Correlation between T-lymphocyte CD4+ and Total Lymphocyte Count (TLC), hemoglobin, Neutrophil to Lymphocyte Ratio (NLR) and T-lymphocyte CD4+/CD8+ ratio in HIV patients at Prof. Dr. I.G.N.G Ngoerah Hospital, Denpasar, Bali, Indonesia

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ABSTRACT

Background: Human Immunodeficiency Virus (HIV) is an RNA virus from the Retroviridae family, Lentiviri subfamily, which attacks the immune system, especially T lymphocytes, namely Cluster of Differentiation 4+ (CD4+) cells. Human Immunodeficiency Virus destroys T lymphocyte CD4+ cells, decreasing immunity against opportunistic infections. For monitoring of HIV, an examination of T lymphocyte CD4+ cells and complete blood counts were conducted.

Methods: A cross-sectional quantitative analytic study was conducted from October 2021 to January 2022 at Prof. Dr. I.G.N.G Ngoerah Hospital. Measurements were made on the total number of lymphocytes, hemoglobin levels, neutrophil-to-lymphocyte ratio (NLR), CD4+ levels and CD4+/CD8+ ratios. We analyze normality data using Kolmogorov-Smirnov and correlation using Pearson and Spearman tests. The p-value < 0.05 was considered significant.

Results: 56 patients met the inclusion and exclusion criteria, with 46 males (82.1%) and 10 females (17.9%). Mean age 38.98±11.58 years, mean total lymphocyte 0.64 x 109/μL, median T lymphocyte CD4+ 133.50 sel/mm3, mean hemoglobin 10.11±1.49 g/dL, median NLR 6.51, median CD4+/CD8+ ratio 0.06. The hemoglobin variable was normally distributed, while the other variables were abnormal. The Pearson Correlation test showed a significant relationship between T lymphocytes CD4+ and hemoglobin with r=0.329 (p=0.013). Spearman’s Correlation test showed a significant relationship between T lymphocytes CD4+ and NLR, total lymphocytes, CD4+/CD8+ ratio with r=0.334 (p=0.012), r=0.777 (p=0.000), r=0.729 (p=0.000) respectively.

Conclusion: There is a strong and significant correlation between the level of T lymphocytes CD4+ and total lymphocyte count and the ratio of CD4+/CD8+, a weak and significant correlation between the level of T lymphocytes CD4+ with hemoglobin and NLR in HIV patients at Prof. Dr. I.G.N.G Ngoerah Hospital.

Keywords: CBC, CD4+, Levels, HIV.


INTRODUCTION

The Human Immunodeficiency Virus (HIV) is an RNA virus belonging to the Retroviridae family, the Lentiviri subfamily, which attacks the immune system, especially T lymphocyte cells called Cluster of Differentiation 4+ (CD4+) cells. The Human Immunodeficiency Virus destroys CD4+ T lymphocyte cells, thus causing a weakening of the body’s immunity against opportunistic infections, such as tuberculosis infections, fungal, viral and bacterial infections. This opportunistic infection is called Acquired Immunodeficiency Syndrome (AIDS).1,2

The Human Immunodeficiency Virus is becoming a global health crisis, with an estimated 1.5 million new HIV infections and 680,000 AIDS-related deaths in 2020. Data on HIV/AIDS cases in Indonesia have continued to increase in the last eleven years, with the number of HIV cases reaching its peak in 2019, namely 50,282 cases.3

The immune system in people with HIV is important in fighting pathogens, infections and diseases. The mechanism of infection with HIV starts from the occurrence of viral replication. After the virus enters the individual’s body, the virus enters lymphocyte cells to various binding receptors found on macrophages, T lymphocytes, dendritic cells and monocytes. The virus binds to certain chemokine receptors and interacts with cell membrane proteins to enter the
host cell. CD4+ T lymphocytes are the main target of HIV infection because the virus has an affinity for CD4+ surface molecules. HIV enters lymphocytes and monocytes via the viral glycoprotein gp120 on the CD4+ cell surface molecule and chemokine receptors, specifically CXCR4 or CCR5. A complex process occurs after the virus fuses with CD4 lymphocyte cells, and new virus particles are formed from those infected.

People with HIV have a strong immune system that helps them fight pathogens, infections, and diseases. The mechanism of HIV infection begins with viral replication after the virus enters the individual's body; the virus enters lymphocyte cells and binds to various binding receptors found on macrophages, T lymphocytes, dendritic cells, and monocytes. The virus enters the host cell by binding to specific chemokine receptors and interacting with cell membrane proteins. Because the virus has an affinity for CD4+ surface molecules, CD4+ T lymphocytes are the primary target of HIV infection. The HIV enters lymphocytes and monocytes by recognizing the viral glycoprotein gp120 on the CD4+ cell surface molecule and chemokine receptors, namely CXCR4 or CCR5. After the virus fuses with CD4 lymphocyte cells, a series of complex processes take place, and new virus particles are formed from those infected.

