Effects of Counseling in CVT Clinic and Black Tea *(Camelia sinensis varietas Assamika)* Supplements in the Improvement of CD4 Profile in HIV Patients Receiving ARV Treatment

Adius Kusnan[a]*, Suryani Asad[b], Agussalim Bukhari[c], Nasir Jidhe[d], Rosdiana Natsir[e] , Andi Asadul Islam[f], Prihantono Prihantono[g], Nasrum Massi[h], La Ode Sura[i], Haruddin[j]

[a,I,j]*Department of Epidemiology, Faculty of Medicine, Haluoleo University, Kendari, Indonesia*
[b,c]*Department of Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[d]*Department of Pharmachology, Faculty of Pharmachology, Hasanuddin University, Makassar, Indonesia*
[e]*Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[f,g]*Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[h]*Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[a]*Email: adius_kusnan@yahoo.com*

Abstract

Incidence of HIV / AIDS in Indonesia tend to increased, as well as in Southeast Sulawesi. HIV / AIDS has a huge impact on patients, their families, and society. Prevention of the spread of infection can be pursued through increased access to treatment and support to patients and their families. Voluntary Counselling and Testing (VCT) clinics is a means to provide care, support and treatment for people with HIV/AIDS.

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* Corresponding author.

Although, people living with HIV/AIDS in Southeast Sulawesi who attending to VCT clinic is still lacking, only 4% who take advantage of this facility. Several studies have shown that counseling have major effect on the health profile of patients with HIV / AIDS. Studies also mentioned that polyphenols in black tea, containing teaflavins inhibit the entry of glycoproteins by blocking HIV-1, thus reducing HIV-1. The aims of this study is to determine the effect of adherence counseling and black tea supplementation in the increasing of CD4 profile in HIV patients. This is a Longitudinal study, random sampling of HIV-AIDS patients in Bahteramas Hospital Southeast Sulawesi. All patients undergoing antiretroviral therapy standards. In the intervention group, counseling and suplementation black tea regularly in daily basis, the control group did not received suplementation of black tea. Detection of CD4 using Cyflow cytometry methods. Data analysis was performed using willcoxon test. Total 70 patients including in this study, analyze the effect of conselling and blacktea supplementation in changed in CD4 profile, Showed that in the intervention group levels of CD4 was increased by 140.07 ± 64.22, and levels of CD4 in the control group was decreased by -14.82 ± 39.29, mean difference of CD4 were 79.05 ± 24.59, this is significant with p = 0.003. Results of this study suggest that adherence counseling and suplementation of black tea proven to increased levels of CD4 of HIV patients taking antiretroviral therapy.

Keywords: Black tea (EGC, teaflavi, tanin); CD4; ARV; VCT; HIV.

1. Introduction

Epidemic of HIV is still a problem in various countries around the world, including Indonesia. Estimated 35.3 million people worldwide were living with HIV in 2012, the number growing by 2.3 million of new cases in the same year. World Health Organization (WHO) stated that the disease as the most destructive epidemics in history, to anticipate this, WHO establish special organizations combating HIV / AIDS called Joint United Nations Programme on HIV/AIDS. Every day more than 6800 people infected with HIV and more than 5700 people died of AIDS, it is because inadequate access to prevention and health services [1].

Since 1993, the government has provided VCT (Voluntary Counseling and Testing) clinics as a voluntary counseling and check their HIV status, but the utilization is still very low. Until the end of December 2015, only 4% of an estimated 193.030 people living with HIV who take advantage of this facility [2].

VCT clinic is a health-care facility that is used in the response to HIV / AIDS cases. The clinic works closely with all parties in the mapping, estimation and search cases as early as possible, especially in high-risk groups (high risk population).

VCT clinic also provides treatment and support for people living with HIV in order not to transmit it to others so that quality of life can be improved. In developed countries, VCT is a key component in HIV programs, while in developing countries, VCT has not become a major strategy in the prevention of HIV / AIDS [3].

In Southeast Sulawesi, until the month of December 2015, in 4 VCT clinic of General Hospital has been serving 10.431 cases of HIV / AIDS. This amount covers all people living with HIV who have not actually need treatment to improve their quality of life. In Southeast Sulawesi province the number of HIV who reached 916

people, consisting of 592 HIV-positive and 324 AIDS patients. The relatively low HIV-positive people who come check-ups and counseling VCT clinic assumed that utilization remains low [4].

