through the Trojan Horse mechanism by crossing the blood-brain barrier riding infected monocytes, which then differentiate into macrophages. This can occur within 1-2 weeks after the virus enters the systemic circulation. Neuronal damage is thought to occur by two mechanisms. The first is through viral protein released from infected monocytes causing neuronal death through the direct interaction of viral proteins with neurons. Second, there is indirect death of neurons mediated by the inflammatory response to the viral protein or the HIV-infected cells [5]. The cascade of activated microglial cell-mediated chemokines and cytokines leads to cell death through decreased neuronal arborization. This process can cause a decrease in cognitive function and progressive behavior changes in patients with HIV [6].

Considering the role of inflammation in the pathogenesis of cognitive impairment, we hypothesized that routine blood biomarkers in cognitively impaired patients could have diagnostic and predictive value. If confirmed, this standard blood biomarker could become an informative plasma marker for diagnosis, stratification, and prediction of disease progression and/or could be used as evidence of response to cognitive impairment interventions.

MATERIALS AND METHODS

This cross-sectional study has obtained ethical eligibility from the ethical commission of Udayana University with number 2022.03.1.0041. The research was conducted in polyclinic Voluntary Counseling and Testing (VCTs). The time of the study was fulfilled in March-June 2022. Inclusion criteria: (1) All patients diagnosed with HIV were positive, (2) Patients aged 18-50 years. (3) Patients receiving ARV therapy with CD4+ 200-350 cells/µl. (4) Patients are cooperative and willing to be included in the study by signing informed consent. Exclusion criteria: (1) Patients with a history of neurological disorders like stroke, seizures, head trauma, intracranial tumors, or Parkinson's. (2) Have risk factors for cognitive disorders such as hypertension, DM, heart disorders, and dyslipidemia. (3) Severe psychiatric disorders (such as schizophrenia) or are being treated with antipsychotic drugs. (4) Not able to perform daily functions independently. Data analysis used an independent T-test with a 95% confidence level (α=0.05). A descriptive study to determine the mean with standard deviation, median, and proportion of the characteristics in the case and control groups. The collected data is processed through the Statistical Package for Social Sciences (SPSS) program.

RESULTS

The study was conducted on 44 people living with HIV, 22 HIV sufferers with cognitive impairment, and 22 people living with HIV without cognitive impairment, with the results of the data characteristics obtained in Table 1.

TABLE 1. Basic characteristics of research subjects

Variable	Cognitive Disorder n (%)	
	Yes	No
Age (years)	39.77+6.24	36.05+7.42
Age group		
18-30 years	1(4,5)	5(22,7)
31-40 years	21(95.5)	17(77.3)
Gender		
Male	16(72.7)	16(72.7)
Female	6(27,3)	6(27,3)
Length of education		
<9 years	7(31,8)	2(9,1)
>9 years	15(68,2)	20(90.9)
CD4 number (cell/µl)		
<200	9(40,9)	7(31,8)
>200	13(59,1)	15(68,2)
ARV therapy duration		
<1 year	4(18,2)	9(40,9)
>1 year	18(81.8)	13(59,1)

The analysis results of differences in mean peripheral blood images from the levels of leukocytes, neutrophils, lymphocytes, hemoglobin, hematocrit, platelets, and neutrophil-lymphocyte ratio in subjects with and without cognitive impairment are presented in Table 2.

DISCUSSION

This study was conducted on 44 people with HIV who were divided into two groups: the group with cognitive impairment, 22 people, and the group without cognitive impairment, 22. The results of Widyastuti's research (2012) also reported that most HIV sufferers who experienced cognitive impairment were aged 30-

TABLE 1. Differences in mean peripheral blood appearance in subjects with and without cognitive impairment

Variable	Cognitive impairment (mean+SD)		p-value
	Yes	No	
Leukocytes	8.66+3.72	6.09+2.45	0.010
Neutrophils	62.93+21.35	50.44+11.07	0.019
Lymphocytes	19.86+13.08	34.76+16.89	0.002
Hemoglobin	13.33+2.49	13.87+2.06	0.443
Hematocrit	40.58+6.50	41.78+6.89	0.557
Platelets	248.72+95.20	263.72+68.28	0.551
Neutrophil Lymphocyte Ratio (NLRR)	4.03+2.59	2.22+2.29	0.018

40 years (64.7%).[7] A study by Njamnshi et al. (2008) on 185 people with HIV aged 18-59 years found that most cognitive disorders occurred at less than 40 years, namely 62% [8]. Research by Meidani et al. (2011) also stated that of 212 HIV-positive subjects, the average age was 27-45 years [9].

