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RESEARCH

VIROLOGICAL AND IMMUNOLOGICAL RESPONSE TO ANTI-RETROVIRAL TREATMENT IN HIV-INFECTED PATIENTS

(Respons Virologis dan Imunologis terhadap Pengobatan Anti-Retroviral di Pasien Terinfeksi HIV)

Umi S. Intansari[1], Yunika Puspa Dewi[1], Mohammad Juffrie[2], Marsetyawan HNE Soesatyo[3], Yanri W Subronto[4], Budi Mulyono[1]

ABSTRAK

Infeksi HIV/AIDS masih menjadi tantangan global. Pengobatan antiretroviral (ART) berperan dalam menurunkan replikasi virus, menurunkan tingkat kematian infeksi oportunistik dan meningkatkan kualitas hidup orang yang hidup dengan HIV/AIDS. Meskipun demikian data terkait respons virologis dan imunologis termasuk aktivasi imun masih sangat terbatas. Penelitian ini bertujuan untuk mengetahui respons virologis dan imunologis pasien HIV setelah 6 bulan memulai pengobatan ARV. Subjek dari penelitian observasional prospektif ini adalah 44 pasien HIV yang belum pernah mendapat pengobatan ARV, yang berobat di RSUP Dr. Sardjito Yogyakarta dan RSUP Dr. Kariadi, Semarang. Sampel darah EDTA sebanyak 6 mL diambil pra dan pasca 6 bulan pengobatan ARV untuk pemeriksaan jumlah sel T CD4$^+$, kadar RNA HIV dan persentase sel T CD8$^+$/38$^+$. Kadar RNA HIV turun secara bermakna sejalan dengan persentase sel T CD8$^+$/38$^+$, sementara jumlah sel T CD4$^+$ meningkat bermakna. Sebanyak 79,5% pasien mengalami pemulihan sel T CD4 optimal (>50 sel/μL) dan kadar RNA HIV turun lebih dari 1 log$_{10}$kopi/mL pada 93% pasien. Pasien dengan respons tidak sesuai antara virologis dan imunologis didapatkan sebanyak 13,6%. Kadar HIV bernasab positif dengan persentase sel T CD8$^+$/38$^+$ (r=0,58, p<0,0001) dan bernasab negatif dengan jumlah sel T CD4$^+$ (r=–0,470 (p<0,0001). Berdasarkan telitian ini, sebagian besar pasien mempunyai respons virologis dan imunonologis yang sesuai 6 bulan setelah ART. Sebanyak 20,45% pasien tidak berespons atau mengalami ketidaksesuaian respons virologis dan imunologis dan memerlukan penilaian dan pengobatan secara terus menerus.

Kata kunci: *Infeksi HIV, ART, respons virologis, respons imunologis*

ABSTRACT

HIV/AIDS remains a significant global challenge. ART has made significant contribution by reducing viral replication, reducing morbidity from opportunistic infection, and improves the life of person living with HIV/AIDS. However there is a very limited data related with virological and immunological response including T cell activation during ART in Indonesia. The purpose of this study were to know the virological and immunological response to ARV therapy in HIV patients pre and 6 months after ART initiation. The subjects in this observational prospective study were 44 ART naive-HIV infected adults patients in RSUP Dr. Sardjito, Yogyakarta and RSUP Dr. Karyadi Hospital, Semarang. Blood samples were collected in 6 mL volume EDTA- tube, before and 6 month after ART initiation to determine the CD4 T cell count, Viral Load (VL) and percentage of CD8$^+$/38$^+$ T cell. Viral load was significantly decreased 6 month after ARV initiation, parallel with the percentage of CD8$^+$/38$^+$, while CD4 T cell was increased (p<0.0001). There were 35 (79.5%) patients obtained optimally CD4 T cells recovery (>50 cell/μL) and 9 patients were sub-optimal, after 6 months of ART initiation. Viral load were decreased more than 1 log$_{10}$ copy/mL in 93% patients. We found 13.6% patients had discordance response. The VL positively correlated with CD8$^+$/38$^+$ cell percentage (r=0.58, p<0.0001) and negatively correlated with CD4 T cell count (r=–0.470 (p<0.0001). Based on this study we concluded, most HIV patients obtained concordance virological and

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immunological response 6 month after ART initiation. There were 20.45% non- responder and discordance responder patients that need to be evaluated and treated intensively.