CD4+ is a glycoprotein found on the surface of immune cells such as T-helper lymphocytes, monocytes, macrophages, and dendritic cells. CD4+ T lymphocytes can aid in the diagnosis and overview of the body's natural immune system. CD4+ T lymphocyte cells, when used in conjunction with clinical assessment, can be an early indicator of disease progression because the number of CD4+ T lymphocytes decreases at the onset of the disease.

Examining CD4+ T lymphocyte count and viral load is the gold standard for HIV testing. Still, it requires expensive equipment and trained technicians and is not always available in several countries and some areas in Indonesia. For this reason, Langford SE et al. have studied several laboratory markers with this aim: simple markers that can be measured to assess disease progression in limited resources, including total lymphocyte count (TLC), hemoglobin, and body mass index (BMI). The purpose of this research was to investigate the relationship between CD4+ T lymphocytes and total lymphocyte count, hemoglobin, neutrophil to lymphocyte ratio (NLR), and the ratio of CD4+ T lymphocytes to CD8+ T lymphocytes in HIV patients at Prof. Dr. IGNG Ngoerah Hospital, Denpasar, Bali, Indonesia.

METHODS

This research is a cross-sectional quantitative analytic study on HIV patients who underwent examination at Prof. Dr. IGNG Ngoerah Hospital Denpasar from October 2021 to January 2022. The data source for this study was from laboratory examination results taken from the Laboratory Information System (LIS) and medical records at Prof. Hospital. Dr. IGNG Ngoerah Denpasar. The inclusion criteria of this study were patients who were HIV positive, had never received ARV therapy, and had examinations at the Voluntary Counseling and Testing (VCT) polyclinic and those who were treated at Prof. Dr. IGNG Ngoerah Hospital, Bali, Indonesia.

Patients under 18 were excluded from this study, including those with incomplete medical record data, a complete blood count (CBC), and an examination of CD4+ T lymphocyte cells. The sample size in this study was calculated using the correlation sample size formula, where the minimum sample required in this study was 51 people; then, the sample size was increased by 10% to make the total sample 56 people. The number of CD4+ T lymphocyte cells in HIV patients was the independent variable in this study; the dependent variable was total lymphocyte count (TLC), hemoglobin, neutrophil to lymphocyte ratio (NLR), and the ratio of CD4+ and CD8+ T lymphocyte cells, and confounding variables included type, gender, and age.

The SPSS software version 25.0 was used for this study. Numerical variables will be shown as mean and standard deviation, while categorical variables will be shown as amounts and percentages. The Kolmogorov-Smirnov Test was used to determine normality. Spearman’s Correlation test analysis is used for non-normally distributed variables, whereas Pearson’s Correlation test analysis is used for normally distributed variables. If the p-value < 0.05, all values are considered significant.

RESULTS

This study was conducted at Prof. Dr. IGNG Ngoerah Hospital, Bali, from October 2021 to January 2022, with 56 research subjects who met the inclusion and exclusion criteria. The gender of the majority of patients was known to be 46 (82.1%) male patients and 10 (17.9%) female patients. Table 1 shows data on research characteristics.

The average age of the research subjects is productive and belongs to late adulthood, as shown in Table 1. The median total lymphocyte count of the subjects in the study was 0.64 x 10^9 μL. The average hemoglobin level of the study subjects was less than 12 g/dL, and the number of CD4+ T lymphocytes in the study subjects was less than 500 cells/mm^3.

Based on the normality test results conducted using the Kolmogorov-Smirnov Test, it is known that the hemoglobin variable is normally distributed while the other variables are not normally distributed. The Pearson Correlation test analysis was used for the hemoglobin variable, while Spearman’s Correlation test was used for the other variables.

