INDONESIAN JOURNAL OF

CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

CONTENTS

RESEARCH

LITERATURE REVIEW

CASE REPORT

CORRELATION OF TOTAL LYMPHOCYTE COUNT WITH CD4 COUNT IN HIV/TB COINFECTED PATIENTS

Herniaty Rampo, Uleng Bahrun, Mansyur Arif

Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia.
E-mail: herniatyrampo24@gmail.com

ABSTRACT

HIV-TB co-infection is a global challenge in the healthcare world. HIV infection which has a role in TB immunity causes CD4 to decrease. CD4 as a marker for HIV progression is expensive and not available in all healthcare facilities, so the WHO recommended Total Lymphocyte Count (TLC) as a substitute for CD4. This study aimed to determine the correlation between TLC and CD4 in HIV-TB co-infected patients. This research was a retrospective study using the medical records of hospitalized patients with HIV-TB co-infection in the Dr.Wahidin Sudirohusodo Hospital. The spearmant test analyzed the correlation. CD4 data were divided into two groups: CD4 < 200 cells/mm^3 and CD4 ≥ 200 cells/mm^3 and Mann-Whitney test was performed. Sensitivity and specificity of TLC were determined by ROC curve analysis. There were 172 patients with a diagnosis of HIV-TB co-infection. Spearman test showed a positive correlation between TLC and CD4 with a moderate correlation strength (p <0.001 and r = 0.56). The Mann-Whitney test showed a significant TLC difference between CD4 < 200 cells/mm^3 and CD4 ≥ 200 cells/mm^3 (p < 0.001). The ROC curve analysis showed that TLC had a good accuracy value for predicting CD4 in CD4< 200 cells/mm^3 (AUC = 0.911). A sensitivity of 88.9% and a specificity of 84.7% was found in TLC 1154.9 cells/mm^3 which predicted CD4 < 200 cells/mm^3.There was a positive correlation between TLC and CD4 with a moderate correlation strength. Total lymphocyte count could be used to predict CD4 in CD4 <200 cells/mm^3. The researcher suggests further study in HIV-TB coinfected patients with a larger sample size grouped into an active phase and latent phase TB.

Key words: HIV-TB co-infection, TLC, CD4

INTRODUCTION

Human Immunodeficiency Virus (HIV)-Tuberculosis (TB) coinfection is a global challenge in healthcare. In 2016, the World Health Organization (WHO) reported that 36.7 million people were suffering from HIV infection. TB is the most prevalent opportunistic infection in HIV patients. It is estimated that 14-15% of TB patients are co-infected with HIV. In 2015, there were 10.4 million TB cases, about 1.2 million of which were people with HIV infection. In 2016, 1.5 million deaths in TB were reported, 1.1 million in HIV-negative TB patients and 0.4 million in HIV-positive TB patients.[1,2]

Tuberculosis in people with HIV has nonspecific clinical features causing difficulty in diagnosis and TB is the most common cause of death in HIV patients. Tuberculosis has been proven to accelerate the course of HIV infection. The mortality rate of HIV-TB coinfection is four times higher than that of non-HIV-infected TB mortality. HIV and tuberculosis infection is a serious problem because it poses a health threat to human kind, which if not handled seriously will cause them uncontrollable.[2]

HIV and TB infectionare intracellular infections and are known to be related to each other. HIV infection causes a decrease in the number of CD4 cells which play a significant role in immunity against TB. HIV infects cells by using envelope glycoproteins called gp120 which primarily bind to CD4 cells. Therefore, the virus mainly infects CD4 cells.[3] Tuberculosis is caused by *Mycobacterium tuberculosis.* Immune response to TB infection consists of non-specific (primary) response starting from the initiation of infection to bacterial phagocytosis by macrophages, and specific (secondary) response initiated by T cell lymphocyte activation. *Mycobacterium tuberculosis* infection stimulates cellular immunity in which T helper lymphocytes or CD4 cell activate macrophages by releasing IFN-γ.[3]