Key words: HIV infection, ART, virological response, immunological response

INTRODUCTION

Human Immunodeficiency Virus (HIV) Infection still becomes a public health problem in the world, including Indonesia with the increasing population of people living with HIV and *acquired immunodeficiency syndroms* (AIDS) ever year. In 2014, the number of people living with HIV/AIDS (PLWHA) in the world reached 36.9 million (34.3 to 41.4 million), or 0.79% of the total world population 34.3 million of whom were PLWHA adults.[1] Each year from 2009 to 2013, there were 230,000 (160,000–370,000) people infected with HIV in Southeast Asia.[2] In Indonesia, the cumulative number of HIV/AIDS cases up to September 2014 was 206 095, or 0.47% of total population, 9796 of whom died. The number of new cases of HIV/AIDS reported from January to September 2014 was 24 645, or 0.06% of the population. The Special Region (DI) of Yogyakarta is a province on the eight rank of the highest prevalence in Indonesia with the prevalence of AIDS cases about 26.49 cases per 100.000 people.[1,3]

A research resulting a mathematical modeling estimates that 80% of ARV therapy and a campaign prevention of HIV infection could reduce the number of HIV-infected patients from 3 million to 1.2 million in 2025.[4] Nowadays, the coverage of *Antiretroviral* therapy (ARV) has been increasingly widespread, ie 12.9 million people getting ARV therapy in the world in 2013, 11.7 million of whom came from poor and developing countries. The percentage also decreased due to the expansion of the criteria for HIV levels changing ARV into CD4 (<350 cells/μL).[1] There were 11.7 million people obtaining ARV therapy, 36% (34-38%) of whom with HIV living in poor and developing countries. Despite considerable progress, the number of deaths due to HIV infection is still high.[2]

The main signs of HIV infection are persistent destruction of immune cells as well as immune system dysfunction and inflammation leading to the occurrence of opportunistic infections and disease progression. Understanding of the loss of $CD4^+$ T cells progressively remains a challenge in HIV research. There are several hypotheses to explain it, including direct mechanism by virus or indirect mechanisms like immune activation, a key characteristic of HIV infection. Immune activation is generally determined from the phenotype of cells that have an increased expression of cell activation markers, such as CD38 and HLA-DR, an increase in T cell proliferation and cell death, secretion of soluble receptors, cytokines, chemokines and inflammatory mediators. Immunological damage, including increased apoptosis, increased naive peripheral T cell division and memory and decreased thymus output was found in chronic infection suspected as a result of the high level of activation. One of the activation markers of cellular immune proved to be better than other markers is the expression of CD38 on CD8 T-cells ($CD8^+$ / 38 $^+$ T cells).[5,6]

Anti-Retroviral (ARV) therapy has effectively inhibited key steps in viral replication with a dramatic response in lowering plasma virus. People with HIV who are successful treated with ARV therapy have a life expectancy equal to the HIV-uninfected people.[7] Patients who get treatment with a low CD4 T cell count have significantly increased their life expectancy if they have a good CD4 recovery response and undetectable HIV viral load.[8] The clinical outcomes of *Highly Active Antiretroviral Therapy* (HAART) in HIV-infected individuals vary greatly. Most of the viral counts decrease to undetectable levels, and reconstitution of the immune system occurs, characterized by an increase in the number of $CD4^+$ T cells. However, there are still some patients experiencing a failure response and 20-40% of them had discordance in their response to the viral load compared to $CD4^+$ T cell recovery.[9] Discordance (not appropriate) of virological and immunological responses in 3–9 months of the treatment influences the clinical outcome and risk of death in long term.[10] It was reported that the increased CD4 T cell count of 50 cells/mL in 120 days of HAART can reduce the risk of opportunistic infections about 60%.[11]

Monitoring of ARV therapy in the first 6 months, consequently, has a very important role because of various cohort studies in Europe and North America concluding that the number of CD4 T cells and VL 6 months after ARV therapy is closely associated with disease progression. But, there was no correlation between the number of CD4 and VL before ARV therapy and disease progression. The ability to achieve long-term immunological response is reflected by an increase in CD4 T cells in 3 to 6 months after ARV therapy.[12] Therefore, this research aimed to determine

the virological and immunological responses to a six-month antiretroviral therapy in HIV patients.