Table 2 shows that the hemoglobin level with the number of CD4+ T lymphocytes has a significant weak positive correlation (r=0.329; p=0.013). The correlation between the neutrophil-to-lymphocyte ratio (NLR) and the number of CD4+ T lymphocytes showed a weak and significant negative relationship (r = -0.334; p = 0.012). A strong positive and significant correlation was found between the total lymphocyte count (TLC) and the number of CD4+ T lymphocytes (r = 0.777; p = 0.000) and between the ratio of CD4+/CD8+ T lymphocytes to the number of CD4+ T lymphocytes with r = 0.729; p = 0.000. The scatter plot graph in Figure 1 shows a correlation between the number of CD4+ T lymphocyte cells and the total lymphocyte count (TLC) (Figure
The findings of this study show that people with HIV in Prof. Dr. IGNG Ngoerah are more likely to be male than female, with an average adult age of 38 years, which is a productive age. This finding is consistent with data from the Ministry of Health of the Republic of Indonesia from 2017, which shows that men have a higher risk of developing HIV than women. According to the 2017 Republic of Indonesia Health Regulations, the highest percentage of infections occur between the ages of 25 and 49, namely 69.6% of those of productive age. Men are more at risk of contracting HIV AIDS, which may be caused by unhealthy sexual behavior, homosexuality, frequent changing of partners and the use of unsterile needles in drug abuse. However, women remain a serious concern because they are susceptible to infection from men and can infect the baby during pregnancy.

A positive correlation of $r=0.777$ was discovered in this study between the total lymphocyte count and the number of CD4+ T lymphocytes. This correlation demonstrates that, in some cases, the total lymphocyte count can be used instead of the CD4+ count. T lymphocyte cells that express CD4+ as a surface marker play an important role in immune system regulation and are the main target of HIV during the disease. The virus binds to and infects CD4+ cells, rendering them inactive. The number of CD4+ T lymphocytes in the blood decreases by 20%-40% during primary HIV infection. The CD4+ count indicates the number of T lymphocyte cells expressing CD4+ in the blood circulation, so if the CD4 count falls, so will the T lymphocyte cells. Githinji N et al. found a positive correlation between CD4+ and total lymphocyte count in HIV patients. In contrast, Stebbing J et al. found that in the absence of CD4+ counting facilities, total lymphocyte count could be used as a predictor of CD4+ in HIV patients. Mwamburi DM et al. discovered the same thing, stating that total lymphocyte count is quite good at predicting CD4+ counts.

Figure 1A), the number of CD4+ T lymphocyte cells and hemoglobin levels (Figure 1B), the number of CD4+ T lymphocyte cells and NLR (Figure 1C), number of CD4+ T lymphocytes with CD4+/CD8+ ratio (Figure 1D).

**Table 1.** Baseline characteristic of respondents

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Characteristics</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years) (mean±SD)</td>
<td>38.98 ± 11.58</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL) (mean±SD)</td>
<td>10.11 ± 1.49</td>
<td>0.058</td>
</tr>
<tr>
<td>Neutrophil to lymphocyte ratio (Median)</td>
<td>6.51</td>
<td>0.000*</td>
</tr>
<tr>
<td>Total lymphocyte count (x10³µL) (Median)</td>
<td>0.64</td>
<td>0.004*</td>
</tr>
<tr>
<td>CD4+/CD8+ T lymphocyte ratio (Median)</td>
<td>0.06</td>
<td>0.012*</td>
</tr>
<tr>
<td>CD4+ T lymphocytes (cells/mm³) (Median)</td>
<td>133.50</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

*Statistically significant if p-value less than 0.05

**Table 2.** Correlation between CD4+ T Lymphocytes and research variables

<table>
<thead>
<tr>
<th>CD4+ T lymphocytes</th>
<th>Coefficient Correlation (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>0.329</td>
<td>0.013*</td>
</tr>
<tr>
<td>NLR</td>
<td>-0.334</td>
<td>0.012*</td>
</tr>
<tr>
<td>TLC</td>
<td>0.777</td>
<td>0.000*</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>0.729</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*Statistically significant if p-value less than 0.05

**DISCUSSION**

The findings of this study show that people with HIV in Prof. Dr. IGNG Ngoerah are more likely to be male than female, with an average adult age of 38 years, which is a productive age. This finding is consistent with data from the Ministry of Health of the Republic of Indonesia from 2017, which shows that men have a higher risk of developing HIV than women. According to the 2017 Republic of Indonesia Health Regulations, the highest percentage of infections occur between the ages of 25 and 49, namely 69.6% of those of productive age. Men are more at risk of contracting HIV AIDS, which may be caused by unhealthy sexual behavior, homosexuality, frequent changing of partners and the use of unsterile needles in drug abuse. However, women remain a serious concern because they are susceptible to infection from men and can infect the baby during pregnancy.