Polyphenols in black tea have galloyl and teaflavins which inhibits the entry of glycoproteins by blocking HIV-1-mediated membrane fusion, the inhibitory activity of polyphenols in tea work together to block the formation of gp41, polyphenolic compounds form theaflavin-3.3 V- digallate (TF3) hydrophobic binding pockets so as to reduce the HIV-1 virus contained on gp41 [5].

The aims of this study is to determine the effect of adherence counseling and and black tea supplementation in the increasing of CD4 profile in HIV patients receiving ARV treatments.

2. Materials and Method

2.1. Collection of Samples

Study was conducted within a population of HIV/AIDS patients who had been diagnosed through clinical and laboratory examination, which entered the Bahteramas Hospital in Kendari, Southeast Sulawesi, Indonesia. All samples who fulfilled inclusion and exclusion criteria and willing to participate in the study and signing informed consent recruited as study samples.

The samples consisted of 70 patients with HIV/AIDS who had been taking antiretroviral therapy for less than two years, consists of two groups, 35 respondents of the intervention group and the control group were 35 respondents.

The details of the therapy is as follows intervention group gained ARV therapy standard dose of Ministry of Health of the Republic of Indonesia, counseling and Additional supplements containing black tea (EGCG, teaflavin, tanins), a dose of 1505mg, in 1000 ml to 1400 ml, equivalent to 5-7 cups / day at a temperature of 90^0C, which has been tested at the research center of tea and quinine Gambung Indonesia West Java license number : 030701.PPTK.III / 2016. The control group taking a standard dose of ARV. Detection of CD4 level conducted prior commencement of treatment and at the end of the study (24 weeks).

2.3. Detection of CD4

Detection of CD4 was done according to Cyflow cytometry method previously describe, whole blood was collected in EDTA vacutainer tubes (Becton-Dickinson, Mt View, California) and aliquoted into two tubes (200 μL in each).

Tube was sent to the Department of Microbiology and Mollecular Bioolgy Hasanuddin university for CD4+ T-lymphocytes using a Cyflow counter and a FACSCount respectively within 6 hours of phlebotomy following manufacturers' instructions. The two independent medical laboratory scientists who conducted the enumeration of the CD4+ T-lymphocytes were blinded to each other's results. Cyflow reagents and consumables were used according to the manufacturer's instructions [6].

Briefly, 20μL of whole EDTA blood was pipetted into a Partec test tube. Ten microlitres of CD4 phycoeryrthrin conjugated monoclonal antibody supplied by Partec was added to the tube containing whole blood and the reactants incubated for 15 minutes at room temperature. Following incubation, 800μL of no lyse buffer, supplied by Partec were added to the tube and gently vortex-mixed. The tube was then plugged into the Cyflow counter for automatic counting. The histogram and absolute counts are displayed and printed automatically. The histogram shows direct counting result in terms of absolute CD4+ T-lymphocytes/μL [7]..

The CD4+ T-lymphocytes with high fluorescence appear in a prominent peak at the right of the histogram, whereas the weaker but also CD4+ monocytes appear to the left without any overlap with CD4+ T-lymphocytes.

For FACS Count analysis, 50μL of whole EDTA blood was added to the CD4 FACS Count reagent tube containing anti-CD3 and anti-CD4 monoclonal antibodies, sample diluent, and reference beads. The samples were then incubated at room temperature for 60 minutes and subsequently run on the FACSCount [8].

2.4. Data Analysis

Data analysis using the SPSS (Statistical Package for Social Science) version 22. Normality of the samples were analyzed using shapiro wilk's test. Analysis of mean difference was performed using willcoxon test, which is used to compare the two groups of intervention.

2.5. Ethical Clearence

Ethical approval for this study was obtained from Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Number; 0666/H.8.4.5.31/PP36-Kometik/2016. Research permit from Bahteramas General Hospital, Southeast Sulawesi Province number: 332 / R & D / RSU / I / 2016.

3. Results

We recruited 70 HIV/AIDS patients during April to September 2016, originated from Bahetramas Hospital. Youngest age of subject was 17 years and the oldest was 54 years old, the mean age of subjects in this research was 34.3 years.

This study shows the differences in CD4 level in both groups, the intervention group who have acquired HIV therapy standard doses prior suplementation of black tea (EGCG, teaflavin and tannins) Mean value of CD4 pre test (week 1) was 360.91 ± 221.20 and after suplementation of black tea, Mean value of CD4 post test (week 24) was 425.14 ± 178.87.

While in the control group which has gained the standard dose ARV therapy Mean value of CD4 pre-test (week 1) was 380.57 ± 78.63 and Mean value of CD4 post test (week 24) was 365.74 ± 81.13.

