CORRELATION BETWEEN TGF- 1 PLASMA LEVEL AND CD4+ T LYMPHOCYTE COUNT IN STAGE I HIV-INFECTED PATIENTS

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ABSTRAK

*Infeksi HIV dan AIDS telah menyebar ke seluruh dunia dan jumlah pasien terus meningkat dari tahun ke tahun. Indonesia merupakan salah satu negara dengan peningkatan prevalensi HIV dan AIDS yang cukup tinggi. Jumlah absolut CD4+ T-limfosit dan persentase pasien terinfeksi HIV dapat digunakan untuk menentukan stage penyakit, perkembangan penyakit, dan untuk memprediksi timbulnya penyakit oportunistik. Kadang-kadang sulit untuk menentukan secara klinis pada kondisi tertentu dan hasil dari jumlah mutlak CD4+ T-limfosit dan persentase masih tinggi sehingga pemeriksaan TGFJ 1 diperlukan untuk memprediksi perjalanan penyakit pada pasien, karena peningkatan perkembangan penyakit ini juga disertai dengan peningkatan kadar TGFJ 1. Penelitian ini menggunakan metode studi observasional cross sectional. Sampel terdiri dari 41 pasien terinfeksi HIV stage I yang dirawat di Rawat Jalan Unit Menengah Perawatan Penyakit Infeksi (UPIPI) RSUD Dr Soetomo sejak Januari hingga Mei 2012. Pemeriksaan TGFJ 1 plasma dilakukan dengan metode ELISA, jumlah absolut dan persentase CD4+ T-limfosit dengan menggunakan flowcytometry (BD FACSCalibur ™). Hasilnya dianalisis secara statistik menggunakan uji korelasi Pearson. Hasil jumlah CD4+ T-limfosit kurang mutlak dan lebih dari 200 sel/uL menunjukkan tidak ada korelasi dengan tingkat plasma dari TGFJ 1 pada tahap I pasien infeksi HIV. Ada korelasi positif yang signifikan antara jumlah CD4+ persentase T -limfosit dengan TGFJ 1 plasma dalam tahap infeksi HIV I.**(FMI 2014;50:92-95)***

Kata Kunci : Tingkat plasma TGFJ 1, T Limfosit CD4+, Infeksi HIV stage 1

ABSTRACT

*HIV infection and AIDS have spread throughout the world and the number of patients continues to increase from year to year. Indonesia is one of the countries with a quite high increase in incidence of HIV and AIDS. The absolute number of CD4+T-lymphocytes and percentage in HIV-infected patients can be used to determine the stage of disease, progression of the disease, and to predict the onset of opportunistic diseases. Sometimes it is difficult to determine clinically in certain circumstances and the results of the absolute number of CD4+ T-lymphocytes and percentage are still high so examination of TGFJ 1 is necessary to predict the course of the disease in the patient, because the increase in disease progression is also accompanied by increased levels of TGFJ 1.This was a cross sectional observational study, the samples consisted of 41 stage I HIV-infected patients treated in the Outpatient Clinic of the Infectious Disease Intermediate Care Unit (UPIPI) Dr. Soetomo Hospital from January to May 2012. Examination of TGFJ 1 plasma was performed by ELISA method, the number of absolute and percentage CD4+T-lymphocyte by using flowcytometry (BD FACSCalibur™). The results were statistically analyzed using a Pearson product moment correlation test.mThe result of the number of CD4+T-lymphocytes of less absolute and more than 200 cells /«L shows no correlation with plasma levels of TGFJ 1 in stage I HIV infection patients. There is a significant positive correlation between the number of CD4+T-lymphocytes percentage with TGFJ 1 plasma in stage I HIV infection.**(FMI 2014;50:92-95)***

*Keywords: TGF-*B1 *plasma levels, CD4+T-lymphocytes, stage I HIV Infection*

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INTRODUCTION

HIV disease and AIDS (Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome), are now spread throughout the world and continues to increase from year to year. Indonesia is a country with an increase in the incidence of HIV and AIDS, people living with HIV and AIDS in Indonesia is currently aged 20 to 29 years as many as 12288 cases. The incidence of AIDS were reported until June 30, 2011 as many as 26 483 cases in 32 provinces and 300 districts and cities, with the number of positive rate of 10.13% and 5056 deaths. HIV is an intracellular obligate retrovirus with complete replication in the host cell

(Global HIV/AIDS Response 2011). HIV infection involves the natural immune system (innate) and adaptive. HIV tends to invade lymphocyte T-helper that has CD4 receptors (Cluster of Differentiation 4) and the CCR5 (Chemokine Receptor 5) co-receptor and CXCR4 (Chemokine receptor type 4). CD4+ receptor is an ideal partner for gp120 (glycoprotein 120) HIV surface (Nasronudin 2007, Abbas & Lichtman 2010)

CD4+ T lymphocyte examination combined with TGF-B1 examination will be more useful to the clinician in predicting the course of HIV disease. Laboratory not having CD4+ T lymphocyte examination facilities with flowcytometry method, TGF-B1 examination can be used as an additional examination. TGF-B1 examination can be done by ELISA (Enzyme Linked Immunosorbent Assay) method using available equipment in most laboratories. Besides, in TGF-B1 examination, samples can last up to several months and are less influenced by diurnal factors. The role of TGF-B1 in HIV patient stage I has not been widely discussed and not yet fully known. In this study, researchers wanted to examine the correlation between TGF−β1 plasma levels with the number of CD4+ T lymphocytes in HIV patients stage I.

