The Correlation of Treg Cells (CD4+CD25+) with Viral Load between Patients and TB-HIV Patients in Semarang, Indonesia

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***Abstract*—**The number of HIV-AIDS patients in Indonesia has considerably increased. The life expectancy of HIV-AIDS patients gets longer in the presence of antiretroviral treatment (ARV). Most opportunistic infections of HIV infection are found to be Tuberculosis (TB-HIV). Antiretroviral and anti-tuberculosis treatment will improve the quality of life of people living with HIV-AIDS (PLWHA). Regulatory T cells (Tregs) were suggested to play a role in immune response of HIV and TB-HIV. This study aims to investigate the response of Tregs of patients with HIV and TB-HIV, their clinical changes, and other immunological aspects such as CD4+ T cells. The viral load in peripheral blood among two groups after receiving antiretroviral treatment (ARV) was also measured.

The cohort study was carried out in Dr Kariadi Hospital Semarang from January 2012 to June 2013 on 52 HIV patients and 40 TB-HIV patients who were observed for one year of antiretroviral and antituberculosis treatment. Five HIV patients and 5 TB-HIV patients died during the studies. The number of Tregs (CD4+CD25+) was assayed by flowcytometer (FACS), viral load was done by measuring HIV-RNA in peripheral blood of patients with HIV and TB-HIV after one year antiretroviral treatment.

The results indicate that the number of Tregs was significantly different between the two groups at the beginning of treatment ($p = 0.001$). The number of Treg was increased in the HIV patients and was decreased in the TB-HIV patients after antiretroviral treatment. However, there was inverse relationship between the number of Tregs (CD4+ CD25+) and CD4+ T cells. There was positive relationship between the number of Tregs with viral load of HIV patients and TB-HIV patients after antiretroviral Treatment.

In conclusions, the number of Tregs (CD4 + CD25 +) may be suggested as a factor to determine progression of the diseases beside CD4+ T cells and viral load

***Keywords*—**Treg cells (CD4 + CD25 +), CD4 T cell, viral load, HIV, TB-HIV.

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I. Introduction

IN Indonesia, HIV-AIDS cases have been increasing every year. On June 2013, there were 108,600 HIV and 43,667 AIDS cases, 8,235 died. From those who died, 50% were TB-HIV [1].

The goals of treatment of PLWHA (people living with HIV-AIDS) are getting three zero: no new cases of HIV, no AIDS deaths, there is no stigma and discrimination. This is expected to improve the quality of life of PLWHA. The number of PLWHA in Indonesia who received antiretroviral therapy until September 2013 was 36,483 people, with 3:1 being the ratio of men and women, and the highest percentage aged 20-29 years old. The use of the regimen was 33,847 (96.82%) using first line drugs, and 1,110 (3.17%) second lines drugs and 4 were unknown. The death rate of PLWHA has declined after ARV therapy from 18.16% (2000) to 0.85% (2013) [1]-[3].

It is unclear how Treg cells play a role in HIV and TB-HIV infection of T cells in response to failures that facilitate the persistence of HIV virus in the body or vice versa, and how Treg cells play a protective role by limiting the activation of the immune system which, in this case, a decrease of Treg cells level will affect the deviation T cell activation [4]-[6].

In HIV infection, Treg cells play different roles, depending on the severity of the disease. In the early stages, the number of Treg cells increases and may reduce the adaptive response of virus-specific T cells, so as to effectively prevent the virus while cleaning the secretion of IFN-γ (pro-inflammatory cytokines) that has increased as a result of HIV antigen stimulation [7]-[9].

The existence of Treg cells play a role in controlling immune activation (plays a role in balancing the immune system) and the progression of the disease. There is an inverse correlation

between the number of Treg cells with progression of disease (low CD4 counts or high viral load) [6]-[9].

The objectives of this study were to assess the response of Treg in the immune status of patients infected with HIV and TB-HIV, and to know the clinical progression and immunological changes (Treg, CD4), and viral load in the peripheral blood of the patients.

II. Material and Method

Subjects were patients (age 18 years or older) with HIV and TB-HIV seeking treatment at the VCT-CST Clinic - Dr Kariadi Hospital Semarang Indonesia. The HIV diagnosis was based on WHO criteria 2010, while the diagnosis of tuberculosis was based on: clinical features, radiological, and/or bacteriological examinations (sputum smear 3x). Patients with HIV received antiretroviral therapy according to WHO 2010, and TB-HIV patients received antituberculosis therapy during the first two weeks, then added antiretroviral therapy to antituberculosis drugs. HIV patients treated with Lamivudine, Zidovudine, and Nevirapine, while TB-HIV patients received Lamivudine, Zidovudine, and Efavirenz. The FDC (fixed drug combination) of Anti TB drugs (Isoniazid, Rifampicin, Ethambutol and Pyrazinamide) is the appropriate treatment program by the Indonesian Ministry of Health.