Most of the research subjects were male in the group with cognitive impairment and in the group without cognitive impairment, which was 72.7%. These results are not much different from the research conducted by Qiao (2019), in which the study found that the number of male subjects was 77%, while women comprised 22% of the study population [10]. Men are more at risk of contracting HIV and AIDS because of their sexual behavior, including having sex with more than one partner, having anal sex, and homosexual behavior. In addition, the awareness rate for men to carry out HIV tests is higher than for women because it is related to the demand for work as employees in several companies requiring periodic health check-up data. In addition, the cause of HIV/AIDS in this group is related to sharing needles among drug addicts, who are more numerous than women [11].

The level of education is an essential factor that determines cognitive ability. Cognitive decline due to normal aging in individuals with higher education is reported to be slower than in individuals with lower levels of education. Education can provide a protective (protective) effect on cognitive function. Different results were obtained in this study that people with HIV with and without cognitive impairment were more common in subjects with more than nine years of education. The survey by Schouten et al. (2020) states that the level of education significantly affects cognitive function in people with HIV, and higher education is protective against the occurrence of HAND [12].

As many as 13(59.1%) of people with HIV with CD4 >200 cells/µl had cognitive impairment, and 15(68.2%) did not experience cognitive impairment. Research conducted by Moreno et al. (2008) in all patients receiving ARV treatment, it was found that cognitive impairment tends to occur more frequently in HIV patients with a CD4 nadir <200 cells/mm3 [13]. Cognitive impairment in the subjects worsened with a decrease in the CD4 nadir. The CD4 nadir number is an irreversible marker due to inflammatory changes in the brain. These changes are related to damage to the immune system due to HIV resulting in lymphocyte dysfunction, which causes brain damage. Cognitive function is more affected by systemic immune suppression, and with ARV therapy, cognitive abilities can improve, but recovery does not occur in all individuals undergoing treatment.

In this study, all the therapies used were the same, namely tenofovir (TDF) + lamivudine (3TC) + dolutegravir (DTG), according to the latest guidelines from the Republic of Indonesia Ministry of Health circular letter for 2021 [11]. ARV therapy duration >1 year was found in 18 (81.8%) in the group with cognitive impairment and 13 (59.1%) in the group without cognitive impairment. Each of these drugs works because lamivudine blocks enzymes that play a role in viral multiplication so that viral loads can be reduced and disease progression is slowed down. Tenofovir works by interfering with HIV DNA synthesis through competitive inhibition of reverse transcriptase and incorporation into viral DNA. Dolutegravir belongs to a class of drugs known as integrase inhibitors, has a low resistance rate, and is relatively well tolerated in the clinical setting [14].

This study showed a significant difference ($p<0.05$) in the mean number of leukocytes, neutrophils, lymphocytes, and neutrophil lymphocytes between groups of HIV patients with cognitive impairment and those without cognitive impairment. Meanwhile, the difference in mean hemoglobin, hematocrit, and platelet counts showed no significant difference ($p>0.05$). Human Immunodeficiency Virus (HIV) is an RNA virus that causes a decrease in the body's immune system, destroying and damaging specific white blood cells called T-helper lymphocytes or lymphocytes carrying the T4 factor (CD4). CD4 is a parameter in HIV-AIDS patients, while NLR is a simple parameter to assess inflammation status [15].

Randomized clinical trials demonstrated a correlation between inflammatory markers such as increased serum NLR in HIV-infected versus non-HIV-infected individuals and disease progression and death [16]. The presence of increased macrophage activation and inflammatory markers can lead to the development of HAND [17]. Serum NLR is a simple parameter used to assess inflammatory status. Serum NLR was obtained from the division of total neutrophils by total lymphocytes. Neutrophils are myeloid precursors that serve as the first line of response to a pathogen and trigger an innate immune response. Neutrophils inhibit microorganisms, espe-

cially bacteria or fungi, by phagocytosing and killing pathogens through mechanisms that require NADPH oxygenase or produce an anti-bacterial protein in the phagosome. In comparison, lymphocytes are lymphoid precursors that function as adaptive immune cells and have a significant role in the immune process because lymphocytes respond specifically to infection with microorganisms. Generally, serum NLR is used to determine the degree of inflammation in malignancy and cardiovascular disease and as a marker of bacterial infection. Leukocyte and neutrophil counts are the most frequently used parameters in diagnosing bacterial infections, but these parameters do not always differentiate between bacterial, fungal, and viral infections.

