Viral load profiles in drug users with asymptomatic HIV infection and normal CD4 cell counts

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Abstrak

*Sejak tahun 2000 jumlah penderita HIV/AIDS di Indonesia meningkat tajam, terutama pada pecandu narkotika suntik. Terapi antiretroviral yang terbukti dapat menurunkan mortalitas dan meningkatkan kualitas hidup pasien, diberikan berdasarkan kondisi klinis, jumlah sel limfosit CD4 dan kadar virus dalam darah. Dalam penelitian ini dilakukan pemeriksaan kadar CD4 dan kadar virus pada 71 pasien HIV asimptomatik yang merupakan pecandu narkotika suntik untuk melihat apakah kadar CD_4 berkorelasi dengan kadar virus HIV. Kadar CD4 diperiksa dengan metode imunofluoresensi indirek menggunakan antibodi monoklonal dan kadar virus menggunakan teknik PCR. Pemeriksaan hitung virus dilakukan pada 56 pasien yang mempunyai kadar CD4 lebih dari 200 sel/mm3 ($\overline{x}$ = 473 $\pm$ 180,6). Sebanyak 30 orang (55,4%) mempunyai kadar virus dalam darah lebih dari 55.000 kopi/ml dan 35,7% kadar virusnya 5.000-55.000 kopi/ml. Korelasi antara kadar CD4 dengan kadar virus dalam darah memberi nilai r: 0,194. Disimpulkan bahwa pada pecandu narkotika kadar CD4 tidak berkorelasi dengan kadar HIV sehingga pemberian antiretroviral sebaiknya didasarkan pada kadar HIV dalam darah. (**Med J Indones 2002; 11: 143-7**)*

Abstract

*Since the year 2000 there has been a steep increased in the number of HIV/AIDS patients in Indonesia , coming mostly from intravenous drug users. Antiretroviral treatment has been proved to decrease mortality and increase quality of life of HIV/AIDS patients. The treatment is given according to clinical condition of the patients, number of CD4 and viral load. In this study, CD4 and viral load were examined in 71 asymptomatic HIV patients originated from injecting-drug users. CD4 counting was performed by indirect immunoflouresence method using monoclonal antibody, and viral load was tested using PCR technique. Among 56 patients who has the number of CD4 more than 200/mm^3, 30 patients (55,4 %) has viral load more than 55,000 copies/ml and 35,7% has viral load 5,000-55,000 copies/ml. Correlation between the number of CD 4 and viral load gave the r value of 0,194. It is concluded that there is no association between the number of CD 4 and viral load in drug user HIV/AIDS patients. The treatment of HIV/AIDS for these patients should be given according to the viral load. (**Med J Indones 2002; 11: 143-7**)*

*Keywords***:** *viral load, CD4, injecting-drug user*

Since the year 2000 there has been a sharp increased in the number of HIV/AIDS patients in Indonesia. It is estimated that between 80.000 and 120.000 persons are currently infected with HIV, coming mostly from intravenous drug users.[1] Treatment with antiretroviral drugs (reverse-transcriptase inhibitors, protease inhibitors etc) when used as part of combination drug regimens, can profoundly suppress viral replication, with consequent repletion of CD4. The combination of anti retroviral drugs has been demonstrated to slow the progression of HIV type 1 (HIV-1) disease dramatically and to lower mortality among adults cases[2], children and adolescents[3] infected with HIV-1. Clinical trials have shown the efficacy of the highly active antiretroviral-drug combinations[3,4,5,6,7,8] by measuring the plasma load of HIV RNA and CD4+ cell counts.