CD4 cell count is a marker to assess the disease progression of HIV-TB co-infected patients. CD4 tests are expensive and not always available in all healthcare facilities. When CD4 test cannot be performed, WHO recommends the use of total

lymphocyte count (TLC) as a marker to start and monitor therapy of HIV. The World Health Organization states that TLC ≤ 1200 cells/mm^3 is equivalent to CD4 cells ≤ 200 cells/mm^3.[4] Reduced CD4 cell count was equivalent to reduced TLC as reported by Alavi et al.[5] A study by Sen showed that TLC had a sensitivity of 70.7% and a specificity of 81.7% in predicting CD4 cell count of 200 cells/mm^3.[6] Jerry et al. found a positive correlation between TLC and CD4 cell count in HIV patients with cognitive impairment. A study conducted by Angelo showed no relationship between TLC and CD4 cell count.[7] CD4 cell count in HIV patients can be affected by some factors, one of which is HIV-TB coinfection. Lymphocytes play a role in cellular response to *Mycobacterium tuberculosis* infection. A study conducted by Kumarasamy et al. and Martin et al. showed a positive correlation (correlation coefficient of 0.7 and 0.74, respectively) between TLC and CD4 cell count in HIV-TB co-infected patients.[8,9]

Studies on the correlation of TLC and CD4 cell counts in HIV had been frequently reported, but the correlation of TLC and CD4 counts in HIV-TB coinfected patients had never been reported in Indonesia, especially in Makassar, so we were interested in conducting this study.

METHODS

This study was conducted retrospectively, with the study population being all adult (>18 years old) patients diagnosed with HIV-TB coinfection in the Dr. Wahidin Sudirohusodo Hospital Makassar. The study samples were the study population tested for TLC and CD4 cell count. Sample collection was done by taking medical record data from January 2015 to June 2017 in the Medical Record Installation of the Dr. Wahidin Sudirohusodo Hospital Makassar. From a total of 195 data of HIV-TB co-infection, 23 data were excluded because the TLC and CD4 cell count data were not obtained at the same time. Patients receiving antiretroviral and antituberculosis treatment were excluded. The ethical clearance was obtained from the Medical Research Ethics Commission, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital Hasanuddin University Hospital, Makassar.

Total lymphocyte count is the number of lymphocytes per mm^3 of blood, measured using Sysmex XN 1000, Sysmex Asia Pacific Pte Ltd and ABX Pentra 80 (Horiba, France). CD4 cell count is the number of CD4 cells per mm^3 of blood, measured with flow cytometry method using Alere® device. The study samples were divided into two groups based on CD4 cell count which was CD4 <200 cells/mm^3 and CD4 ≥200 cells/mm^3.

Spearman correlation test was performed to assess the correlation between TLC and CD4 cell count. Mann-Whitney test was performed to assess the TLC difference between CD4 <200 cells/mm^3 and CD4 ≥ 200 cells/mm^3 groups. Results were considered significant if p was<0.05. Receiver Operating Characteristic (ROC) curves analysis was performed to assess TLC accuracy in predicting CD4 cell count.

RESULTS AND DISCUSSION

A total of 172 data of patients were included in this study, with data characteristics as shown in Table 1. The data showed that HIV-TB coinfected patients were more prevalent in males (80.2%) in the age group 31-40 years (45.9%). The youngest patient was 21 years old, and the oldest patient was 57 years old.

Table 1. Sample characteristics

Variable	N = 172	Percentage (%)
Sex		
Male	138	80.2
Female	34	19.8
Age		
19-30	38	22.1
31-40	79	45.9
41-50	42	24.4
>50	13	7.6
CD4 (cells/mm^3)		
<200	169	98
≥200	3	1.7

Gender and age distribution in this study was consistent with the study of Wang et al. who reported that the incidence of HIV-TB co-infection was more common in males than females (61.1%: 38.9%) with the highest frequency in the 30-45 years group.[10]

Table 2. TLC dan CD4 correlation

	Median (min-max)	p*	r*
TLC (cells/mm^3)	726 (73-3217)	< 0.001	0.56
CD4 (cells/mm^3)	24 (1-441)		

*Spearman correlation test

Spearman correlation test showed a significant positive correlation between TLC and CD4 cell count with moderate correlation strength (p <0.001 and r = 0.56). This study was consistent with a study conducted by Kumarasamy et al. and Martin et al. which showed a positive correlation (correlation

coefficient of 0.7 and 0.74 respectively) between TLC and CD4 cell count in HIV-TB co-infected patients.[8] This suggested that the decrease in TLC cell count was followed by a decrease in finding CD4 cell count. CD4 is the type of T lymphocyte cells formed in the thymus gland in the form of naive CD4 cells. Lymphocytes consist of two subtypes: B lymphocytes, which are the primary cells of the humoral immunity and T lymphocytes, which are the primary cells of the cellular immunity. Lymphocytes consist of 3% - 21% B cells and 20% - 80% T cells. T cells consist of 34% -67% of CD4 cells and 10% - 42% of CD8 cells.[3]

Table 3. Comparison of TLC in CD4 groups (<200 and ≥200)