Several studies have reported the important role of chronic inflammation in developing chronic neurodegenerative diseases in the CNS. NLR is a marker of peripheral inflammation in chronic disease that can provide data on disease course and treatment response [18]. Patients with cognitive impairment or Alzheimer's dementia are reported to have an increased NLR compared to normal controls [19,20]. In adults over 65, the average NLR value in people with Alzheimer's dementia is higher than cognitively usual. Increased NLR is independently associated with an increased risk of Alzheimer's dementia. NLR, with a cutoff ratio of 2.48, has a sensitivity of 70% and a specificity of 80% [5]. Dong et al.'s study reported significantly different NLR values between the healthy control group and the AD and MCI groups but found no significant differences in AD and MCI sufferers. The optimal NLR cutoff point was 2.35 to differentiate AD sufferers from healthy controls, with a specificity of 54 % and 83% sensitivity [21,22]. As Reported in several studies, patients with serum NLR> 5 are at risk of experiencing cognitive impairment more than patients with NLR <5. NLR is better than neutrophil or lymphocyte alone for reflecting the association of systemic inflammation with cognitive impairment [23].

CONCLUSION

There are differences in the peripheral blood picture in HIV patients with cognitive impairment and without cognitive impairment in Prof. Dr. IGNG Ngoerah Denpasar Hospital. Significant differences in the mean number of leukocytes, neutrophils, lymphocytes, and neutrophil-lymphocyte ratio ($p<0.05$) between HIV patients who experienced cognitive impairment and those who did not experience cognitive impairment. There were no significant differences ($p>0.05$) in the mean hemoglobin, hematocrit, and platelet counts between groups of HIV patients who experienced cognitive impairment and those who did not experience cognitive impairment.

Conflict of interest: None

Author's contributions

Conceptualization, K.W., I.M.S.U and A.A.A.P.L.; methodology, K.W., I.M.S.U and A.A.A.P.L.; software, K.W.; validation, K.W., I.M.S.U and A.A.A.P.L.; formal analysis, K.W.; investigation, K.W., I.M.S.U and A.A.A.P.L.; resources, K.W., I.M.S.U and A.A.A.P.L.; data curation, K.W., I.M.S.U and A.A.A.P.L.; writing—original draft preparation, K.W., I.M.S.U and A.A.A.P.L.; writing—review and editing, K.W., I.M.S.U and A.A.A.P.L.; visualization, K.W., I.M.S.U and A.A.A.P.L.; supervision, K.W.; project administration, K.W.; funding acquisition, K.W. All authors have read and agreed to the published version of the manuscript".

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REFERENCES

1. Shah A, Gangwani MR, Chaudhari NS, Glazyrin A, Bhat HK, Kumar A. Neurotoxicity in the Post-HAART Era: Caution for the Antiretroviral Therapeutics. *Neurotox Res.* 2016;30:677–97. doi: 10.1007/s12640-016-9646-0.
2. Gde Putra D, Ketut W, AAA. Putri L. Hiv-Associated Neurocognitive Disorder (Hand) Pada Pasien Dengan Hiv Tanpa Infeksi Oportunistik. *Callosum Neurol.* 2019;2:11–2. doi: 10.29342/cnj.v2i3.86.
3. Widyastuti K, Susilawathi NM, Utama IMS, Laksmidewi AAAP. Differences In Mean Cd4 Lymphocytes Of Hiv Patients With And Without Verbal Memory Impairment. *Neurona*. 2017;34.
4. Estiasari R, Imran J, Lastri DN, Prawiroharjo P, Price P. Cognitive impairment among Indonesia HIV naïve patients. *Neurol Asia.* 2015;20:155–60.
5. Kuyumcu ME, Yesil Y, Oztürk ZA, Kizilarslanoğlu C, Etgül S, Halil M, et al. The evaluation of neutrophil-lymphocyte ratio in Alzheimer's disease. *Dement Geriatr Cogn Disord.* 2012;34:69–74. doi: 10.1159/000341583.
6. Watkins CC, Treisman GJ. Cognitive impairment in patients with Aids – Prevalence and severity. *HIV/AIDS Res Palliat Care.* 2015;7:35–47. doi: 10.2147/HIV.S39665.
7. Widyastuti K, Adnyana IMO, Sudewi AAR. Angka Cd4 Nadir Dan Current Rendah Sebagai Faktor Risiko Gangguan Kognitif Pada Penderita Hiv Di Rsup Sanglah Denpasar. *Neurona* 2012;29:1–8.
8. Njamnshi AK, Djientcheu VDP, Fonsah JY, Yepnjio FN, Njamnshi DM, Muna WF. The international HIV dementia scale is a useful screening tool for HIV-associated dementia/cognitive impairment in HIV-infected adults in Yaoundé - Cameroon. *J Acquir Immune Defic Syndr.* 2008;49:393–7. doi: 10.1097/QAI.0b013e318183a9df.
9. Askarian Z, Meidani M, Ahmadi N, Jamali B. Relative frequency of Human T-cell Lymphotropic Virus I/II in HIV/AIDS patients. *Adv Biomed Res.* 2014;3:8. doi: 10.4103/2277-9175.124635.
10. Qiao X, Lin H, Chen X, Ning C, Wang K, Shen W, et al. Sex differences in neurocognitive screening among adults living with HIV in China. *JNV*. 2019;25:363–71. doi: 10.1007/s13365-019-00727-0.
11. Kemenkes RI. Infodatin HIV AIDS. *Kementeri Kesehat Republik Indones.* 2020:1–8.
12. Schouten J, Cinque P, Gisslen M, Reiss P, Portegies P, Gao C, et al. HIV-Associated Neurocognitive Disorder (HAND): Relative Risk Factors. *Arch Gerontol Geriatr.* 2020;88:401–26. doi: 10.1016/j.yexmp.2019.03.008.