It was important to identify subgroups of HIV infections, in which the anti retroviral treatment should be started (Table 1). The first clinical cathegory is symptomatic HIV patients (wasting, prolonged fever, thrush). There was consensus that these patients should be treated with antiretroviral therapy.[9] The second clinical category is HIV infected patients without clinical symptoms. Consideration for starting antiretroviral therapy in the second group is

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more complex. The risk of opportunistic infections increases markedly when CD4 cell count less than 200 per cubic millimeter and it is strongly supported that this group of patients should be offered antiretroviral therapy.[5,9] There was theoretical benefit of antiretroviral therapy for patients with CD4 above 200 per cubic millimeter. The other clinical category is HIV patients with more than 350 CD4 cells/mm3 but plasma HIV RNA is more than 55,000 copies/ml. This group should be offered antiretroviral therapy, because the risk of progression to AIDS is very high.[10]

Table 1. When to start antiretroviral therapy

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 1 - AIDS
 2 - asymptomatic HIV + CD4 <200 cells/mm3
 3 - acute HIV infection, Post Exposure Prophylaxis
 4 - mother to child transmission
 5 - asymptomatic HIV, CD4 > 200, Viral Load >55,000
     copies/ml

The purpose of this study was to evaluate whether there is a subgroup of asymptomatic HIV infection in drug users with normal CD4 cells count who might benefit from early antiretroviral therapy. The correlation between CD4 and viral load among drug users with normal CD4 cells count (more than 200 cells/mm^3) was performed in order to know whether CD4 count can be used as predictors of viral load.

METHODS

Patients with asymptomatic HIV-1 infection from drug users and having more than 200 CD4+ lymphocytes per cubic millimeter were enrolled in this study. The patients were recruited from various hospital in Jakarta between November 1999 till August 2001. CD4 and viral load were examined in 56 HIV/AIDS patients. CD4 testing was performed by indirect immuno-flouresence method using monoclonal antibody, and viral load was tested using PCR technique (Amplicor HIV-1 Monitor assay version 1.5, Roche Molecular System, Branchburg NJ). All the samples for a given patient were retrieved and analyzed at the Hematology-Medical Oncology Department of Internal Medicine, University of Indonesia.

HIV-1 RNA PCR

Plasma specimens were collected and stored at -20°C. Two hundred microlitres of each plasma samples were

assayed on the same plate with three controls (negative, low positive and high positive control) in each assay. RNA was extracted from the plasma sample by the standard specimen preparation procedure, RNA is isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol. Copy numbers of each plasma sample were calculated on the basis of the optical density readout of the final ELISA hybridization detection of amplicon and then adjusted for the efficiency of extraction, reverse trancription, and amplification of an internal target control sequence. The optical density (OD) was measured at 450 nm. The HIV-1 RNA   level is calculated according to the equation:

$$\frac{\text{Total HIV-1 OD}}{\text{Total QS OD}} \times \text{Input HIV-1 QS copies/PCR} \times 40 = \text{HIV-1 RNA copies/ml}$$

Data Collection and Statistical Analysis

The plasma HIV-1 detection range was 400-750,000 copies/ml (OD values 0.20-2.0). Test results less than 400 was reported as "HIV-1 RNA not detected", less than 400 copies/ml". Test results greater than 750,000 was reported as "greater than 750,000 copies/ml". In this case, the original plasma specimen should be diluted with HIV negative human plasma and retested to know the quantitative results.

We used Spearman correlation to investigate the association between the level of CD4 count and viral load count. All data was analyzed using SPSS 10.0 for Windows.

RESULTS

Of 71 asymptomatic HIV-infected patients, 57 patients has the number of CD4 more than 200 cells/mm^3. One patient was drop out because the viral load measurement result was more than 750,000 copies ml and we could not repeated the test because at that time there was no reagents anymore. Among 56 patients who has the number of CD4 more than 200/mm^3 (x = 473 $\pm$ 180,6), 55,4% (30/56) has viral load more than 55,000 copies/ml and 35,7% has viral load 5,000-55,000 copies/ml (Table 2). The mean plasma HIV RNA concentration was 128,894 copies per milliliter, and the values ranged between 636-

