

Effect of N-Acetylcysteine on IL-10 and Total Lymphocyte Count in HIV Infected Patients Undergoing Antiretroviral Treatment

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Abstract:

Objective: To determine NAC oral administration's effect on changes in IL-10 levels and total lymphocyte count (TLC) in patients with HIV/AIDS in Dr Mohammad Hoesin Hospital, Palembang.

Material and Methods: This study was a double-blind, randomized clinical trial. A total of 32 HIV/AIDS patients undergoing ARV treatment were randomly divided into two groups: the placebo and NAC groups. In the placebo group, patients were given capsules containing lactose at a dose of 3x1 capsules/day, while the NAC group, were given NAC at a dose of 3x200 mg/day. Each group was treated for 12 weeks.

Results: NAC administration significantly reduced IL-10 levels $P= 0.038$ but could not significantly increase TLC after treatment $P= 0.376$. However, TLC on the NAC group remained higher when compared with TLC on the placebo group.

Conclusion: NAC administration significantly reduced levels of IL-10 and increased TLC; therefore, NAC has potential effects of increasing the effectiveness of antiretroviral therapy in HIV/AIDS patients, although it still needs to be studied further.

Keywords: HIV/AIDS, interleukin 10, NAC, total lymphocyte count.



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Introduction:

Human Immunodeficiency Virus (HIV) infection is still a health problem for most countries in the world. In 2017, WHO estimates that HIV has infected 36.9 million people worldwide. In Indonesia, HIV is estimated to have infected 640.000 people, specifically in the province of South Sumatra until the end of 2017, HIV patients recorded as many as 2.810 patients, making it the fourth most HIV-infected province in Sumatra after North Sumatra, Riau Islands, and Riau.¹

HIV infects CD4⁺ cells, which play a crucial role in the immune system, causing a decrease in the number of these cells in the blood—resulting in a progressive decline of the immune system. Besides being driven by a reduction in CD4⁺ cells, the decline in the immune system in HIV patients is also

caused by the disruption of cytokine regulation that affects various immune cells' functions. Increased interleukin 10 (IL-10) is one of the leading causes of dysregulation. Increased IL-10 causes immunosuppressive conditions by inhibiting the response of CD4⁺ cells to HIV infection.²⁻⁴ Ex vivo studies that have been conducted show inhibition of IL-10 previously can increase CD4⁺ or CD8⁺ cell activity. Therefore, inhibition of IL-10 is expected to restore T cell function and improve its ability to control the virus.⁵⁻⁷

Also, HIV infection can be found in a condition of oxidative imbalance, characterized by reduced levels of glutathione (GSH) in plasma. GSH levels in erythrocytes and T cells also decreased in line with the progression of HIV.^{8,9}

N-acetylcysteine, as a variant of acetylated L-cysteine, is a cysteine source in the biosynthesis of GSH.¹⁰ This compound can increase GSH levels in HIV patients.^{8,11} Several studies have shown that this compound can inhibit HIV replication and affect cytokine production in mononuclear cell culture from peripheral blood circulation. This compound has been shown to reduce anti-inflammatory cytokines, such as IL-10, and increase proinflammatory cytokines, such as IL-2 and IL-12, in peripheral blood culture mononuclear cells (PBMC).¹²

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Several in vitro studies showed that T lymphocyte cells originating from HIV patients tend to undergo apoptosis more rapidly.^{13,14} Interestingly, NAC administration was able to reduce apoptosis.^{15,16} Based on these data, NAC administration as an additional supplement is thought to provide other benefits to HIV/AIDS patients receiving antiretroviral (ARV) therapy. There have not been many publications regarding the benefits of NAC administration in Indonesia, especially its effects on IL-10 and the TLC in HIV/AIDS patients.

This study aims to determine NAC administration's effect at a dose of 600 mg/day in HIV patients undergoing ARV treatment by testing the IL-10 level and TLC of the patient.

Methods:

Study Design

This research was a double-blind, randomized clinical trial conducted in July 2019 - February 2020 at the Dr Mohammad Hoesin Hospital, Palembang. Thirty-two HIV patients, aged 20-60 years, received ARV treatment, with no history of allergy to NAC and taking antioxidant drugs other than NAC were enrolled.

All participants were randomly divided into two groups, placebo and NAC groups; each group numbered 16 people. Patients in the NAC group were given capsules containing NAC at a dose of 3x200 mg/day, while the placebo group were given capsules containing lactose at a dose of 3x200 mg/day. All participants received treatment for 12 weeks.

Written informed consent was obtained from all individual participants before enrolling in the study. This study was approved by the ethics committee of the Faculty of Medicine, Sriwijaya University and Dr Mohammad Hoesin Hospital, Palembang (No. 552/kepkrsmhfkunsri/2019).

Sample Collection

The peripheral blood from all participants will be collected before starting treatment and after completing treatment with the drugs. Blood from each participant was drawn into the two blood collection tubes, one tube containing EDTA and another no anticoagulant. The non-anticoagulant tubes were centrifuged to obtain the serum for IL-10 levels measurement, while the EDTA tubes were used for TLC measurement.

