CORRELATION BETWEEN PLASMA LEVELS OF INTERFERONJ¥ AND VIRAL LOAD IN PATIENTS WITH HIV STAGE 1

Hermi Indita Malewa[1], Endang Retnowati[2], Erwin Astha Triyono[3]
[1]Resident at the Department of Clinical Pathology
[2]Department of Clinical Pathology, [3]Department of Internal Medicine
Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya

ABSTRAK

Angka kejadian HIV di Indonesia terus meningkat dan di Asia, Indonesia merupakan yang paling cepat. Salah satu alat diagnosa HIV adalah viral load. Limfosit T-CD8+ mengeluarkan IFN-γ akan mencegah perubahan dari virus HIV melalui induksi protein ativiral dan memicu respon imun yang akan membunuh sel yg terinfeksi. Pengujian dari plasma IFN-γ dan viral load akan lebih menyakinkan untuk pengobatan dan/atau untuk mengetahui perkembangan dari HIV&AIDS. Tujuan dari penelitian ini adalah untuk mengetahui hubungan antara plasma IFN-γ dan viral load pada pasien HIV stadium 1. Empat puluh dua sampel dari pasien HIV dikumpulkan pada Unit Pelayanan Cepat dan Penyakit Infeksi, RSU dr. Soetomo, surabaya dari April hingga Juni 2011. Kandungan dari plama IFN-γ dihitung menggunakan metode ELISA (eBioscience) dan jumlah viral load pada pasien HIV stadium 1. Level dari plasma IFN-γ adalah 11.4 pg/ml sampai 576 pg/ml dan level dari viral load adalah 589 salinan/ml hingga 510.000 salinan/ml. Analisis statik menunjukkan tidak ada hubungan signifikan (p>0.05) antara plasma IFN-γ level dan viral load pada pasien HIV stadium 1. Tidak ada hubungan yang ditemukan antara plasma IFN-γ dan viral load pada pasien HIV stadium 1. ***(FMI 2014;50:67-72)***

Kata Kunci: *IFN-γ, viral load, HIV stadium 1*

ABSTRACT

The incidence of HIV in Indonesia is on the increase and in Asia, Indonesia is considered as the most rapid. One of the diagnostic tools for diagnosing HIV is viral load. Lymphocyte T-CD8 + secreted IFN-γ will inhibit replication of HIV virus through induction of antiviral protein and host immune response which kills infected cells. Examination of IFN-γ plasma and viral load will be more confincing for the treatment and/or to knowing the progressiveness of HIV & AIDS. The aim of the study is to know the correlation between IFN-γ plasma and viral load in stage 1 HIV patients. Forty two samples from HIV patients were collected at the Intermediate Care and Infectious Disease Unit, Dr. Soetomo Hospital, Surabaya from April to June 2011. The concentration of IFN-γ plasma was measured by ELISA (eBioscence) method and amount of viral load was measured using PCR Cobas Amplicor (Roche Diagnostics). The level of IFN-γ plasma was 11.4 pg/ml to 576 pg/ml and the level of viral load was 589 copies/ml to 510,000 copies/ml. The statistical analysis showed no significant correlation (p>0.05) between IFN-γ plasma level and viral load in stage 1 HIV patients. No correlation was found between IFN-γ plasma and viral load in stage 1HIV patients. ***(FMI 2014;50:67-72)***

Keywords: *IFN-γ, viral load, stage 1 HIV*

Correspondence: Hermi Indita Malewa,Resident at the Department of Clinical Pathology, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya

