

# Differences of Homocysteine Levels in HIV Patients with CD4<200 Cells/ $\mu$ L and CD4>200 Cells/ $\mu$ L

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**Abstract:** HIV patients with CD4 counts <200 cells/ $\mu$ L had a greater risk of thrombosis than HIV patients with higher CD4 counts. A variety of coagulation disorders can predispose to hypercoagulability in HIV, including elevated homocysteine. Several studies have shown that homocysteine is increased in HIV-infected patients and is significantly associated with increased cardiovascular risk. This study aimed to determine the differences of homocysteine levels in HIV patients with CD4 <200 cells/ $\mu$ L and CD4  $\geq$ 200 cell/ $\mu$ L. This study was an observational analytic study, with cross-sectional method. The subjects of this study were HIV patients at Haji Adam Malik Medan General Hospital who had met the inclusion and exclusion criteria. Homocysteine levels were examined in HIV patients with CD4 <200 cells/ $\mu$ L and CD4  $\geq$ 200 cells /  $\mu$ L. From 30 samples, it was found that homocysteine levels were increased in HIV patients (median homocysteine = 20.67  $\mu$ mol/L). In this study, it was found that homocysteine levels in HIV patients with CD4<200 cells/ $\mu$ L were significantly higher than homocysteine levels in HIV patients with CD4  $\geq$ 200 cells/ $\mu$ L ( $p=0.019$ ). It was concluded that there is significant difference in homocysteine levels between HIV patients with CD4<200 cells/ $\mu$ L and HIV patients with CD4  $\geq$ 200 cells/ $\mu$ L.

**Keywords:** Homocysteine, CD4<200 Cells/ $\mu$ L, CD4>200 Cells/ $\mu$ L, HIV

## 1. Introduction

HIV (Human Immunodeficiency Virus) is a virus that is known as the cause of AIDS (Acquired Immune Deficiency Syndrome). HIV damages the body's immune system, thereby reducing the ability of people with this disease to defend them against other disease.<sup>1</sup>

CD4 lymphocytes play an important role in maintaining immune integrity. CD4 is also the main target of the HIV virus. Pathogenesis of HIV infection is mostly caused by a decrease in the number of CD4 lymphocytes, where the immunological status of HIV patients can be measured from CD4 values to classify the level of immunosuppression caused by HIV infection. WHO itself classifies the immunological status of HIV patients based on CD4 values. HIV patients with CD4 values of 350-499 cells/ $\mu$ L were categorized as mild immunosuppression, CD4 values of 200-349 cells/ $\mu$ L were categorized as moderately immunosuppression, and CD4 values <200 cells/ $\mu$ L were categorized as severe immunosuppression. A CD4 value <200 in the WHO classification indicates that there are clinical signs and symptoms of AIDS. Meanwhile, HIV patients with CD4 values <200 indicate the need for faster and more appropriate help.<sup>2,3</sup>

Various hematological manifestations can occur in HIV patients. Several clinical studies have reported a higher risk of thrombotic complications in HIV-infected patients. The risk of venous thrombotic events has been described as 6.5 times to 10 times greater in HIV patients than in the general population, and autopsy studies have demonstrated a high frequency of previously undiagnosed thromboembolism among patients with HIV-AIDS. HIV patients with CD4 counts <200 cells/L have a greater risk of thrombosis compared to HIV patients with higher CD4 counts. A variety of coagulation disorders may predispose to hypercoagulable states in HIV disease, including elevated homocysteine levels.<sup>4,5</sup>

Based on these data, this study aimed to determine the differences of homocysteine levels in HIV patients with CD4 <200 cells/ $\mu$ L and CD4  $\geq$ 200 cell/ $\mu$ L

## 2. Methods

This study is an observational analytic study, with a cross-sectional method. The subjects of this study were HIV patients at Haji Adam Malik General Hospital Medan from November 2019 until January 2020. Inclusion criteria in this study were HIV positive patients with CD4 <200 cells/ $\mu$ L and CD4  $\geq$ 200 cells/ $\mu$ L and willing to participate in the study. Exclusion criteria included HIV patients with pregnancy, pediatric patients, and patients with kidney disorders. The sample in this study was 15 people in each group.

The CD4 count was checked by flowcytometry method using BD FACS Calibur. Homocysteine examination was carried out by competitive immunoassay method using CMIA plasma technology in Abbott Architect Plus C1 4100. The data were analyzed using a computer statistical application. All statistical tests with p value < 0.05 were considered significant.

Ethical clearance was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Sumatera Utara University/Adam Malik General Hospital, Medan with number 116/TGL/KEPK FK USU-RSUP HAM/2019.