13. Muñoz-Moreno JA, Fumaz CR, Ferrer MJ, Prats A, Negredo E, Garolera M, et al. Nadir CD4 cell count predicts neurocognitive impairment in HIV-infected patients. *AIDS Res Hum Retroviruses.* 2008;24:1301–7. doi: 10.1089/aid.2007.0310.
14. Patel PR, Louie S. Drug Interactions in Infectious Diseases: Antimicrobial Drug Interactions. Cham: Springer International Publishing; 2018. doi: 10.1007/978-3-319-72416-4.
15. Bianconi V, Schiaroli E, Mannarino MR, Paciosi F, Benedetti S, Marini E, et al. Expert Review of Anti-infective Therapy The association between neutrophil to lymphocyte ratio and endothelial dysfunction in people living with HIV on stable antiretroviral therapy. *Expert Rev Anti Infect Ther.* 2021;00:1–8. doi: 10.1080/14787210.2021.1933438.
16. Rubin LH, Benning L, Keating SM, Norris PJ, Burke-Miller J, Savarese A, et al. Variability in C-reactive protein is associated with cognitive impairment in women living with and without HIV: a longitudinal study. *J Neurovirol.* 2018;24:41–51. doi: 10.1007/s13365-017-0590-4.
17. de Jager CPC, Wever PC, Gemen EFA, Kusters R, van Gageldonk-Lafeber AB, van der Poll T, et al. The Neutrophil-Lymphocyte Count Ratio in Patients with Community-Acquired Pneumonia. *PLoS One.* 2012;7:4–11. doi: 10.1371/journal.pone.0046561.
18. Kara SP, Altunan B, Unal A. Investigation of the peripheral inflammation (neutrophil–lymphocyte ratio) in two neurodegenerative diseases of the central nervous system. *Neurol Sci.* 2021. doi: 10.1007/s10072-021-05507-5.
19. Liu J, Zhang Y, Ma Q, Sun H, Xu Y, Pan C. Elevated blood neutrophil to lymphocyte ratio in older adults with cognitive impairment. *Arch Gerontol Geriatr.* 2020;88:104041. doi: 10.1016/j.archger.2020.104041.
20. Kalelioglu T, Yuruyen M, Gultekin G, Yavuzer H, Özturk Y, Kurt M, et al. Neutrophil and platelet to lymphocyte ratios in people with subjective, mild cognitive impairment and early Alzheimer's disease. *Psychogeriatrics*. 2017;17:506–8. doi: 10.1111/psyg.12260.
21. Dong X, Nao J, Shi J, Zheng D. Predictive Value of Routine Peripheral Blood Biomarkers in Alzheimer's Disease. *Front Aging Neurosci.* 2019;11:1–9. doi: 10.3389/fnagi.2019.00332.
22. Sayed A, Bahbah EI, Kamel S, Barreto GE. The neutrophil-to-lymphocyte ratio in Alzheimer's disease: Current understanding and potential applications. *J Neuroimmunol.* 2020;349:577398. doi: 10.1016/j.jneuroim.2020.577398.
23. Halazun HJ, Mergeche JL, Mallon KA, Connolly ES, Heyer EJ, York N. Neutrophil-lymphocyte ratio as a predictor of cognitive dysfunction in carotid endarterectomy patients. *J Vasc Surg.* 2012;59:768–73. doi: 10.1016/j.jvs.2013.08.095.