

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



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ABSTRACT

Background: Acquired immunodeficiency syndrome (AIDS) is caused by the Human immunodeficiency virus (HIV) infection and characterized by a progressive decrease in the immune system function which eventually leads to the development of opportunistic infections and other complications. In order to monitor the disease progression the CD4 examination and additional Complete Blood Count (CBC) were commonly used.

Objectives: To determine the correlation of total lymphocyte count, hemoglobin level, lymphocyte/leukocyte ratio (LLR) and lymphocyte/neutrophil ratio (LNR) to CD4 levels in patients with HIV infection.

Methods: This a retrospective cross-sectional analytic study conducted from July to August 2017. The total lymphocyte count, hemoglobin level, lymphocyte/leukocyte ratio (LLR) and lymphocyte/neutrophil ratio (LNR) and CD4 level were measured in the study. The data normality and the correlation were analyzed using the Kolmogorov-Smirnov test and the Pearson Correlation test, respectively, performed in SPSS version 14. The p-value less than 0.05 was considered significant for the results.

Results: A total of 60 samples conducted CD4 and complete blood counts (CBC) examinations during the period July-August 2017. The mean CD4 levels were 341.73 ± 243.48 cells/ μ L, the total leukocyte count was $6.98 \pm 2.93 \times 10^3$ / μ L, the total lymphocyte count was $2.09 \pm 0.87 \times 10^3$ / μ L, hemoglobin level 13.38 ± 2.09 g/dL, lymphocyte/leukocyte ratio (LLR) 0.31 ± 0.11 , lymphocyte/neutrophil ratio (LNR) 0.67 ± 0.40 . Statistical analysis showed that data is normally distributed. Pearson correlation analysis showed that there was a significant relationship between CD4 levels with total lymphocytes counts, hemoglobin levels, RLL, RLN with correlation coefficients of 0.571 ($p = 0.000$), 0.324 ($p = 0.012$), 0.509 ($p = 0.000$), 0.463 ($p = 0.006$), respectively. There was no significant correlation between CD4 levels and total leukocytes with a correlation coefficient of 0.171 ($p = 0.19$).

Conclusion: There is a positive correlation between CD4 levels and total lymphocytes, hemoglobin levels, RLL and RLN in patients with HIV at Sanglah Hospital.

Keywords: CD4 levels, HIV infection, complete blood counts.

Cite this Article: Wandu, I.N., Fuadi, M.R., Hadi, S. 2019. 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 Medical Journal* 8(2): 337-341. DOI:10.15562/bmj.v8i2.1408

BACKGROUND

From January to March 2017 the number of HIV infections reported was 10.376 people. The highest percentage of HIV infections was reported in the age group 25-49 years (69,6 %), followed by the age group 20-24 years (17.6%), and the age group ≥ 50 years (6.7%). The HIV ratio between men and women is 2:1. The highest percentage of HIV risk factors was in homosexuals (28%), heterosexuals (24%), others (9%) and the use of non-sterile needles (2%). The number of AIDS during January to March 2017 was reported as 637 people with the highest percentage of AIDS in the 30-39 years age group (38.6%), followed by the age group 20-29 years (29.3%) and the age group 40-49 year (16.5%). The AIDS ratio between men and women is 2: 1. The highest percentage of AIDS risk factors is risk sex

for heterosexuals (67%), homosexuals (23%), perinatal (2%) and use of nonsterile needles (2%).¹

HIV infection has affected around 60 million people to date. In 2009, 33.3 million people were living with HIV worldwide; 2.6 million new cases were presented, and 1.8 million deaths were due to AIDS in the same year. In 2009, Sub-Saharan Africa was the world's leading region for deaths caused by AIDS, carrying 1.3 million cases. Even though AIDS is a global problem, countries with fewer resources have a greater impact.^{2,3}

HIV is a retrovirus that mainly affects T cells and cells that express CD4, such as macrophages, follicle dendritic cells, and lymph nodes. In a history of natural HIV infection, there is an initial decrease in TCD4 lymphocyte count associated with primary

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Received: 2018-12-01
Accepted: 2019-04-01
Published: 2019-08-01

clinical infection (2 weeks after infection) then a partial recovery occurs, due to atypical lymphocytes and an increase in CD8 T cell lymphocytes (3-4 weeks after exposure). Finally, the lymphocyte count decreases again; slowly during a latent period and faster during the final stage characterized by immunodeficiency known as the decrease in the number of CD4 T cells below 500 CD4/ μ L.