The purposes of this study were measuring TGF−β1 plasma levels in HIV-infected patients stage I, measuring the number of CD4+ T lymphocytes in absolute and percentage of HIV-infected patients stage I, explaining the correlation between plasma levels of TGF−β1 with the number of CD4+ T lymphocytes in absolute and percentage of HIV-infected patients stage I. The results of this study are expected to provide an overview of the role of TGF−β1 pathology in patients infected with HIV, in addition to the results of this study can be used as the basis of further immunological studies of the pathogenesis of TGF−β1 in HIV infection stage I.

MATERIALS AND METHODS

This research was an observational analysis with cross-sectional design. Samples taken in this research were limited to the adult (17-55 years old) patients of stage I HIV in Intermediate Treatment Infectious Disease Unit of Dr. Soetomo Hospital Surabaya. With an estimated correlation coefficient r: -0.42 based on the results of previous research (Wiercinska et al. 2004), significance level zα = 1.960 and power zβ = 0.842, obtained 41 samples. The number of CD4+absolute T lymphocytes and percentage were calculated using flowcytometry. Afterwards, the data would be interpreted based on CD4+ T lymphocytes value. Pearson correlation test (Pearson Product Moment Correlation) was used to determine the correlation between the two variables (TGF−β1 plasma level with the number of CD4+ absolute T lymphocytes and percentage of patients of HIV stage I.

RESULTS

TGFJ 1 Plasma levels in Patients Infected HIV Stage I

TGF−β1 Plasma levels in Patients Infected HIV Stage I found in this research increased with an average value of 19121 pg/ml. One hundred percent of the samples had higher levels of TGF−β1 plasma above 8000 pg/ml. TGF−β1 plasma levels of the lowest in this study were 8147 pg/ml and the highest levels were 48 345 pg/ml. Research conducted by the Wiercinska DA et al., comparing the TGF−β1 levels in patients infected HIV with normal controls indicated that the levels of TGF−β1 in normal individuals was 6.1 ± 1.5 ng/ml, whereas levels of TGF−β1 in patients with asymptomatic stage of HIV infection was 7.9 ± 8.6 ng/ml. Between TGF−β1 levels in patients infected HIV and normal controls found a significant difference (Wiercińska-Drapalo et al 2004)

The sample in this research was limited to patients with HIV infection stage I. HIV infection is established based on positive HIV test results using three different methods. Data on the clinical state of the patient is only based on history and physical examination. Physical examination conducted to determine whether there are signs and symptoms of opportunistic infections. Another laboratory examination to rule out any infections other than HIV, such as checks of anti HCV, HBsAg, which may also be suffered by patients remembering the equal transmission of the disease is not done. This can affect the levels of TGF−β1 obtained in this research. Navikas et al research found that TGF-β is a cytokine that has functions as an autocrine and paracrine so TGF-β has a very short half-life. This situation also leads to TGF-β is unstable (Navikas et al 1994). There is another limitation that this research only examined one of the Th2 cytokines, thus it cannot be compared with the Th1 cytokine response to see immune respons disruption.

The number of CD4+ T Lymphocytes Absolute and Percentage in Patients Infected with HIV Stage I

Based on the number of T lymphocytes, CD4+ absolute, most of the samples (28 samples) has a lower number of CD4+ T lymphocytes than the normal value (normal value CD4+ T lymphocytes absolute 410 is 1590 cells/mL by reference from the BD FACS Calibur ™

and the National Committee for Clinical Laboratory Standards 2000). The virus tends to attack lymphocyte-T helper in HIV infection because there is CD4 receptors on T-helper lymphocyte surface which is an ideal partner of gp120 (glycoprotein 120) surface on the outer surface of HIV (enveloped). Besides, lymphocyte-T-helper also has two co-receptors (CCR5 and CXCR4) that help the virus internalization into monocytes/ macrophages (Zerhouni et al 2004, Nasronudin 2007). The decrease in the number of CD4+ T lymphocytes not only influenced by biological factors but also influenced by psychosocial factors and the decrease can take place through several mechanisms. One mechanism of reduction in the number of CD4+ T lymphocytes is through a process of programmed cell death (apoptosis) (Nasronudin 2007).

The samples examined were HIV stage I, the results of the number of T lymphocytes, CD4+ obtained in this research are very varied and most of the samples have a number of CD4+ T lymphocytes absolute less than 300 cells/mL and the number of CD4+ T lymphocytes percentage less than 30 %. That situation can be attributed to many factors that influence the calculated number of CD4+ T lymphocytes, such as stress, physical activity, fatigue, and diurnal factors. Counting the number of CD4+ T lymphocytes by flowcytometry method is able to check the number of CD4+ T lymphocytes absolute and CD4+ T-lymphocyte percentage. The number of CD4+ T lymphocytes absolute indicated the number of CD4+ T lymphocytes circulating in the blood functional, whereas CD4+ T-lymphocyte percentage is the percentage of CD4+ T lymphocytes to the total lymphocyte count.