INTRODUCTION

Incidence of HIV infection is increasing in Indonesia. AIDS cases reached 21.770 cases in June 2010, while the HIV positive cases in June 2010 reached 44.292 cases from 32 level mask and 300 districts/cities. One of HIV diagnosis is the examination of viral load. It measures the amount of HIV virus in each millimeter of blood. Combined with the examination of T-lymphocytes CD4+ benefits include knowing how the body fights HIV, used as a diagnosis because it can detect the content of viral load at any time after infection, estimated risks toward AIDS, and determine the effectiveness of the therapy (Ministry of Health Republic of Indonesia 2010). Guidelines for the initiation of therapy and determination of HIV and AIDS progression in clinical is the amount of viral load and T-lymphocyte CD4 at certain circumstances, sometimes difficult to be determined, and the results of the T-lymphocytes CD4+ number still high and not in the limits of therapy of Anti-Retro Virus (ARV) considering there are a lot of factors that affect the

results of T-lymphocyte CD that situation of IFN-γ examination needs to be done to describe the patient's level of disease progression. Examination of viral load combined with IFN-γ examination would be more convincing and will be useful in providing management and determining the rate of progression of HIV and AIDS. The role of IFN-γ in patients infected by HIV have not been widely discussed and understood completely. In this study, researchers want to examine the correlation between plasma levels of IFN-γ with viral load.

MATERIALS AND METHODS

This type of research is observational analytic cross-sectional design. The population in this study were patients with HIV, adults (age> 12 years) treated at the Infectious Disease Care Unit intermediates (UPIPI) Dr. Soetomo General Hospital Surabaya. The sample size in this study was calculated based on a sample size formula for the correlation coefficient in a single sample. Total sample comprised 35 people. In this study, samples taken were limited to HIV-infected patients with stage 1: 1) positive HIV test results using three different kinds of methods of examination, 2) the clinical picture were only asymptomatic generalized lymphadenopathy; 3) able to move normally; 4) willing enrolled in the study and signed an informed consent. Criteria for sample rejection of HIV-infected patients: 1) Patients with HIV infection, who had received corticosteroid therapy, had also received antiretroviral therapy; 2) other than HIV infection, and 3) patients who refused to participate in this study.

Location of the research done in 2 location which are Intermediates Care Unit of Infectious Diseases Dr. Soetomo General Hospital Surabaya for the selection of patients and blood samples as well as in the Department of Clinical Pathology, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital, Surabaya, for plasma separation, inspection and examination of viral load levels of IFN-γ plasma. Research began in March 2011 until finished. The results of research using Pearson correlation test (Pearson Product Moment correlation) determined the correlation between the two variables, in this case to see the correlation between plasma levels of IFN-γ with viral load in HIV-infected patients.

RESULTS

Quality assurance for examination of plasma levels of IFN-γ was done by measuring imprecision. “Within run” imprecision in this study was the SD of 0.47 pg/ml and a CV of 4.7%. “Between run” impression for inspection between four different plasma samples got a SD of 1.12 pg/ml and 4.9% CV. This suggested that the quality of the results of IFN-γ levels was quite well. Quality assurance of viral load test results performed whenever operating the tool by entering the value of the lot-specific standard quantitation copy number (copy number standard of PCR) in IU/PCR and Amplicor HIV-limit value and a low positive control positive control in the CA high HIV Monitor test data cards. One of the control values in this study was the low positive control with a value range of 1.1 to 1.2 X103 X103 copies/ml, with a high positive control value range of 1.7 to 7.5 X103 X103 copies/ml. The results of the control should be included in the value of the range before performing the examination of patient samples.

A total of 42 HIV-infected patients who had met the criteria for acceptance and rejection of the sample, 22 patients (52.4%) were male and 20 patients (47.6) were women. Thirty-five (85.4%) classified as heterosexual, and 7 (16.6%) including a homosexual. Patient age range between 21-47 years (mean = 32 years, standard deviation (SD) = 7.2 years). Based on the job, 22 patients (53.7 %) worked in the private sector, 13 (31%) as a housewife, two (4.8%) work as commercial sex workers (CSWs), two students (4.8%), one person was farmer (2.4%), while two (4.8%) patients not currently have a job. Biggest risk factors that facilitate HIV transmission in patients were infected by their partner (husband/wife was a patient with HIV&AIDS) that was equal to 16 patients (8.2%). The second is the biggest risk of sexual promiscuity (sex) by 14 patients (33.3%) followed by transmission through sexual intercourse with the same sex (homosexual) by 7 patients (16.7%) and the latter through the use of intravenous drug users (IDUs) by 5 patients (11.5%). Married couple were 26 samples (63.4%).