For this reason, both the percentage of CD4 T cell lymphocytes and the occurrence of opportunistic infections are used to stage HIV infection and establish treatment guidelines. In monitoring the development of HIV infection, there are three stages of laboratory examination, namely: calculate the total leukocyte count, the percentage of the number of lymphocytes or differential count, and calculate the percentage of lymphocyte counts of CD4 T cells.⁴ In this study, we wanted to find out whether there was a relationship between the results of a complete blood test (DL) and the level of CD4 T cells in patients who have HIV. Complete blood test results include total leukocytes, total lymphocyte count, lymphocyte/leukocyte ratio (LLR), lymphocyte/neutrophil ratio (LNR) and hemoglobin levels. Moreover, the parameters which can estimate levels CD4 T cells in complete blood tests will be explored.

METHODS

This study is a retrospective cross-sectional analytical study. The subject in this study was taken in the period July to August 2017 which carried out a complete blood examination and CD4. Complete blood examination using a *CELL-DYN Ruby Hematology Analyzer Abbott Diagnostics*®, while, the CD4 levels examination using a *BD FACSCount™ System*®. The lymphocyte/leukocyte ratio is obtained by dividing the total number of lymphocyte counts by the total number of leukocytes. The lymphocyte/neutrophil ratio is obtained by dividing the total number of lymphocyte counts by the total number of neutrophil counts.

Statistic analysis

The data normality was analyzed using Kolmogorov-Smirnov test and correlation analysis using Pearson Correlation test. This analysis was conducted using SPSS version 14. The p-value <0.05 was considered as significant.

RESULTS

During the study, the number of samples that fulfilled the criteria were 60 patients who performed a complete blood count examination (CBC) and examination of lymphocyte CD4 T-cells. There were 32 (53.33%) male patients and 28 (46.67%) female patients. The following are the characteristics of the research subject during the period July 2017 to August 2017.

In [Table 1](#), it shows that the age of research subjects suffering from HIV is productive age and classified as young. The mean total lymphocyte count is still within normal limits. The mean CD4 level of HIV positive subjects was low (less than 500 cells/ μ L). The mean hemoglobin level of the study subjects was within normal limits (above 12 g/dL).

[Table 2](#) showed that the total number of leukocytes with CD4 levels has a weak correlation ($r = 0.027$) and the significance level is not significant ($p = 0.835$). There is a correlation with the total number of lymphocytes with CD4 levels in the blood, with a moderate strength ($r = 0.564$) and a significant level of significance ($p = 0.000$).