METHODS

This research was an analytic observational study with prospective design. Subjects of this research were adult patients infected with HIV. Those patients, who were treated at the Edelweis Tropical Infectious Diseases Clinic in Dr. Sardjito Hospital, Yogyakarta and at the Tropical Infectious Diseases Clinic in Dr. Kariadi Hospital, Semarang, had not received previous antiretroviral therapy. Both of the health care facilities are a reference to HIV services in DI Yogyakarta and Central Java.

A total of 6 mL of EDTA blood was taken before ARV therapy and 6 months after the therapy for a complete blood test to measure the number of CD4 T cells, the percentage of $CD8^+/38^+$ T cells, and HIV RNA levels. Complete blood test was performed using an *automatic hematology analyzer*. The number of CD4 T cells and the activation of CD8 T-cells then were measured using *flow cytometer* by adding 10 uL of monoclonal fluorescent-antibody mixture, namely anti-CD3 FITC/CD4 PE/CD45perCP and anti-CD8 FITC/CD38 PE/CD45 PerCP at each of 50 uL EDTA blood. Afterwards, incubation was conducted for 15 minutes, and then 450 uL of erythrocyte pelisis solution was added and reincubated for 15 minutes in a dark room at the room temperature. The percentage of $CD8^+/CD38^+$ T cells and $CD4^+$ T cells was determined by FACS Calibur and Cell-Quest program.

Anti-retroviral therapy used in this research, is anti-retroviral drugs approved by the WHO including CCR5 antagonist class, Fusion inhibitors, *nucleotide/nucleoside reverse transcriptase inhibitors* (NRTIs), *non-nucleotide/nucleoside reverse transcriptase inhibitors* (NNRTIs), INSTI and PI.[13] Anti-retroviral selection decision was submitted to the doctors and be free from the influence of the researcher. HIV patients included in this research were patients diagnosed with HIV by doctors, and then confirmed by laboratory results according to the definition of HIV in the literature.[14]

HIV RNA levels (VL), furthermore, were measured in EDTA plasma by Real time-PCR using Cobas ampliprep/TaqMan with a limit of detection of 20 copies/mL and expressed in units of copies/mL or Log10 copies/mL.[15] EDTA Blood then was rotated at a speed of 3000 rpm for 15 minutes to obtain plasma. Plasma formed was stored in a freezer at -80°C until the sample size was met and then sent to the Dharmais Cancer Hospital in Jakarta for HIV RNA levels measurement.

Virological response was defined as a decrease in HIV RNA levels into undetectable levels in 3–6 months, or a decline more than 1 log10 copies/mL from the beginning of therapy.[15,16] Immunological response is defined as improvement of the immune system in HIV-infected patients after HAARV therapy assessed from an increase in the number and the percentage of CD4 T cells in the circulating blood. However, there is still no agreement in optimal immunological response after 6 months of the therapy. This research used the criteria of an increase in $CD4^+$ T cell count, about ≥50 cells/mL, 6 months after the ARV therapy as the optimal immunological response as used by some previous researchers.[10,17,18]

Finally, some statistical analyses, T test and Mann Whitney test, were performed to determine differences in the levels of HIV RNA, CD4 T cell count, and the percentage of $CD8^+/CD38^+$ T cells between before and after ARV treatment. Correlation among the variables was analyzed using Spearman test. This research had received approval from the Ethics Committee of the Medical and Health Research, Faculty of Medicine, University of Gajah Mada.

RESULTS AND DISCUSSION

There were 44 subjects enrolled in this study and observed for 6 months. All of the subjects got a first-line Fixed Dosed Combination (FDC) ARV therapy. Twenty-six (26) subjects (59.1%) took *Tenofovir, Hiviral and Efavirenz* (THE), 15 subjects (34.1%) took *Atripla* containing *Efavirenz, Emtricitabine*, and *Tenofovir* and the others took a combination of *Duviral (Zidovudine* dan *Lamivudine)* and *Neviral*.