The results indicated a significant positive correlation between plasma levels of TGF–β1 and CD4+ T-lymphocyte percentage. However, there was no correlation between plasma levels of TGF–β1 and the number of CD4+ T lymphocytes, both in absolute number of cells more or less than 200 cells/mL. This indicated that CD4+ T-lymphocyte percentage's quality was not reduced compared with the number of CD4+ T lymphocytes in determining the absolute rate of disease progression. The ability of the number of CD4+ T lymphocytes absolute and percentage in determining immune status and the level of disease progression in HIV infection is still controversial. Gebo et al research (2004) stated that the number of CD4+ T-lymphocyte absolute more important in determining immune status and therapy decisions than CD4+ T-lymphocyte percentage.

Pirzada et al conducted a research to predict the onset of AIDS using the number of CD4+ T lymphocytes, the absolute and percentage. The results of the research stated that the CD4+ T-lymphocyte percentage is better or almost equal to the number of CD4+ T-lymphocyte absolute to predict the occurrence of opportunistic infections associated with AIDS. The number of CD4+ T lymphocytes is the absolute best predictor, but the lymphocyte T CD4+ percentage is still an accurate predictor (Pirzada et al 2006).

DISCUSSION

The results indicated there is no correlation between TGF–β1 plasma level with the number of CD4+ T lymphocytes absolute less than 200 cells/mL in patients with HIV infection stage I with $r = 0.104$ and $p = 0.682$, and also for the number of CD4+ T lymphocytes absolute more of 200 cells/mL $r = 0.410$ and $p = 0.052$. Between TGF–β1 plasma levels and the number of CD4+ T-lymphocyte percentage there is a significant positive correlation with $r = 0.326$ and $p = 0.037$. Elrefaei et al (2006) stated the results of the research of a number of HIV-infected patients showing that there is an increasing in the production of TGF–β1 on increasing progression of the disease and an increase in HIV replication.

Immune response to HIV infection which was originally located in a state of balance between Th1 and Th2 will experience a shift with continued infection. Immune response will be disturbed and shifted to Th2. The shift causes increased Th2 cell differentiation suppresses Th1 activity. A number of cytokines produced by Th1 such as IL-2, IL-12, IFN-γ will decrease and the amount of cytokines produced by Th2 such as IL-4, IL-5, IL-6, TGF–β1 will increase. Cytokines produced by Th1 suppressive apoptosis of CD4+ T lymphocytes, whereas produced by Th2 is proapoptosis or accelerate the process of apoptosis of CD4+ T lymphocytes. Th 2 strong response will increase levels of TGF–β1 that is proapoptosis to the CD4+ T-lymphocytes, resulting in TGF–β1 level increasing followed by the number of CD4+ T lymphocytes decreasing. TGF–β1 plasma levels increasing and the number of CD4+T lymphocytes decreasing is in line with disease progression increasing (Clerici et al 1997, Badley et al 2000, Alimonti et al 2003, Perfettini et al 2005).

Besides the decline in the number of CD4+ T lymphocytes, enhancement of disease progression was also an increase in the levels of TGF–β1. The number of viruses is also important because it can reduce the number of CD4+ T lymphocytes, because the HIV virus can directly damage the CD4+ T lymphocytes. It has been found in this research between the two variables that have a significant positive correlation is weak. This can be useful as a consideration either by the clinician

or by laboratories that do not have facilities with flowcytometry method to examine TGF−β1 as additional checks when checking the number of CD4+ T lymphocytes cannot be done. In certain circumstances which led to the doubtful results of the examination of the number of CD4+ T lymphocytes, besides examined in healthy controls, the examination of TGF−β1 may be considered as an additional examination in order to assist clinicians in predicting the course of disease in patients with late-stage HIV I.

CONCLUSION

TGF−β1 level increasing has been found in patients infected with HIV stage I, with the mean level of 19 121 pg/ml and SD 9444 pg/ml (showing the results around the mean were more varied). The decrease of the number of CD4+ T lymphocytes absolute found in patients infected with HIV stage I, with the number of cells less than 200 cells/μl r = 0.104 and p = 0682, while the number of cells greater than 200 cells/μl r = 0.410 and p = 0052, the average of 304.26 cells/μL (SD 228.76 cells/μL). The mean number of CD4+ T-lymphocyte percentage of 13:52% (SD 7.46% respectively). There is a significant positive correlation between TGF−β1 plasma rate and the number of CD4+ T-lymphocyte percentage in HIV-infected patients. Meanwhile, there was no correlation between the rate of TGF−β1 plasma and the amount of CD4+ T-lymphocyte absolute (less or more than 200 cells/μl).

ACKNOWLEDGMENTS

We thank to Laurentius Andre for his assistance in making the layout of the article.

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