600,341 copies per milliliter (Table 2, Figure 1). The correlation between the number of CD 4 and viral load gave the correlation coefficient (r value) of 0,194 and the p value of 0,152 (Figure 2).
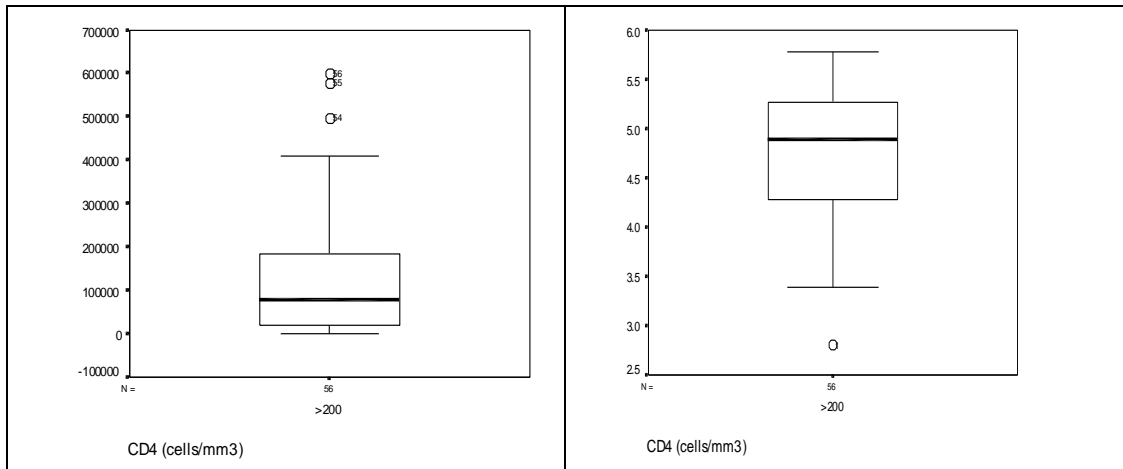

Figure 1. Distribution of plasma HIV-1 RNA in asymptomatic HIV patients with more than 200 CD4 cells/mm³, in copies/ml (a), log copies/ml (b)

Table 2. Viral Load in patients with more than 200 CD4 cells/mm³

| No.	Viral Load (copies/ml)	CD4 (cells/mm3)	No.	Viral Load (copies/ml)	CD4 (cells/mm3)
1	636	276	29	76597	332
2	2451	243	30	78344	382
3	2639	399	31	83105	610
4	3754	564	32	83106	387
5	3981	441	33	89071	675
6	7233	646	34	106882	250
7	7962	450	35	141266	608
8	9400	286	36	146832	436
9	11439	397	37	153369	223
10	12569	554	38	161600	303
11	12912	489	39	164347	252
12	15113	258	40	168364	652
13	16381	390	41	172415	241
14	17410	274	42	182021	940
15	21487	653	43	187800	458
16	23136	837	44	191045	652
17	23406	364	45	228328	211
18	24013	360	46	240877	535
19	29007	439	47	250242	374
20	33306	690	48	262929	717
21	37649	297	49	275833	647
22	47794	485	50	291507	675
23	48507	584	51	294064	865
24	53396	567	52	377790	613
25	53854	522	53	410356	433
26	56495	296	54	496988	327
27	72651	266	55	578641	771
28	75405	547	56	600341	395
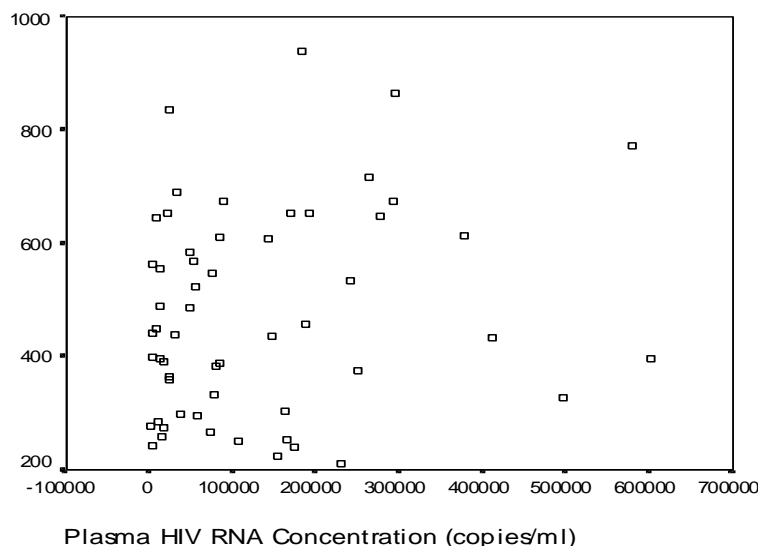
Figure 2. *Correlation between the number of CD4 and Viral Load, p=0.152, r = 0,194*