Measurement of IL-10 Levels

Measurement of IL-10 levels was carried out at the Prodia Clinical Laboratory in Central Jakarta. The examination of IL 10 levels used the ELISA method (Human IL-10 High Sensitivity Elisa Kit from Thermo Fisher Scientific, Cat. Number BMS215HS).

Measurement of TLC

Measurement of TLC was carried out at the Clinical Pathology Laboratory Dr Mohammad Hoesin Hospital, Palembang. The TLC was calculated using the flow cytometry method by the Sysmex XN 1000i Hematology Analyzer (DCL Cellpack reagents).

Data Analysis

Data of IL-10 levels and TLC will be tested using unpaired T-test or Mann Whitney test between the NAC and placebo group. In contrast, the difference in the average IL-10 levels and TLC before and after treatment will be tested using the paired T-test or Wilcoxon test. All tests were performed at a 95% confidence level, using SPSS software.

Results:

General Characteristics

Most of the placebo and NAC group samples were male and were in the age range of 31-40. Most of the patients were in stage 1, had no comorbidities, and had only been on ARV treatment for <1 year.

There were no significant differences in sample characteristics between the placebo and NAC groups (Table 1).

Table-I. General Characteristic of Samples

Variable	Number of samples		P
	Placebo	NAC	
Gender			
- Male	12 (75%)	14 (87.5%)	0,654^a
- Female	4 (25%)	2 (12.5%)	
Age (Years)	35,63±1,45	37,06±1,79	0,629 ^b
- 20 - 30 years	5 (31.3%)	3 (18.8%)	
- 31 - 40 years	7 (43.8%)	9 (56.3%)	
- 41 - 50 years	3 (18.8%)	3 (18.8%)	
- > 50 years	1 (6.3%)	1 (6.3%)	
Duration of treatment			
- < 1 years	6 (37.5%)	5 (31.3%)	0,719^c
- 1 - 3 years	3 (18.8%)	5 (31.3%)	
- 3 - 5 years	2 (12.5%)	3 (18.8%)	
- > 5 years	5 (31.3%)	3 (18.8%)	
Stadium			
- Stadium 1	10 (62.5%)	12 (75%)	0,446^c
- Stadium 2	4 (25%)	2 (12.5%)	
- Stadium 3	2 (12.5%)	2 (12.5%)	
Comorbidity			
- No Comorbidity	12 (75%)	14 (87.5%)	0,500^a
- Comorbidity	4 (25%)	2 (12.5%)	

Exp: ^a p-value based on Fisher's test; ^b p-value is based on the independent T-test;

^c p-value based on the Chi-Square test; All tests are carried out at α 5%.

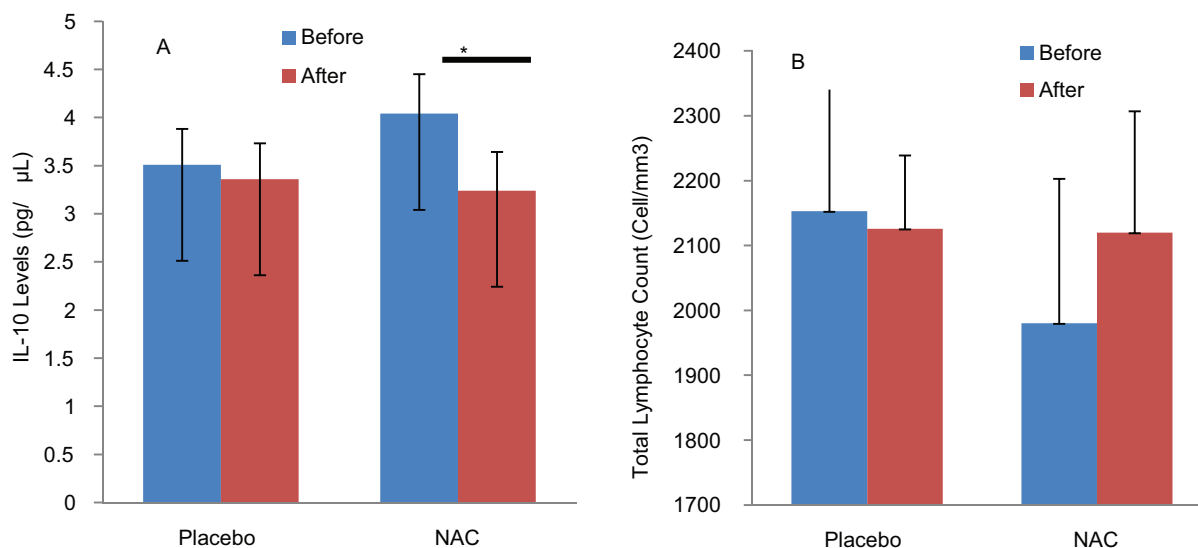


Figure 1. A. IL-10 mean levels; B. Total lymphocyte samples mean levels. Exp: * indicates $p < 0.05$ based on paired T-test.