	TLC (cells/mm^3) Median (min - max)	p*
CD4 (cells/mm^3)		
< 200	698.7 (73 - 2570)	< 0.001
≥ 200	1687.2 (902 - 3217)	

*Mann-Whitney test

Mann-Whitney test showed that there was astatistically significant difference in TLC between the CD4 <200 cells/mm^3 and the CD4 ≥ 200 cells /mm^3 groups (p <0.001). Human immunodeficiency virus infections cause severe immune function disorders characterized by decreased CD4 cells and the emergence of opportunistic infections. The virus enters the body by infecting the rectum or vaginal mucosal Langerhans cells. The virus is then spread through viremia accompanied by the early acute syndrome. The virus infects cells by using envelope glycoproteins called gp120 which primarily bind to CD4 cells. Therefore, the virus mainly infects CD4 cells. Macrophages and dendritic cells can also be infected. The early acute syndrome phase will be followed by the latent phase. In this phase, the immune response (CD8 and anti-HIV antibodies) occurs, resulting in a decrease of viral load. Although the level of virus is low in the latent phase, the destruction of CD4 cells continues in the lymphoid tissue. As a result, the number of CD4 cells in the circulation decreases. This phase may take many years before turning to a chronic progressive phase, and the patient becomes susceptible to various infections and is called Acquired Immunodeficiency Syndrome (AIDS). From the various studies obtained, patients began to have AIDS with a CD4 cell count <200 cells/mm^3.[3]

Lymphocytes play a role in the immune response to *Mycobacterium tuberculosis* infection. T lymphocytes will be activated and proliferate in the acute phase of *Mycobacterium tuberculosis*

infection. Therefore, lymphocytosis can occur early in the infection and in the latent phase. However, in the active phase lymphopenia can occur (decreased lymphocyte count below 2,000/mm^3) which shows the active tuberculosis process as a result of CD4 cell decline. The study conducted by Zuri *et al.* found a significant difference between CD4 counts in latent HIV-TB coinfected patients with active phase HIV-TB. Successful treatment of tuberculosis can be assessed by the number of lymphocytes that return to normal or sometimes increase.[11,12]
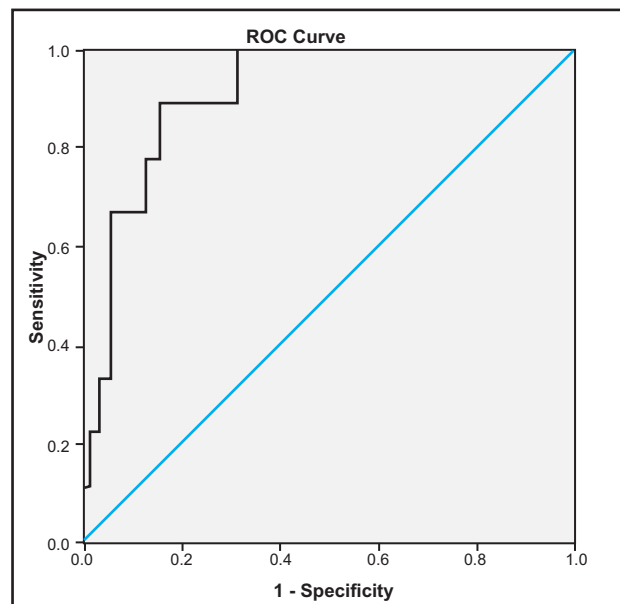

Figure 1. ROC curve for TLC to predict CD4 <200 cells/mm^3

The ROC curve analysis showed the Area Under Curve (AUC) of 0.911. It suggested that TLC had good accuracy for predicting CD4 cell count<200 cells /mm^3 in HIV-TB co-infected patients (Figure 1).

Table 4. Sensitivity and specificity from various TLC cut-off values to predict CD4 <200/mm^3

TLC (cells/mm^3)	Sensitivity	Specificity
896.8	100	68.7
1154.9	88.9	84.7
1266.2	77.8	87.1
1648.6	66.7	94.5
2043.3	33.3	96.9
2277.9	22.2	98.8
2894.1	11.1	100

The various cut-off was tested for sensitivity and specificity through curve analysis (Table 4). The highest sensitivity was 100% obtained from TLC cut-off of 896.8 cells/mm^3 with a specificityof 68,7%. The highest specificity was 100% derived from cut-off 2894.1 cells/mm^3 with a sensitivity of 11.1%.