The basic characteristics of the subjects in this research are shown in Table 1. The age of the subjects ranged from 19 years to 51 years. Data from the Health Ministry show that patterns of transmission of HIV infection within the last 5 years have not changed much. The age of 25–49 years is the age range of the most recent incident of HIV infection (72%), while the others at the age of 20–24 years (16%). The percentage of male subjects also known to be higher than the female ones. In the year of 2014 the percentage of male HIV patients were about 58%.[19]

Sixty-one point 4 percent of (61.4%) the subjects had a risk factor associated with Man Sex with Men (MSM), 43.2% had a risk factor associated with heterosexual and the rest had other risk factors,

Table 1. Characteristics of the research subjects

No	Parameter	N	%	Mean ± SD Med (Min-Max)
1.	Age (years)	44		28 (19–51)
2.	Sex			
	Male, n (%)	38	(86.4%)	
	Female, n (%)	6	(13.6%)	
3.	Clinical stage			
	1	25	(56.8%)	
	2	15	(34.1%)	
	3	4	(9.1%)	
	4	0	0	
4.	Risk factors:			
	MSM	27	(61.4%)	
	Heterosexuals	19	(43.2%)	
	Others	3	(6.8%)	
5.	Laboratory results			
	Levels of HIV RNA (copy/mL)			62700 (596–982000)
	Levels of HIV RNA (log_{10} copy/mL)			4.80 (2.77–5.99)
	$CD4^+$ T cell counts (cell/μL)			290±133
	Percentage of $CD8^+$ / 38^+ T cells (%)			46.78±14.35

MSM: Man sex with Men

including injecting drug users. On the contrary, data from the Ministry of Health showed that the highest risk factor was associated with heterosexual (50.75%), followed by other groups and MSM as much as 14.32%.[20] Nevertheless, the pattern of transmission in this research is similar to the pattern of transmission in other studies conducted in 10 countries, including Canada, Australia and countries in Europe showing that the most risk factor is associated with homosexual (71.9%).[21] Some studies also suggest that the pattern of transmission does not affect the progression of the disease.[22,23]

Anti-retroviral therapy, moreover, has effectively suppressed viral replication although there is still controversy regarding both the rate of decline in the number of virus and their normalization. Virological and immunological responses six months after the initiation of antiretroviral therapy are presented in Table 2.

HIV RNA levels 6 months after the ARV therapy had decreased significantly compared to those before ARV therapy, which means the antiretroviral therapy is highly effective to suppresses viral replication and the majority of the subjects in previous researches also respond well to ARV therapy.[10,18,24] The median of decreased HIV RNA levels within 6 months of the ARV therapy was -3.20 (-4.69– -0.21) log10 copies/mL. The result is not much different from another research

Table 2. Differences in mean/median of the parameters before ARV therapy and six months after ARV therapy

Laboratory parameters	Before ARV therapy Mean±SD Median (min-max)	Six months after ARV therapy Mean±SD Median (min-max)	P
CD4+ T cell counts (cell/μL)**	290±133	432±150	<0.0001
Percentage of CD4+ T cells (%)**	15.18±5.95	19.10±5.81	<0.0001
Percentage of CD8+ / 38+ T cells (%)*	44.08 (18.5–87.9)	29.56 (12.5–69.14)	<0.0001
Levels of HIV RNA (copy/mL)*	62700 (596–982000)	25 (20–263000)	<0.0001
Levels of HIV RNA (log)*	4.8 (2.77–5.99)	1.4 (1.3–5.42)	<0.0001

*tested with Wilcoxon
**tested with *Paired T Test*

reporting that a median reduction in HIV RNA levels within 6 months of ARV therapy was -2.8 (-3.4– -1.9) log10 copies/mL.[18] Based on the results of HIV RNA levels before and 6 months after the ARV therapy, 41 (93.18%) patients had a virological response, 21 of whom (47.73%) achieved undetectable HIV RNA levels (less than 20 copies/mL) and 20 (45.45%) of whom had decreased HIV RNA levels, more than 1 log10 copies/mL. The other 3 (6.8%) patients did not have virological response.