Analyze the effect of adherence counseling and blacktea supplementation in the increasing of CD4 profile in both groups for 24 weeks, showed in the intervention group CD4 levels increased by 140.07 ± 64.22 and in the

control group, levels of CD4 decreased by -14.82 ± 39.29, mean difference in CD4 level was 79.05 ± 24.59, this is significant with p = 0.003.

Table 1: Responder characteristics

Characteristic	Intervention n (35)	Intervention %	Control n 35)	Control %	Total n(70)	Total %	p
Age (Years)							
17-29	10	28,6	12	34,3	22	31,4	0,257
30-39	13	37,1	17	48,6	30	42,9	
≥ 40	12	34,3	6	17,1	18	25,7	
Sex							
Male	19	54,3	16	45,7	35	50,0	0,663
Female	16	45,7	19	54,3	35	50,0	
Marital Status							
Not Married	10	28,6	6	17,1	16	22,9	0,257
Separated	3	8,6	1	2,9	4	5,7	
Married	22	62,9	28	80,0	50	71,4	
Employee							
Not working	14	40,0	15	42,9	29	41,4	0,850
Private	12	34,3	13	37,1	25	35,7	
Government	9	25,7	7	20,0	16	22,9	
Compliance to counselling (Pre)							
Not obey	11	31,4	13	37,1	24	34,3	0,801
Submissive	24	68,6	22	62,9	46	65,7	
Compliance to counselling (Post)							
Not obey	10	28,6	14	40,0	24	34,3	0,450
Submissive	25	71,4	21	60,0	46	65,7	

Table 2: Comparisons of CD4 level pre and post intervention (n=70)

Groups	Pre (Mean±SD)	Post (Mean±SD)	Mean differennce (Mean±SD)	p*
Intervention	360,91±221,20	425,14±178,87	64,22±140,07	0,003
Control	380,57±78,63	365,74±81,13	-14,82±39,29	

Independen Sample T-Test

In correlation analysis found a significant correlation between adherence counseling to changes in CD4 cell count in HIV patients in intervention group and the control group with a value of r = -0.451, significant at p = 0.000.
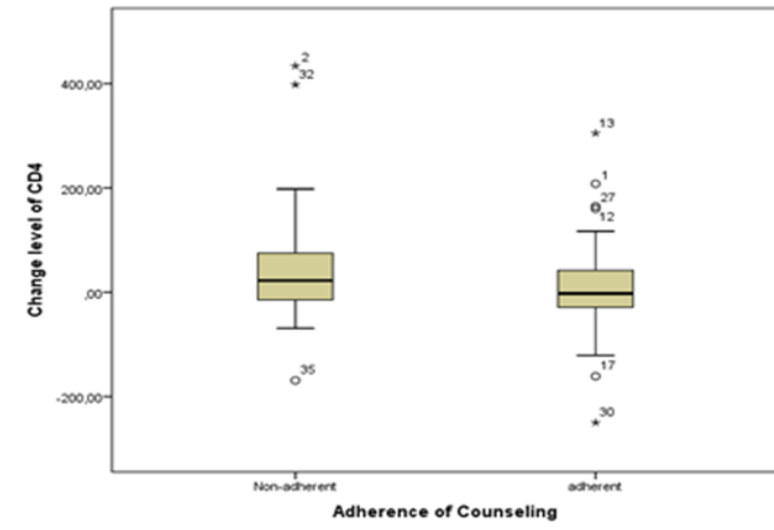
Picture 1: Graph Box Plot Adherence of Counselling and Change in CD4 level

Table 3: Correlations of Counselling and CD4 level pre and post intervention (n=70)

| Variable | CD4 Level | | | | | |
| | Pre | | Post | | Differences | |
	R	p*	r	p*	r	p*
Compliance to Counseling (Pre)	0,302	0,011	0,113	0,351	-0,176	0,145
Compliace to Counseling (Post)	-0,315	0,008	-0,032	0,792	0,451	**0,000**

*Spearman Correlation

4. Discussion

Anti-retroviral therapy (ARV) aims to increase levels of lymphocytes T-CD4 + by pressing the HIV / AIDS virus. ARVs work by blocking the ability of the virus to replicate and infect the new cells, which in turn slows the progression of the disease. T-cell count of CD4 + lymphocytes is an indicator for the prognosis and course of HIV infection, so it is often used to monitor whether a given treatment is working or not. If ART is working then the number of lymphocytes T CD4 + increases above 50 / uL in a few weeks and then increased by 50 / uL-100μL/year, until the limit value is achieved [9].