Before the Elisa test conducted on samples of the study, in the first place we created a standard curve. With the initial concentration of standard solutions had already known, conducted Elisa test, so that the absorbance of the standard was known. Stock standard solution that used for serial dilutions to obtain standard solutions with high levels of IFN-γ were 100 pg/ml, 50 pg/ml, 10 pg/ml, 25 pg/ml, 12.5 pg/ml, 6.35 pg/ml, 3.1 pg/ml and 1.6 pg/ml. “A” diluent solution was used as the zero standard (0 pg/ml). The reading of the results was done by Elisa reader at 450 nm that the results obtained in the form of absorbance values (optical density/OD at 450 nm). Table 5.2 presents data from the absorbance of standard IFN-γ.Determination of the concentration of IFN-γ can be calculated with the help of a standard curve (Figure 1).

Absorbance values obtained from a horizontal line drawn that it intersects the curve, then a vertical line drawn down (X axis direction) in order to obtain the concentration of IFN-γ. Determination of plasma levels of IFN-γ could also be calculated directly with a standard curve by Elisa reader in accordance with the magnitude of the absorbance. IFN-γ levels of plasma HIV-infected patients ranged from 11.4 pg/ml to 576.2 pg/ml with a mean of 39.3 pg/ml and 85.31 SD pg/ml. Viral load in HIV-infected patients ranged from 589 copies/ml up to 510,000 copies/ml with a mean of 131,213.55 copies/ml and a standard deviation of 146,657.84 copies/ml. From the data results of the plasma levels of IFN-γ and viral load test performed statistical analysis using Pearson correlation (Pearson Product Moment Correlation) to determine the correlation between plasma levels of IFN-γ with viral load in patients infected with HIV. The analysis showed no significant correlation ($p>0.05$) between the levels of IFN-γ plasma and viral load in patients infected with HIV.

Table 1. IFN-γ absorbance values obtained with the Elisa test against various concentrations of IFN-γ standards

Standard	Concentration (pg/ml)	*Absorbance* (OD = 450)
1	1.6	-0.079
2	3.1	-0.056
3	6.3	-0.044
4	12.5	0.006
5	25.0	0.073
6	50.0	0.165
7	100.0	0.202