IL-10 Levels

IL-10 levels were measured both before and after treatment in the two test groups. The test results showed a significant decrease in IL-10 levels in the NAC group after treatment $P = 0.038$. Still, there was no significant difference in IL-10 levels in the placebo group before and after treatment $P = 0.691$ (Figure 1A).

Total Lymphocyte Count

Similar to IL-10 levels, TLC was also measured before and after treatment. The test results showed no significant differences between TLC before and after treatment, both in the NAC $P = 0.376$ and placebo group $P = 0.845$. However, the TLC in the NAC group showed an increasing trend of 6.6%, while the TLC in the placebo group showed a declining trend, by 1.7%, after treatment (Figure 1B).

Discussion:

In HIV infection, interleukin 10 plays a role in reducing the ability of T lymphocyte cells by decreasing the ability of monocytes and dendritic cells to present specific antigens to T lymphocytes. As a result, it cannot optimally respond to virus infection.¹⁷

In the present study, NAC administration reduced IL-10 levels in HIV patients treated with ARVs significantly. These results are consistent with previous studies indicating that NAC can reduce IL-10 levels in PBMC cells and macrophages under oxidative stress conditions, a condition that is often found in HIV infection.^{12,18} This is related to the ability of NAC as a precursor of GSH. NAC can increase GSH levels in HIV patients while giving GSH can reduce IL-10 levels in HIV patients.^{17,19}

N-acetylcysteine reduces IL-10 levels through two mechanisms. First, as an antioxidant, NAC can inhibit IL-10 transcription by decreasing the amount of active NF- κ B.^{17,18,20} The second mechanism is to reduce the replication of HIV through a decrease in the number of active NF- κ B and TNF- α ,²²⁻²⁴ and inhibition of the reverse transcriptase enzyme, which is required in the replication process.^{25,26} Several studies have found a strong positive correlation between increased levels of IL-10 and HIV viral load.²¹ Therefore, a decrease in the amount of virus will also reduce levels of IL-10.

Inhibition of IL-10 can increase the response of CD4+ and CD8+ T lymphocytes. Inhibition of IL-10 has been shown to increase the proliferation of CD4+ T cells against the HIV p24 antigen and directly enhance the effector function of CD4+ T cells. As a result, there is an increase in the clearance of persistent viral infections and the formation of memory T cells against the virus.^{5,27,28}

The present study showed that the decrease in IL-10 levels occurred in the NAC and placebo groups. This decrease is thought to be caused more by the ARV treatment that has been given. The ARV treatment can reduce IL-10 levels due to a reduction in the number of viruses.²⁸ However, the administration of NAC can increase the effectiveness of the ARVs given. This increase of effectiveness is evidenced by the percentage decrease in IL-10 levels in the NAC group (24.7%) higher than the placebo group (4.5%). In the NAC group, the reduction of IL-10 levels also still occurred in the sample group with ARV treatment for less than five years, while in the placebo group, the decrease in IL-10 levels only

happened in the sample with ARV treatment less than three years.

In HIV infection, lymphocyte cells, especially CD4+ T cells, will experience a very significant decrease. In this study, the administration of NAC could increase the total lymphocyte count of the sample but not significantly. The results also showed no significant difference in the mean total lymphocyte count between NAC and placebo. These results are consistent with a study conducted by Treitinger et al., who found that administering NAC to HIV patients who had been given ARVs did not have a significant additional effect on increasing the number of CD4+ T lymphocytes and overall lymphocyte cell viability.²⁹

However, the administration of NAC was able to increase the mean total lymphocyte count of the sample by 6.6%, higher than placebo, which decreased by 1.7% after treatment (Figure 1). It indicates the need for larger doses of NAC to be more effective in increasing glutathione levels, inhibiting apoptosis and increasing lymphocyte cell viability in HIV patients. Research by Arkelund et al. showed that administering NAC at a dose of 800 mg/day can normalize GSH and cysteine levels, reduce TNF- α levels and slow down the decrease in the number of CD4+ T lymphocytes.¹⁵ Other studies also showed an increase in the percentage of CD4+ T lymphocytes in HIV patients who were given a combination of NAC and sodium selenite at 1,800 mg/day. However, it was lower when compared to ARVs.³⁰ Other studies have shown that administration of NAC at a dose of 1 g/day for seven days can increase GSH levels in HIV patients who have received ARVs.¹¹

Although NAC administration in high doses appears beneficial, it can also cause side effects for HIV patients. Side effects include nausea, vomiting, indigestion, rashes, angioderma, bronchospasm, tachycardia, hypotension, or hypertension.³¹⁻³³ Some of the side effects of using NAC in high doses, similar to the side effects of the first use of ARV drugs. Therefore, it was feared that the use of NAC in high doses and ARV would worsen the side effects felt by HIV patients.

Conclusion:

Provision of NAC in patients with HIV / AIDS undergoing ARV treatment has been shown to reduce IL-10 levels significantly. Although a significant increase did not follow the decrease in IL-10 in the NAC group in the number of total lymphocytes, the number remained higher when compared to the group of patients given the placebo. This result shows that NAC administration can increase ARV treatment effectiveness in people with HIV.

Conflict of interest:

The author declares no financial or non-conflict of interest.

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