Virological response to antiretroviral therapy is actually influenced by viral factors, drug factors and HIV control programs including ARV adherence. Resistance to antiretroviral drugs in developing countries triggers an increase in 9–22% of the patients who had ARV therapy.[25] Some factors that may affect the immunological response are age, early CD4 T cell count, stage 4 during the treatment and HCV co-infection. Further researches on patients who did not respond virologically, consequently, are needed to determine the possibility of ARV resistance.

In line with the decrease in HIV RNA levels because of the antiretroviral therapy, the mean and percentage of $CD4^+$ T cells within 6 months of ARV therapy had increased very significantly. The mean of $CD4^+$ T cell count increased into 142 cells/uL. Overall, HIV RNA levels were negatively correlated with $CD4^+$ T cell count (r= -0.470; p<0.0001; 95% CI -0.62– -0.289) (see Figure 1). The negative correlation between moderate to weak levels of HIV RNA and the number of $CD4^+$ T cells are also shown by many other researchers, varied ie r= -0.5726, 0.3427 and -0,29.28.

Thirty-five (35) {(79.5%)} patients had optimal immunological response, 9 (20.5%) patients had sub-optimal response and 6 patients (13.6%) had a discrepancy (discordance) of the virological and immunological response (see Table 2). These patients did not experience immunologically optimal recovery (immunological non-responders) although those patients experienced a significant decrease in HIV RNA levels during the therapy (virological responders). The sub-optimal immunological response at least 6 months after the treatment in the previous studies varied in different populations, such as 8.7%[10] 19%[18] 21%[17] and 29%.[29] This variation may be caused by differences in research populations, monitoring duration, research design including inclusion criteria and immunological response criteria. Multi-center researches in countries with limited resources also reported the discordance in virological and immunological response (33.87%) within 6 months of therapy[18], while the longitudinal research in Birmingham reported a mismatch response (24.5%) over the 3–9 months of ARV therapy.

The increase in the number of $CD4^+$ T cells in 3 to 6 months of ARV therapy actually reflects the lost capacity of the immune system to restore CD4 T lymphocytes.[12] Meanwhile, the increase in the number of $CD4^+$ T cells (<50 cells/μL) in 8 months of effective antiretroviral therapy has a risk of low number of $CD4^+$ T cells until the next 5 years. The risk of clinical

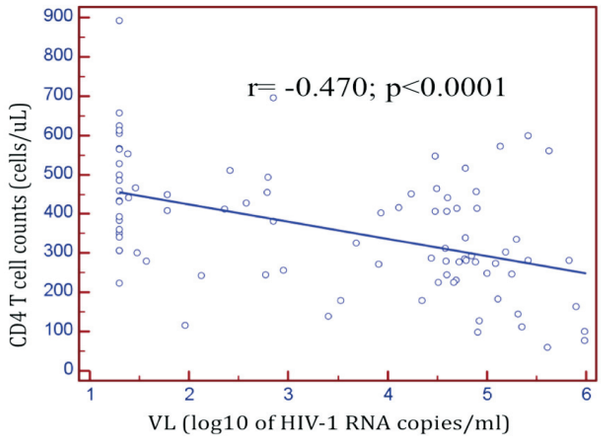

Figure 1. The correlation between HIV RNA levels and $CD4^+$ T cell counts. The high levels of HIV RNA correlated inversely with the number of $CD4^+$ T cells. It means that patients with high HIV RNA levels tend to have low CD4 T cell counts.