Polyphenols in tea, especially black that have galloyl, theaflavins more potent derivative inhibits the entry of glycoproteins by blocking HIV-1-mediated membrane fusion, the inhibitory activity of polyphenols in tea work together to block the formation of gp41, polyphenolic compounds form theaflavin-3.3 V- digallate (TF3) hydrophobic binding pockets so as to reduce the HIV-1 virus contained on gp41 [10].

Hydroxyl group (OH) contained in teaflavin function as free radical or antioxidants. The more hydroxy compound, then his ability as an antioxidant compound, the better[11]. The results of the study, the polyphenols

contained in black tea may increase the body's defense system, giving the effect of an increase in lymphocyte proliferation, increases the production of IL-12, increase phagocytosis. The results of previous studies of TNF-ἀ and can stimulate IFN-ɤ eppigallocatechin-3-Galates (EGCG), while the polyphenol compounds with the active ingredient may provide a beneficial effect as an immunomodulator [12].

Another study was conducted to determine the antioxidant activity, the ability to be able to reduce free radicals by using DPPH [13]. Total catechins in fresh tea leaves as an antioxidant catechin content of 13.5 to 31% and Camelia sinensis varieties Assamika more than Camenlia sinensis varieties Sinensis, the results of the study (2007) University of Kansas American Chemical Sosiety EGCG is more effective to neutralize radical free of the vitamin C and 25 times more potent than vitamin E [14].

In this study, the intervention group of HIV patients given ARV therapy, which can inhibit the replication of HIV-1, results of evaluation of clinical pathology laboratory examinations General Hospital Wahiddin Sudirohusodo Makassar after 24 weeks or 6 months results TCD4 + average increase of 150 / ml in HIV intervention group.

In accordance with our hypothesis whether there is influence adherence counseling in utilizing the clinic VCT HIV-AIDS with treatment compliance taking ARVs standard doses, based on the statistical analysis of the research hypotheses we received indicated the intervention group with a value (p = 0.003), thus it can be concluded that there is influence the utilization of adherence counseling VCT HIV-AIDS interventions ARV therapy, the changing levels of CD4 in which the intervention group can increase levels of CD4 (64, 22 ± 140.07) compared to the control group who ate a standard dose of ARV therapy so that CD4 less likely to experience a significant increase or decrease (-14.82 ± 39.29), as well as not utilizing VCT HIV-AIDS clinic fullest.

Based on the results of the analysis, in accordance with operational research to improved adherence counseling ARV treatment at Mitra Masyarakat Hospital, showed 35 respondents at the beginning of the intervention there were 25 respondents who followed the intervention until the research is completed with the always present to receive ARV adherence counseling 8.32 fold with OR = 8.32 p = 0.0000 (95% CI: 0.66-1.09), compared to respondents who were not present and did not receive adherence counseling [15].

Effect of medication adherence counseling to the level of compliance has been demonstrated on several studies, argues that health care providers prepare accurate information about compliance, have plenty of time to dialogue with people living with HIV in order to improve compliance ARVs [16]. compliance Counseling can help patients find a way out of the difficulties that may arise from the provision of treatment and would affect treatment adherence in running [17].

Family Health International (FHI) reported a study conducted conjunction adherence counseling first program in Ghana, Kenya and Rwanda in a detailed report, all programs require at least one and more than three treatment adherence counseling, conducted by nurses who generally receive training on compliance for two up to three days. In Rwanda, the patients also received treatment-related brochures in local languages, including a card with

a picture of each drug and the schedule wear [18]. In our study, in addition to involving people living with HIV to follow compliance counseling against taking ARV therapy, also involving the families of people with HIV, in spite of the processed data is statistically no effect statistically, it is known if families were included in this study, the possibility of support 4 times better adherence to therapy than respondents consume whose status is unknown and no family support. Thus the results of this study in accordance with guidelines for antiretroviral treatment according to the moh, couples and families are expected to provide support and care. The basic principle of couples and families here are the closest people living with HIV [19].

Research on family support in Indonesia is a participatory research shows that most provide support for people living with HIV are female sexual partners and close friends and know their status. While men are more supportive of parents and also know their status [20].

4. Conclusion

Adherence counseling in utilizing VCT HIV-AIDS clinic and blactea suplementation plays an important role so that the intervention group of HIV patients taking antiretroviral therapy and want to supplement black tea (EGCG, teaflavin, tanins) regularly since proven to significantly increase levels of CD4. It can provide new insights into the importance of regular counseling about treatment with HIV-AIDS VCT uptake.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest

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