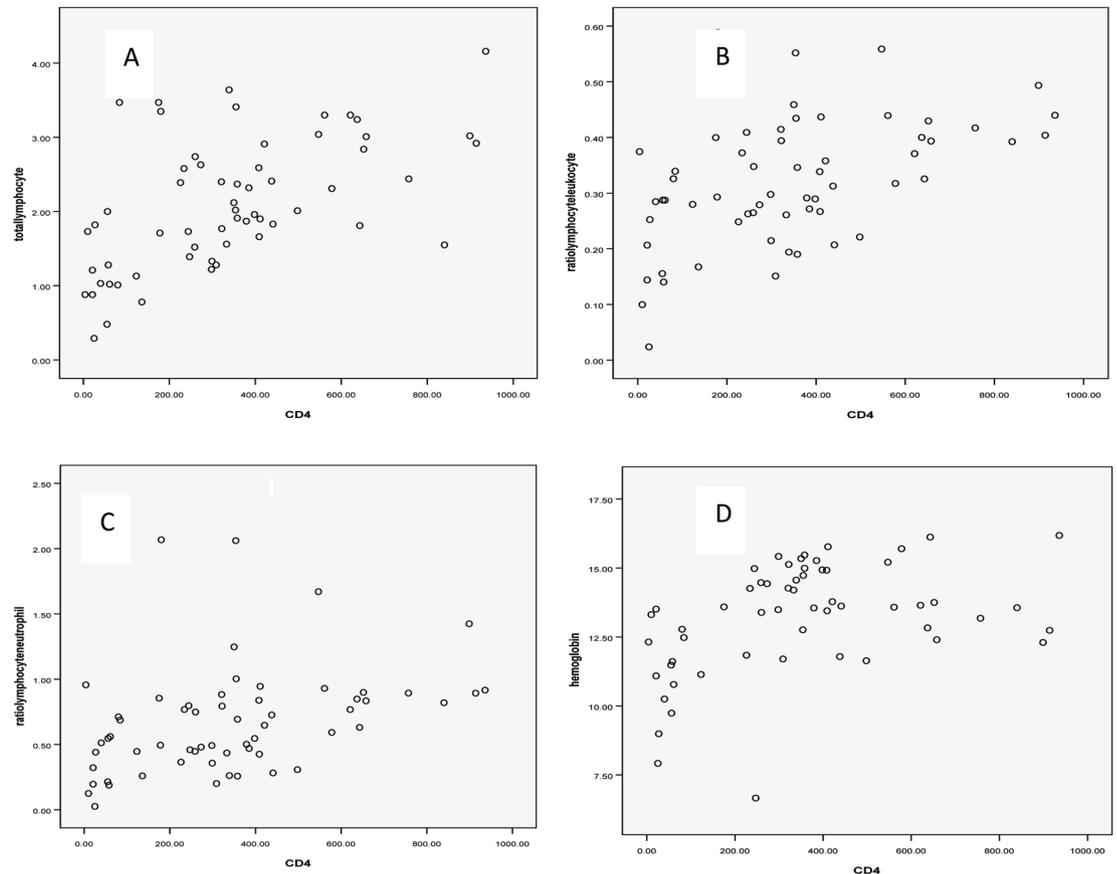
The results of the *Pearson* correlation test analysis showed that there was a moderate correlation between the ratio of total lymphocyte/total leukocytes and the CD4 T-cell levels ($r = 0.506$, $p=0.000$). While the correlation between the ratio of total lymphocyte/neutrophil and level CD4 T cells, showed a weak $p=0.003$). A weak unidirectional association also observed for the correlation between the hemoglobin level and CD4 T cell levels, ($r = 0.324$, p -value = 0.012).

Table 1 Characteristics of sample

Characteristics of subjects	Mean \pm SD
Age (years)	34.40 \pm 1.51
Total Leucocyte ($\times 10^3/\mu$ L)	6.98 \pm 2.93
Total Lymphocyte($\times 10^3/\mu$ L)	2.10 \pm 0.87
Lymphocyte/leucocyte ratio (LLR)	0.32 \pm 0.11
Lymphocyte/neutrophil ratio (LNR)	0.67 \pm 0.41
Haemoglobin level(g/dL)	13.38 \pm 2.09
CD4 level (cell/ μ L)	341.73 \pm 243.48

Table 2 Results of the *Pearson* correlation test between research variables

No.	Research variable	The coefficient of correlation (r)	Direction of correlation	P value
1	Total leukocytes with CD4 levels	0.027 (weak)	Positive (in the same direction)	0.835
2	Total lymphocytes with CD4 levels	0.564 (medium)	Positive (in the same direction)	0,000
3	Total lymphocyte / total leukocyte ratio with CD4 level	0,506 (medium)	Positive (in the same direction)	0,000
4	Total lymphocyte / neutrophil ratio with CD4 level	0.375 (weak)	Positive (in the same direction)	0.003
5	Hemoglobin level with CD4 level	0.324 (weak)	Positive (in the same direction)	0.012

**Picture 1** Scatterplot diagram showing the correlation between (A) total lymphocytes with CD4 T cell levels, (B) lymphocyte/leukocyte ratio with CD4 T cells, (C) lymphocyte/neutrophil ratio with CD4 T cell levels, and (D) hemoglobin level with CD4 T cell levels

DISCUSSION

The results of this study suggest that the number of people with HIV in men is higher compared to women. This finding is similar with data from the Republic of Indonesia Ministry of Health which states that the ratio of HIV among men with the women of 2:1. The average age of people living with HIV in this study was 34 years (productive age). The data from the Republic of Indonesia Ministry of Health in 2017 support our findings. It show that the highest percentage of infections is around 25-49 years old (69.6%). This situation may be resulted from risky sexual behavior for heterosexuals and homosexuals and the use of nonsterile needles on drug abuse.¹

The HIV infection has a cytotoxic effect on T-helper lymphocytes, which in turn causes B cell dysregulation and changes in cytokine release. T cells infected with HIV directly suppress the growth of the spinal cord, thereby suppressing hemopoiesis. CD4 is a cell receptor found on the cell surface that is the target of HIV, carried by T-helper lymphocytes, monocytes and microvascular endothelial cells commonly present in the bone marrow. Monocyte cell infections in the bone marrow that further alter the release of cytokines, which indirectly suppress the capacity of hemopoietic progenitor cells to respond adequately to anemia and other

peripheral cytopenias. This explains why many patients experience pancytopenia at the advanced stage of HIV.⁵