TLC cut-off of 1,154.9 cells/mm^3 which had a sensitivity of 88.9% and specificity of 84.7% as the best cut-off to predict CD4 cell count <200 cells/mm^3 considering the specificity and sensitivity obtained from ROC analysis. This result was consistent with the WHO recommendations which stated that TLC≤ 1200 cells/mm^3 is equivalent to CD4≤200 cells/mm^3.[4] A study conducted by Blatt *et al*. found a 98% sensitivity in TLC<1000 cells/mm^3 in predicting CD4 <200 cells/mm^3.[13] Other study conducted by Martin *et al*. concluded that TLC<1,250 cells/mm^3 and CD4 <200 cells/mm^3 were the same predictors in HIV-TB coinfected patients.[9]

Limitations of this study were the assessment of the correlation of TLC and CD4 cell counts in HIV-TB coinfected patients unable to distinguish between latent phase TB and active phase TB.

CONCLUSION AND SUGGESTION

The correlation between TLC and CD4 cell counts was found to be significantly positive with moderate correlation strength. There were significant differences in TLC between CD4 <200 cells/mm^3 and CD4 ≥ 200 cells/mm^3 groups. TLC can be used to predict CD4 cell count in HIV-TB co-infected patients. TLC <1154.9 cells/mm^3 can predict CD4 <200 cells/mm^3 with a sensitivity of 88.9% and a specificity of 84.7%. The researcher suggests a study in HIV-TB coinfected patients with a larger sample size which are grouped into an active phase and latent phase TB.

REFERENCES

1. World Health Organization. Global HIV statistics. 2016 [cited on May 10,2017]. Available at: http://www.unaids.org/en/resources/fact-sheet.
2. World Health Organization. Global tuberculosis report. 2016 [cited at May 10, 2017]. Available at: http://apps.who.int/iris/bitstream/1n0665/2441/1/9789241565394-eng.pdf.
3. Baratawidjaja KG. Defisiensi imun dalam imunologi dasar. Ed ke-11., Jakarta, Badan Penerbit FKUH. 2014; 449-57.
4. WHO. Guidelines for HIV diagnosis and monitoring of anti retroviral therapy.[cited on May 4, 2017]. Available at: www.who.int/hiv/pub/guidelines/pdf.
5. Alavi SM, Ahmadi F, Farhadi M. Correlation between total lymphocyte count, hemoglobin, hematocrit and CD4 count in HIV/AIDS patients. Acta Medica Iranica, 2009; 47(1): 1-4.
6. Sen LCS, Vyas A, Sanghi LCS, Shanmuganandan K, Gupta RM, *et al*. Correlation of CD4+ T cell count with total lymphocyte count hemoglobin and erythrocyte sedimentation rate levels in human immunodeficiency virus type-1 disease. MJAFI, 2011; 67: 15-20.
7. Angelo AL, Angelo CD, TorresAJ, Ramos AM, Lima M, *et al*. Evaluating total lymphocyte counts as a substitute for CD4 counts in the follow-up of AIDS patients. BJID, 2007; 11(5): 466-70.
8. Kumarasamy N, Mahajan AP, Flanigan TP, Hemalatha R, Mayer KH, *et al*. Total Lymphocyte Count (TLC) is a useful tool for the timing of opportunistic infection prophylaxis in India and other resource-constrained countries. J Acquir Immune Defic Syndr, 2002; 31(4): 378-83.
9. Martin DJ, Sim JGM, Sole GJ, Rymer L, Shalekoff S, *et al*. CD4+ lymphocyte count in African patients co-infected with HIV and tuberculosis. J Acquir Immune Defic Syndr Human Retrovirol, 1995; 8(4): 386-91.
10. Wang W, Zhang W, Alayi A, Yan Ci, Zhang WW, Chao MQ. The Characteristics of TB epidemic and TB/HIV co-infection epidemic: A 2007–2013 retrospective study in Urumqi, Xinjiang Province, China. PLoS One Journal, 2016; 11(10): e0164947.
11. Mabrouk ME. Differential count and total white blood cells among tuberculosis patients under treatment attending Kenana Hospital in White Nile State. J. Blood Disorder, 2017; 4(1): id1045.
12. Sullivan ZA, Wong EB, Ndung'u T, Kasprowicz VO, Bishai WR. Latent and active tuberculosis infection increase immune activation in individuals co-infected with HIV. Ebio Medicine Journal, 2015; 2(4): 334-340.
13. Blatt SP, Lucey CR, Butzin CA, Hendrix CW, Lucey DR. Total lymphocyte count as a predictor of absolute CD4+ count and CD4+ percentage in HIV-infected persons. JAMA, 1993; 269(5): 622-6.