All parameters will be compared between the two groups after ARV treatment. The study design was a cohort study to examine the relationship between clinical stages, Treg, and viral load, with the progression of HIV disease.

At the start of ARVs, the study subjects were examined for Treg cells, CD4, viral load, and clinical stages (stage I-II and III-IV). After one-year observation, the number of Treg cells, CD4, viral load, and clinical stages were reevaluated. The HIV tests were carried out in the Laboratory of Clinical Pathology, Dr. Kariadi Hospital Semarang Indonesia. Treg and CD4 T cells were counted flow cytometrically using fluorochrome labeled monoclonal antibodies: anti CD3 FITC, CD25 PE, CD4 perCP and CD3 FITC, CD4 PE and CD45 PerCP, viral load were carried out in the Laboratory of Clinical Pathology, Faculty of Medicine, University of Gadjah Mada Yogyakarta Indonesia and Laboratory of Clinical Microbiology Dr Kariadi Hospital Semarang Indonesia

III. Result and Discussion

During the study period, 52 HIV and 40 HIV-TB patients were included. After 1 year of antiretroviral therapy, 10 patients died: 5 patients with HIV and 5 patients with TB-HIV. The remaining 82 patients consisted of 47 HIV patients (28 males, 19 females) and 35 HIV-TB patients (24 males, 11 females). The mean of patients with HIV was 35 ± 6 years, and of TB-HIV patients was 36 ± 6 years.

The baseline CD4 of HIV patients after antiretroviral treatment was higher than TB-HIV (HIV CD4: 224 cells / mL; TB-HIV: 163 cells / mL) (p = 0.00). The CD4 HIV group increased 57.29 ± 97.04 cells / mL, while TB-HIV increased 75.07 ± 11 cells / mL. (p = 0.001).

The number of Treg cells in HIV patients before treatment is lower than that of TB-HIV: 18.8 versus 31.1 (p=0.001). The number of Tregs increased in the HIV but decreased in TB-HIV (p = 0.001).

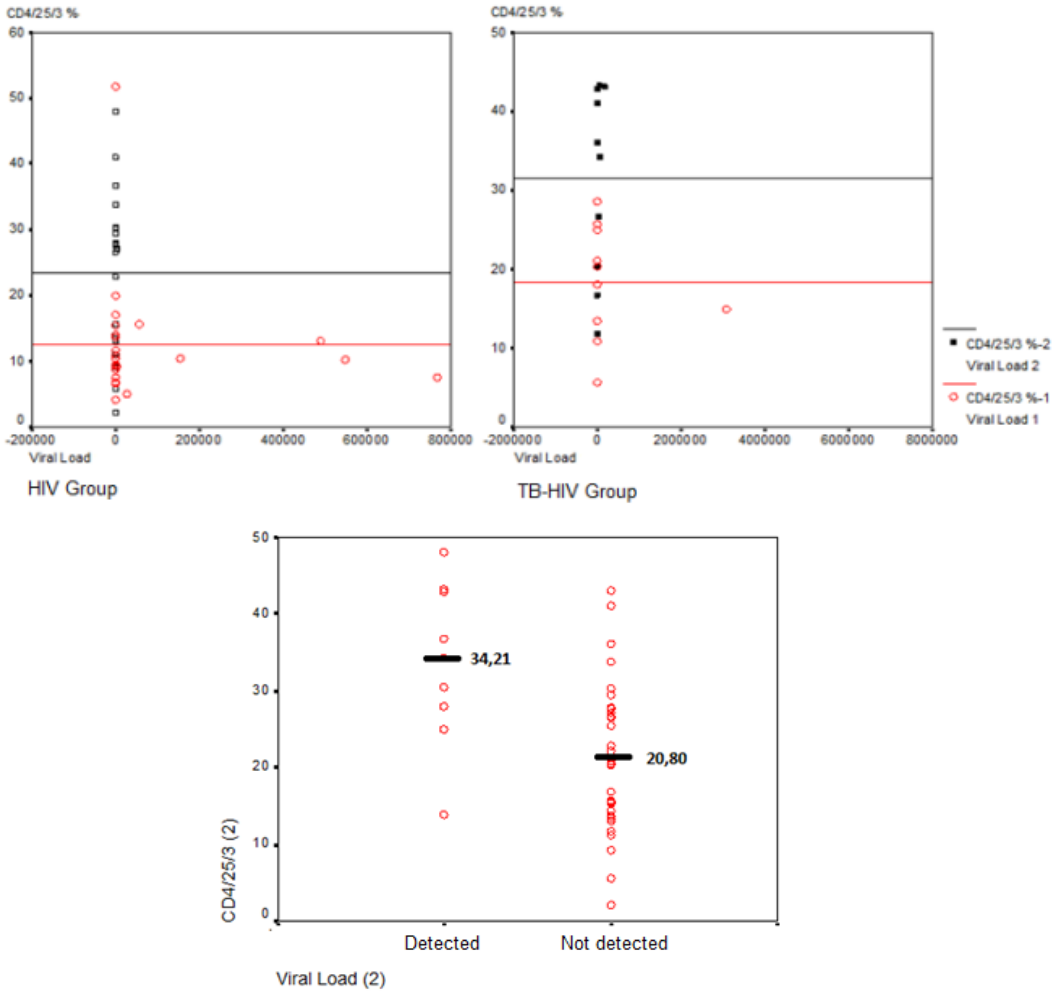
A significant inverse relationship between the increased number of Treg and CD4 decline in both groups was found. There was positive correlation of Treg and viral load in both groups.