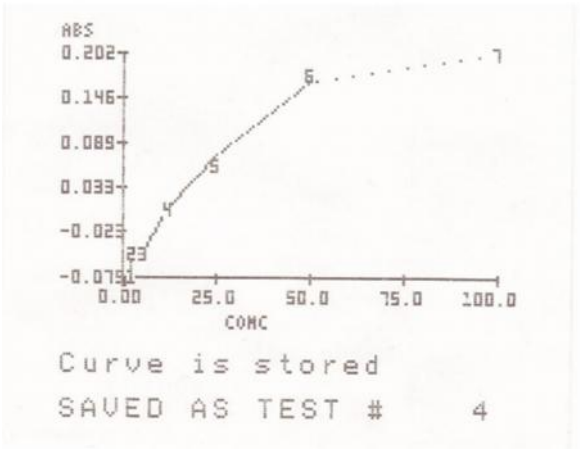

Figure 1. IFN-γ standard curve

DISCUSSION

Quality Assurance and Reliability of Samples

Quality assurance test results levels of IFN-γ in this study had been done by calculating imprecision "within a run" and "between runs". In imprecision "within a run" of the 10 samples obtained SD value of 0.47 pg/ml and a CV of 4.7% Imprecision run for inspection between four different plasma samples got a SD of 1.12 pg/ml and 4.9 % CV. This study had a CV<5%. This suggested that the quality of the results of IFN-γ levels is quite well. Quality assurance of viral load test results performed whenever operating the tool by entering the value of the lot-specific standard quantitation copy number (copy number standard PCR) in IU/PCR and Amplicor HIV-value range low positive control and a positive control in the CA high HIV Monitor test data cards. One of the control values in this study was the low positive control with a value range of 1.1 to 1.2 X103 X103 copies/ml, with a high positive control value range of 1.7 to 7.5 X103 X103 copies/ml.

In this study, a group of HIV-infected patients were limited to patients with clinical stage I and did not have complaints, in hopes of reducing the bias against the results of IFN-γ levels of plasma or viral load, considering at a more advanced stage the opportunistic infections occurred. Group of patients also had never received antiretroviral therapy and corticosteroids/ immunosuppressants. The giving of drugs and cortico-steroids can suppress the synthesis of IFN-γ, which able to decrease the levels of IFN-γ and cause the results of IFN-γ does not reflect the real situation.

Immunoassays to cytokines such as IFN-γ can be performed on a sample of local network (eg. from skin scrapings of HIV-infected patients with dermatitis), culture supernatant PBMCs (peripheral blood mononuclear cells), plasma and serum. It was said that the immunoassay on the best local tissue samples among other samples. PBMC culture supernatant plasma samples followed the second best and the last was the serum. In patients with HIV infection, tissue sampling and sample handling are difficult. Local network that would be taken was generally caused by infections other than HIV or opportunistic infections form. Another difficulty was very infectious HIV, could not be cured, and infectious. All equipment used for sample handling should be disposable. Serum samples had many disadvantages, such as the half-life was very short, which was activated leukocytes during blood clotting, could produce cytokines and proteases in the presence of serum that it might interfere the examination. Plasma was said to be better than serum, but must be processed quickly and immediately while

stored frozen quickly. PBMC culture supernatant samples able to avoid the drawbacks of serum and plasma samples above, also provide a more precise measure of the ability of cells to produce cytokines and could measure several types of cytokines, but the drawback was wearing PBMC requires quite a long time (Handojo 2003).

In this study, heparin plasma samples had been processed quickly to address weaknesses in the inspection of cytokine plasma samples. After collection, blood samples were immediately centrifuged to obtain heparin plasma and stored at -70^{o}C. This study was an initial study to determine the role of IFN-γ in relation to HIV disease activity in patients with a limited population of HIV-infected asymptomatic stage I in Outpatient Unit of Dr. Soetomo General Hospital (UPIPI). In vitro studies needed to be done through the examination of cytokines or cultured PBMCs local network to identify the profile of cytokines IFN-γ closer to the real situation, however, it was quite useful that this research would provide information about the plasma IFN-γ in patients with HIV infection. Another advantages, measurement of plasma levels of IFN-γ was easier and more practical than using a tissue sample of local or PBMC culture supernatants.

Examination of the Reliability Methods Plasma levels of IFN-¥ and Viral Load Number Used in This Research

In this study, plasma levels of IFN-γ were measured using ELISA. In addition to the ELISA method, cytokine levels could be measured by radioimmunoassay method (RIA) and bioassay. Measurement of cytokines by ELISA method had several advantages. Specificity of ELISA methods was higher, reagents and kits were available in the form of test results obtained in a few hours that the ELISA method was preferred. The weakness of the ELISA method is it was less sensitive than the bioassay method, but bioassay method was less specific because it could only trace levels of IFN-γ that were active. While RIA was becoming obsolete because it requires exposure to radioactive substances and radiation risk (Handojo 2003).