DISCUSSION

Ongoing HIV replication leads to immune system damage and progression to AIDS. HIV infection is always harmful, and without treatment, true long-term survival, free of clinically significant immune dysfunction, is unusual. Plasma HIV RNA levels indicate the magnitude of HIV replication and is associated with the rate of CD4+ T cell destruction, whereas CD4+ T cell counts indicate the extent of HIV-induced immune damage.[11]

In this study, we evaluated that there was no negative correlation between plasma HIV-1 RNA viral load and CD4 cells count in drug users group. More than half of the drug users with asymptomatic HIV infection (55.4%) with normal CD4 counts (more than 200 cells/mm³) had high plasma HIV RNA level. ACTG 175, a multicenter study in HIV patients with 200-500 CD4 cells/mm³ conducted by Lathey et al, showed that viral load was inversely corelated with CD4 cell number.[12] In this multicenter study the patients were not grouped according to the way of transmission.

Previous studies showed that there were different underlying conditions in drug users group, e.g. co-infection with Hepatitis C and B virus, tuberculosis and endocarditis. These conditions might facilitate high replication of HIV by direct interaction between

2 viruses[13] or secondary effects resulted from the release of cellular factors in response to infection.[14,15]

Understanding the contribution of virologic measurement and CD4+ cells count as prognostic factor of the disease is very important for determining when to start antiretroviral therapy, as well as for evaluating the outcome of antiretroviral therapy. CD4+ cell counts are good indicators of immune function in HIV infection. Plasma HIV-1 RNA assays (viral load assays) are useful as indicator of the prognosis of HIV infection, for indicating when asymptomatic patients should be treated and as a reference for subsequent monitoring of the virological response to therapy.[16] Palella et.al showed that plasma HIV RNA levels provide more powerful predictors of risk of progression to AIDS and death than do CD4+ T cell levels; however, the combined measurement of the two values provides an even more accurate method to assess the prognosis of HIV-infected persons.[2] There is a relationship between baseline HIV RNA levels, measured in a large cohort of HIV-infected adults, and their subsequent rate of CD4+ T cell decline.[2] Progressive loss of CD4+ T cells is observed in all strata of baseline plasma HIV RNA concentrations, but substantially more rapid decline are seen in persons with higher baseline plasma HIV RNA level. Likewise, a clear gradient in risk for disease progression and death is seen with increasing baseline plasma HIV RNA levels.[2,10,17] Regular, periodic

measurement of plasma HIV RNA levels and CD4^{+} T cell counts is necessary to determine the risk for disease progression in an HIV-infected person and to determine when to initiate or modify antiretroviral treatment regimens. As rates of disease progression differ among HIV-infected persons, treatment decisions should be individualized by level of risk indicated by plasma HIV RNA levels and CD4^{+} T cell counts.

Our study showed that majority of drug users with more than 200 CD4 cells/mm3 had high plasma HIV RNA level. Based on this fact we should offered the majority of HIV patients derived from drug users group to take antiretroviral therapy, although they still had high CD4 cells count level. We can suggest that in people living with HIV (PLWH) due to drug users, antiretroviral drugs should be started early although they have good clinical condition and had high level of CD4^{+} cells number.

Plasma HIV-1 viral load should be used to monitor the outcome of antiretroviral therapy. It had better value than CD4+ cell count in predicting the prognosis of HIV patients coming from injecting drug users.[18]

CONCLUSION

The management of PLWH came from drug user is different from the PLWH who had HIV from sexual transmission. The majority (56,14%) of asymptomatic HIV patients with normal CD4 counts, had high plasma HIV RNA level. Early anti retroviral treatment is suggested in those patients. The viral load examination is very important to monitor the result of the treatment.

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