Various factors are influencing the decrease in the number of CD4 T lymphocyte cells, which causes patients with HIV to be very susceptible to infection. These factors are the direct cytopathic effects of HIV on CD4 lymphocyte cells and their progenitors, induction of apoptosis through immune system activation, destruction of stem cells and bone marrow stromal cells, cytokine cytotoxicity, destruction of lymphoid tissues including the thymus gland so that the production of new cells is not happening. The destruction of stem cells and bone marrow stroma can have an impact on the function and number of neutrophils. Neutrophil dysfunction can cause a person susceptible to bacterial infection.⁶

Research conducted by Angelo *et al.*, 2007, showed that there is a strong correlation between the total lymphocyte count and the number of CD4⁺ T cells ($r=0.581$) in the group, but there is a weak correlation when patients are grouped according to the number of their CD4⁺ T cells. There is a correlation between the total lymphocyte count and CD4 T cells for the whole group, as well as for the subgroup. There was no significant correlation ($r = -0.019$) between the percentage of total lymphocytes and CD4⁺ T cells for the entire group. Interestingly, when observations were grouped based on the number of CD4⁺T cells, a strong negative correlation emerged. In contrast, a strong positive correlation ($r = 0.763$) was indicated between the total lymphocyte count and the number of CD8⁺T cells for the entire group. Weak correlations were also found between TC D4⁺cells and CD8⁺ T cells ($r = 0.280$).⁷

This study found that there is a moderate positive correlation between the total lymphocyte count and CD4 T cell levels and the ratio of total lymphocytes/leukocytes to CD4 T cell levels. The result shows that the CD4 T-cells level is increased following the increase in the total number of lymphocyte cells and the ratio of lymphocytes/leukocytes. This is in line with Angelo *et al.*, 2007 study, which demonstrated a correlation between total lymphocyte counts and CD4 T lymphocyte levels.

The CD4 T-cells play an essential role in controlling the replication and development of HIV into AIDS. However, HIV-1 infection is associated with progressive loss of T cell functional capacity including decreased response to antigenic sensitization, reduced ability to produce cytokines and reduced proliferative and cytotoxic activity. Previous research has found the correlation between the neutrophil dysfunction and superoxide radical

decline to the severity of HIV infected subjects, oxidative stress enzyme activity and the absence of differences in respiratory enzyme activity in HIV-1 infection by sex.^{8,9}

This study showed that there is a weak positive correlation between the ratio of leukocytes/neutrophils to CD4 T lymphocyte levels and hemoglobin levels with CD4 T lymphocyte levels ($p<0.05$). These indicate that CD4 T lymphocyte levels are positively related to the amount of lymphocyte cell production. The higher level of CD4 T lymphocyte cells reduces the anemia in HIV patients. Anemia is one of the most common hematological disorders in people with HIV, and HIV patients are also often found to have low levels of hematocrit.^{10,11}

Research conducted by Emokpae *et al.*, 2017 showed that the ratio of neutrophil/lymphocytes in patients with HIV-1 infection was higher than the control group healthy individuals ($p <0.001$). This suggests that patients with HIV infection have lower lymphocyte proportions compared to healthy individuals.¹²

CONCLUSION

There is a moderate positive correlation between total lymphocyte count and CD4 T cell lymphocyte level and lymphocyte/leukocyte ratio (LLR) with CD4 T cell lymphocytes. A weak positive correlation between the ratio of lymphocyte/neutrophil (LNR) with levels of CD4 T lymphocytes and hemoglobin levels with CD4 T cell lymphocytes. Not found a significant correlation between total leukocyte levels with levels of CD4 T cell lymphocytes.

ETHICAL CLEARANCE

This study has been received ethical approval by Ethics Committee of Udayana University, Bali, Indonesia.

CONFLICT OF INTEREST

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

FUNDING

The authors are responsible for study funding without any involvement of grant, sponsorship, or any other resources of funding.

AUTHOR'S CONTRIBUTION

I Nyoman Wandu and Muhamad Robi'ul Fuadi conceived of the presented idea. I Nyoman Wandu

developed the theory and Solichul Hadi verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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