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Anemia, particularly severe anemia, is common in HIV patients. Bleeding is the most obvious cause of anemia in HIV patients. Bleeding is linked to neoplastic
diseases such as Kaposi's sarcoma of the gastrointestinal tract and gastrointestinal lesions caused by opportunistic cytomegalovirus infection. HIV/AIDS-related anemia is most likely caused by three mechanisms: decreased erythrocyte cell production, increased erythrocyte cell destruction, and ineffective erythrocyte production. Decreased erythrocyte cell production may be due to decreased endogenous erythropoietin production and the use of myelosuppressive drugs such as Zidovudine. Hemolytic anemia and the use of various medications can cause an increase in the destruction of erythrocyte cells, for the generation of ineffective erythrocyte cells as a result of nutritional deficiencies such as iron, folic acid, or vitamin B12. Anemia symptoms account for nearly 80% of HIV-related complications. The activation of proapoptotic genes caused by decreased levels of CD4+ T lymphocytes and an increase in monocytes causes the average decrease in hemoglobin levels in HIV patients. HIV can directly affect cytokine secretion and bone marrow stromal cells. Soluble factors such as HIV protein and cytokines are thought to inhibit the growth of hematopoietic cells in HIV/AIDS patients’ bone marrow.

According to a previous study, one of the most common hematological complications in people with HIV/AIDS is anemia or low hemoglobin levels in patients. With \( r = 0.329 \) and \( p = 0.013 \), this study discovered a weak positive correlation between hemoglobin and CD4+ T lymphocyte levels. Marin JM et al. discovered a strong significant correlation between hemoglobin and CD4+ T lymphocytes while decreasing the CD8+ T lymphocyte ratio. This is because neutrophil dysfunction are more vulnerable to bacterial infections. According to Liew and Kubes’ study findings, NLR has no clear evidence of deterioration in HIV patients, but NLR is frequently used as a marker of inflammation.\(^\text{11}\) This study discovered a significant but weak negative correlation between NLR and CD4+ T lymphocytes (\( r = -0.334; p = 0.012 \)). These findings are consistent with Emokpae MA et al. research in Africa, in which NLR values in HIV patients were higher than in the control group of healthy patients (\( p < 0.001 \)), while the number of CD4+ T lymphocyte cells in HIV patients was lower (\( p < 0.001 \)).\(^\text{16}\) The neutrophil to lymphocyte ratio (NLR) can be used to assess the severity of opportunistic infections and as a marker of inflammation in HIV infection. The inflammatory process causes neutrophil apoptosis, which can interfere with neutrophil function and number, making HIV patients vulnerable to bacterial infections.\(^\text{18-20}\)

In HIV infection, the CD4+/CD8+ T lymphocyte ratio indirectly marks immune activation and inflammation. A low CD4+/CD8+ T lymphocyte ratio, according to Saracinoa A et al., predicts reduced immunity and is associated with increased morbidity and mortality in HIV-infected adults.\(^\text{21,22}\) A strong and significant correlation was discovered in this study between the CD4+/CD8+ T lymphocyte ratio and the level of CD4+ T lymphocytes (\( r = 0.729; p = 0.000 \)). Mendez TD et al. found that giving antiretroviral therapy to some patients can restore the CD4+ count while decreasing the CD8+ count, resulting in a normal CD4+/CD8+ T lymphocyte ratio.\(^\text{23-24}\) This study has limitations; because of the study’s small sample size, more prospective research with a larger sample size is required.

**CONCLUSION**

There is a correlation between the number of CD4+ T lymphocyte cells and total lymphocyte count (\( r = 0.777; p = 0.000 \)), hemoglobin (\( r = 0.329; p = 0.013 \)), neutrophil to lymphocyte ratio (\( r = -0.334; p = 0.012 \)), and CD4+/CD8+ ratio (\( r = 0.729; p = 0.000 \)) in this study. To assist in HIV monitoring, health workers with limited health facilities can estimate the number of CD4+ T cells based on total lymphocyte count, hemoglobin, and neutrophil-to-lymphocyte ratio. A low CD4+/CD8+ T lymphocyte ratio can predict the presence of decreased immunity and is linked to increased morbidity and mortality in patients.

**CONFLICT OF INTEREST**

The authors declare that there is no competing interest regarding the manuscript.

**ETHICAL CONSIDERATION**

This research was conducted based on the ethical conduct of research from the Ethics Committee of the Medical Faculty, Universitas Udayana, Prof I.G.N.G Ngoerah Hospital Denpasar and have received permission from the Research and Development Unit (R & D) of Universitas Udayana, Prof I.G.N.G Ngoerah Hospital Denpasar.

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**AUTHOR CONTRIBUTION**

All authors contributed to the study from the conceptual framework, data gathering, and analysis until the study’s results were interpreted upon publication.

**REFERENCES**

1. Simonetti FR, Dewar R, Maldarelli F. Diagnosis of Human Immunodeficiency Virus Infection. Eighth Edi, Mandell, Douglas, and Bennett's