Characteristic	HIV groups (n=52)	TB-HIV groups (n=40)
Age	35 ± 6 year	36 ± 6 year
Gender		
Male	28 (53.8%)	24 (60.0%)
Female	24 (46.2%)	16 (40,0%)
CD4		
< 200 sel/mL	19 (27.0%)	28 (70.0%)
>200 sel/mL	33 (63.0%)	12 (30.0%)
Clinical stadium		
Stadium 1,2	24 (46.0%)	0 (0.0%)
Stadium 3,4	28 (54.0%)	40 (100.0%)
Viral Load		
≤ 400 copy/ml	17 (33.0%)	15 (37.0%)
> 400 copy/ml	35 (67.0%)	25 (63.0%)

The patients with progressive disease had higher number of Treg cells (Treg > 17.8) than that of the non-progressive (Treg < 17.8) after antiretroviral treatment. Therefore, the cutoff point of Treg was 17.8; patients with Treg above 17.8 have a tendency to be progressive.

In this study, there were significant differences in the number of Treg cells before and after antiretroviral treatment in both groups. The number of Tregs increased in the HIV group but decreased in the TB-HIV group. Changes in the number of the two Treg groups was significantly different (p = 0.001). The increasing Treg number on HIV patients and declining Treg number on TB-HIV patients after antiretroviral treatment demonstrated the difference in the ability in suppressing the body's immune system. In the HIV group, there is only HIV virus infection, whereas in TB-HIV group, there are HIV virus and *M. tuberculosis*. The role of Treg in chronic viral infections such as HIV and TB-HIV is related to Treg ability to perform immunosuppression (cellular immunity suppression) [10]-[13].

In the HIV patients starting antiretroviral treatment with low CD4 counts (< 200 cells / µL), there was an increase in the proportion of Treg higher than those with high CD4 (CD4 > 200 cells / µL) [12]-[16].



Treg level in peripheral blood of patients with HIV-AIDS stages 3-4 is high. The success of antiretroviral treatment leads to a limited increase of Treg number, but it will decrease in 1-2 months later [12]-[16].

Treg can do up- and down-regulation of chronic HIV infection of immune activation, thus Treg is a major factor in the interaction between the host immune system with the HIV virus. Therefore, with high Treg found on advanced HIV infection, Treg is expected to be the up- and down regulators of immune activation. Treg in untreated and progressive HIV patients will be increasing, and relatively low in non-progressive HIV infection. In the non-progressive HIV infection, there is suppression of Treg activity [11],[17]-[20].

The immune response (after 12 months of antiretroviral treatment) shows clinical improvement which is indicated by improvements in viral load. This suggests that Treg and viral load can be used practically to evaluate the therapeutic efficacy on HIV-AIDS patients [21]-[25].

IV. Conclusion

The number of Treg can be used as evaluation of disease progression of HIV-AIDS, beside viral load and CD4. In practice, Treg examination is cheaper than viral load. Thus the new HIV patients should be considered to examine Treg in addition to CD4 and Viral Load.

There was difference in the number of Treg cells at the beginning of antiretroviral treatment on HIV and TB-HIV. After antiretroviral treatment, there was an increase of Treg cells? But it will decrease in 1-2 months later in HIV patient and a decrease in TB-HIV. There is no change in the levels of IL-10 and IFN-γ. There is significant corelation between levels of Treg cells and viral load levels of the two groups.

Further research is needed by adding the examination of Treg between the beginning and after one year antiretroviral treatment. A further study on innate immunity, not only adaptive immunity, is important to give a more comprehensive understanding of the body's immune response to HIV and TB-HIV infection.

In monitoring of antiretroviral treatment of HIV dan TB-HIV patients, Treg examination can be considered as additional test to CD4 and viral load of HIV-AIDS disease progression.

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