Reagents used in this study was a kit from eBioscience (Human IFN-γ Elisa Platinum) with the principle of double antibody sandwich examination streptavidine biotin test that was capable of detecting levels of IFN-γ to 1.6 pg/ml. The use of a double antibody could guarantee the reliability of the sensitivity of the examination result and the use of anti-human IFN-γ monoclonal antibody could guarantee the specificity and the reliability of the IFN-γ levels results.

Examination of viral load was determined by PCR method using a Cobas®Amplicor. Its units were copies/ml or in the calculation of logarithms (logs). PCR method was a technology that could create double knockoff of a specific nucleotide sequence of the target organism. PCR method provides a mechanism to detect the target organisms in very small concentrations with high specificity. In the field of clinical diagnostics only took a very little amount of sample, transfer of genetic material and the presence or absence of specific viruses and bacteria could be detected because of the nucleotide sequences of clone-specific doubles. The stages included pre-PCR process consisted of the preparation of PCR reagents and preparation of specimens for isolation/purification of RNA; PCR amplification was a process consisting of denaturation (separation of RNA chain), annealing (annealing) and extension (elongation by the enzyme); Post PCR a detection/analysis of PCR results.

Characteristics of Research Subjects

The proportion between the samples of men and women in this study was almost the same and most of the samples were heterosexual. Seven samples were homosexual men, who come from all groups were classified as transvestites and male samples. All samples included in the productive age with most 21-47 year age range. This suggested that HIV infection tends to occur at a young age (reproductive age), because a young age are more likely to have risk factors associated with HIV transmission. More than 50% of the sample were married and working in the private sector. Most of the risk factors that lead to HIV infection in the sample were infected by their partner (husband/wife). Lowest risk of transmission was through the use of intravenous drug users (IDUs). This condition occurred because the majority of the sample was the husband/wife who was waiting for her partner treated in UPIPI. All the husband/wife with HIV and AIDS were treated in UPIPI undergo counseling for HIV examination. If it had met the inclusion and exclusion criteria were included in this study. The small number of samples with the use of IDU risk factors was likely due to the limited sampling sites and generally patients with risk factors for IDU users more closed and were suffering status against HIV infection.

Plasma levels of IFN-¥ in HIV Infected Patients with Stage 1

Plasma levels of IFN-γ in patients with HIV infection found in this study ranged from 11.40 pg/ml and 51.1

pg/ml. More than 73 % of patients had levels above 20 pg/ml. And there was one patient that IFN-γ levels reached 576 pg/mL. High levels of IFN-γ in these patients were in accordance to the number of T-lymphocytes CD4 (770 sel/cmm3).

In this study, the samples were limited to HIV-infected patients with stage I. Evidence of HIV infection by HIV-positive test results using three different methods. Data on the clinical state of the patient was only based on history and physical examination. This was one limitation of this study because the data provided by the patient was not 100% correct and the results of the tests were not supported by other tests to rule out any infections other than HIV. For example, examination of anti-HCV, HBsAg, which may also be suffered by patients considering equally a viral infection and provide the same immune response. It was at least affected the levels of IFN-γ that were obtained in this study.

Number of Viral Load in Patients Infected with HIV Stage 1

The number of viral load in this study ranged between 589 copies/ml up to 510,000 copies/ml. A total of 44 % had a viral load over 100,000 copies/ml. Stimulation that can lead to viral replication is still unclear, presumably occurs due to the influence of cytokines. Not all cytokines could stimulate viral replication since some cytokines inhibit the replication instead. Cytokines stimulated cytokine that is generally participate regulating immune responses, such as IL-1, IL-3, IL-6, tumor necrosis factor a and ß, interferon-γ, granulocyte-macrophage colony-stimulating factor. Thus which ablt to inhibit interleukin 4 is a, transforming growth factor ß and interferon-α and ß. Another thing that could spur replication was the presence of co-factors such as DNA viral infections such as Epstein-Barr virus, cytomegalovirus, hepatitis B virus, herpes simplex virus or bacterial infection such as mycoplasma. Cytokines could be established and worked in the local network without entering into circulation, then concentration did not always have to be high to cause the effect on HIV replication or expression in the tissue. Therefore, the state of immunology interference, on the network, especially in the lymph nodes in the network could still occur viral replication or expression (Nasronudin 2007).