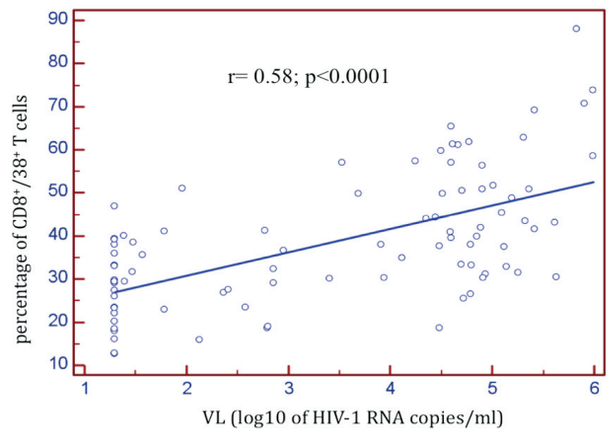

Figure 2. The percentage of $CD8^+/38^+$ T cells correlated with high levels of HIV RNA (log10 copies/mL). The percentage of activated CD8 T cells correlated with HIV RNA levels. It means that a high percentage of $CD8^+/38^+$ T cells was in line with the high levels of HIV RNA in HIV patients. Variations in the percentage of $CD8^+/38^+$ T cells were found in some patients with low levels of RNA.

outcome in the form of AIDS related, non-AIDS related and death in patients with poor recovery (<50 cells/μL) is about 5.8/100 people per year, compared to a recovery (≥50 cells/μL) of 2.7/100 people per year.[30]

In total, there were 20.45% of patients who did not respond and had a discrepancy of virological and immunological response. Some factors may affect the immunological and virological response, including age, early CD4 T cell count, stage 4 during the therapy, and HCV co-infection.[31] Other studies have reported that virological and immunological response was greater in young female patients with high naive CD4 T cell count and high number of initial virus.[26] However, *systematic review* concluded that there was no correlation between gender and both disease progression as well as virological and immunological responses against ARV therapy.[32]

In addition, $CD8^+$ T cell activation indicated by CD38 expressions also decreased 6 months after the ARV therapy due to lower levels of HIV RNA and increasing number of $CD4^+$ T cells. Similarly, the percentage of $CD8^+/38^+$ T cells decreased significantly 6 months after the antiretroviral therapy (a median of 44.08% to 29.56%) although it was still higher than in the healthy population, ie around 12%. The results support previous studies showing that ARV therapy has an ability to inhibit viral replication, so it will reduce the percentage of $CD8^+/38^+$ T cells.[23,24]

HIV replication rate is actually the main factor triggering $CD8^+$ T cell activation. In line with that, the results of this research show that the overall height of immune activation was correlated positively with HIV RNA levels ($r= 0.58$, $p<0.0001$ CI:.42-.71). These results correspond with previous researches[33-35] with a cross-sectional study showing that there is a strong correlation ($r=0.76$, $p<0.01$) between the levels of HIV RNA and the percentage of $CD8^+/38^+$ T cells.

Previous studies show the exaggerated expression of $CD8^+/38^+$ T cells, i.e >13% was found in 40% of aviremic patients with a $CD4^+$ T cell count of <200 cells/μL. There was also a significant correlation expression of $CD8^+/38^+$ T cells with changes in plasma HIV RNA levels at a particular group, especially a $CD4^+$ T cell count of >200 cells/μL. Although positively correlated, decreased $CD8^+/38^+$ T cells at six months after antiretroviral therapy still cannot predict a decrease in HIV RNA levels.[36] It is caused by great individual variation found in the patients undergoing treatment with a variety of responses to decreased levels of RNA HIV.[33] It reflects the variability of the interaction between *host* and virus.

Nevertheless, there are still some limitations in this research. The very few subjects that were at an advanced stage will probably give different responses due to opportunistic infections. Thus, further researches need to be done to determine whether there are differences in virological and immunological responses to antiretroviral therapy at different stages of severity of HIV infection.

CONCLUSION AND SUGGESTION

Six months after ARV therapy, as many as 93.18% of HIV patients had a good virological response and 79.5% had an optimal immunological response. 20.45% of the patients did not respond or had a discrepancy (discordance) of virological and immunological responses. Decreased immune activation after ARV therapy was positively correlated with the levels of HIV RNA.

Based on this research, therefore, it can be concluded that monitoring of ARV therapy, both virological and immunological ones, needs to be done on each HIV patient to determine any discrepancies response to treatment. As a result, an alternative therapeutic strategy can be performed to improve the immunity of patients. Further researches also need to be done to determine whether there are differences in virological and immunological responses to antiretroviral therapy at different stages of severity of HIV infection.

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