The correlation between plasma levels of IFN-γ and Number Viral Load in Patients Infected with HIV Stage 1

The results showed no correlation between plasma levels of IFN-γ and viral load, it was likely caused by an immune response that was different for each patient. This difference caused the immune response of HIV viral replication was also different for each patient. In patients with a number of T-lymphocyte CD4+ who still had high immune response was optimal for fighting viral infections, so the levels of IFN-γ was still relatively high, but in patients with a number of T-lymphocyte CD4+ low (<350 cells/mL) usually occurs impaired immune response, and IFN-γ levels obtained were relatively low.

Research conducted by Novitsky et al (2003) says that in the some of previous studies there were differences in the results of the correlation between CTL response (Cytotoxic T lymphocytes) that produce IFN-γ in viral load. Some studies suggested a positive correlation between CTL that produce IFN-γ in viral load, in contrast to some studies said negative correlation. Several recent studies said there was no correlation between CTL responses with viral load. Positive correlations were obtained in patients who had received Highly Active Antiretroviral Therapy (HAAT), whereas a negative correlation was found in the rapid viral rebound or a relatively high number of CD4+. The difference of this correlation could be caused by differences in methodology examination, targeted a variety of epitopes on the viral genome, differences in stage of disease and the differences of population groups. It also said that the differences in the geographical distribution of HLA alleles play a role in the restriction of the presentation of MHC class 1 epitopes, resulting in a difference of each population or ethnic group. It was agreed that HLAA 29 and HLA-B22 associated with rapid progression, whereas HLA B14 and HLA C8 are associated with slow progression. In the research we did, it was limited to patients with stage 1 HIV who had not received treatment, which had a number of T-lymphocytes CD4+ varied reflecting the immune status of the patient, so that the levels of IFN-γ of HIV patient also varies.

Research conducted by Gab et al (2010) conducted in Korea concluded that the response of IFN-γ produced by activated CTL against HIV replication in people with Primary HIV Infection Korea (PHI) showed no constant correlation and remains controversial. This study said that at this stage of pre-seroconversion obtained negative correlation ($r = -0.22$ to $r = -0.33$) because it was not sufficiently activated CTL responses in controlling viral replication. After this stage, the correlation changed from negative to positive ($r = 0.132$ until 0852) due to increased CTL responses to control viral rebound. During the period of seroconversion to the viral set point, where in the concentration of the virus was maintained, the correlation between viral replication and host immune response dynamic change

(r = -0.195 to r = -407) because the CTL response and viral load during the progression of disease associated. Thus, each individual had a distinct correlation between CTL response and viral load in the IRC. Clinical stages of each subject were an important factor for CTL responses in controlling viral replication and also correlation to viral load. In the study done by Gab et al (2010), used cryopreserved PBMC samples, whereas the studies we conducted using heparin plasma samples, so results may also vary.

Research conducted by Peretz et al (2005) said that IFN-γ was not a potential mechanism as a control mechanism. Secretion of effector molecules such as IL-2, TNF-α and IFN-γ progressively reduced IFN-γ levels but most slightly reduced. This study concluded that that the HIV-specific IFN-γ secretion was not correlated with a decrease in T- lymphocytes CD4+ and viral load that HIV-specific to IFN-γ secretion was not associated with disease control and progression.

CONCLUSION

IFN-γ levels in this study was 11.40 pg/ml to 576.2 pg/ml, the majority (> 73%) had levels above 20 pg/ml. The number of viral load in this study was 589 copies/ml up to 510,000 copies/ml, 44% had a number above 100,000 copies/ml. There was no correlation between the levels of IFN-γ with viral load in HIV-infected patients with stage 1.

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