## 3. Results

From the 30 HIV patients who took part in the study, 26 of the total sample were men (86.7%) and the remaining 4 (13.3%) were women. Of all study participants, the mean age was 33.5 years old. (Table 1)

**Table 1:** Demographic characteristics of subject

| Variable             |                      |
|----------------------|----------------------|
| Gender               |                      |
| Male, <i>n</i> (%)   | 26 (86.7%)           |
| Female, <i>n</i> (%) | 4 (13.3%)            |
| Age (mean $\pm$ SD)  | 33.5 $\pm$ 9.6 tahun |

In the measurement, the median value of CD4 cell count in this study was 193 cells/ $\mu$ L with a minimum value was 5 cells/ $\mu$ L and a maximum value was 576 cells/ $\mu$ L. The median homocysteine level obtained from this study was 20.67  $\mu$ mol/L with a minimum value was 9.72  $\mu$ mol/L and a maximum value was 45.95  $\mu$ mol/L. (Table 2)

**Table 2:** CD4 Count and Homocysteine Levels in Subjects

| Variable                    | n  | Median | Min - Max    |
|-----------------------------|----|--------|--------------|
| CD4 (cells/ $\mu$ L)        | 30 | 193    | 5 - 576      |
| Homocysteine ( $\mu$ mol/L) | 30 | 20,67  | 9,72 – 45,95 |

**Table 3:** Homocysteine Levels based on CD4 Count

| Variabel                   | Mean $\pm$ SD               |                      | <i>p</i> |
|----------------------------|-----------------------------|----------------------|----------|
|                            | CD4 $\geq$ 200 sel/ $\mu$ L | CD4<200 sel/ $\mu$ L |          |
| Homosistein ( $\mu$ mol/L) | 18.11 $\pm$ 4.90            | 25.93 $\pm$ 10.8     | 0.019    |

Table 3 above shows that the average homocysteine level in the group of HIV patients with CD4  $\geq$ 200 cells/ $\mu$ L was 18.11  $\pm$  4.90 mol/L, while the average homocysteine level in the group of HIV patients with CD4 < 200 cells/ $\mu$ L was 25.93  $\pm$  10.8 mol/L. The T test showed that there is significant difference in homocysteine levels between HIV patients with CD4<200 cells/ $\mu$ L and HIV patients with CD4  $\geq$ 200 cells/ $\mu$ L ( $p=0.019$ ).

#### 4. Discussion

In this study, the median value of CD4 cell count was 193 cells/ $\mu$ L. The decrease in CD4 cell count in this study is consistent with a study by Damanik which showed that there was a decrease in CD4 cell count in HIV patients.<sup>6</sup> Another study by Khare et al also showed low CD4 cell count in HIV patients compared to normal controls significantly.<sup>7</sup>

CD4+ lymphocytes (T helper or Th cells) are the main targets of HIV infection because the virus has an affinity for the CD4 surface molecule. Various factors play a role in decreasing the number of CD4 lymphocyte cells in HIV patients. These factors are direct cytopathic effect of HIV on CD4 lymphocyte cells and their progenitors, induction of apoptosis through immune activation, stem cell destruction, cytokine cytotoxicity, destruction of lymphoid tissue including the thymus gland so that new cell production does not occur.<sup>8</sup>

In this study, the median value of homocysteine was 19.99 mol/L. These results are consistent with a study by Khare et al which compared 30 HIV patients and 30 control subjects in India. In this study, it was found that HIV homocysteine levels were significantly higher ( $p < 0.001$ ) in HIV patients (23.63  $\pm$  9.16 mol/L) compared to control subjects (9.23  $\pm$  3.26 mol/L).<sup>7</sup> Another study by Deminice et al also found that homocysteine levels were significantly higher in HIV patients than control subjects and it was found that the prevalence of hyperhomocysteinemia in HIV patients was 30.4%.<sup>9</sup>

Various pathophysiological processes may contribute to hyperhomocysteinemia in HIV patients, including the effect of HIV infection and its replication on host metabolism, the effect of antiretroviral drugs on homocysteine metabolism and other drugs administered to HIV-infected patients, HIV comorbidities, folate deficiency due to inadequate dietary intake; and/or vitamin B6 or B12 deficiency due to insufficient intake or altered metabolism.<sup>10,11</sup>

In this study, it was found that homocysteine levels in HIV patients with CD4 <200 cells/ $\mu$ L were significantly higher than homocysteine levels in HIV patients with CD4  $\geq$ 200 cells/ $\mu$ L ( $p = 0.019$ ). This is consistent with a study by Khare et al which found that homocysteine levels in HIV patients with CD4 <200 cells/ $\mu$ L (35.00  $\pm$  4.60 mol/ml) were significantly higher than homocysteine levels in HIV patients with CD4 200-500 cells/ $\mu$ L (23.07 $\pm$ 7.99 mol/ml) and HIV patients with CD4 >500 cells/ $\mu$ L (17.00 $\pm$ 5.68 mol/ml). Another study in 80 HIV patients in Portugal also found HIV patients with hyperhomocysteinemia had lower CD4 cell counts, although no significant difference was found.<sup>7,10</sup>

Increased homocysteine concentrations contribute to increased production of reactive oxygen species because the sulfhydryl groups of homocysteine are believed to act catalytically with cupric or ferric reactions to produce hydrogen peroxide and various homocysteine radicals. Thus, it can be assumed that the increase in circulating homocysteine concentrations in HIV patients may be one of several factors contributing to the increased production of reactive oxygen species, which may lead to stimulation of HIV replication through NF- $\kappa$ B activation. This causes the number of viruses to become very large and the number of CD4 T-cells to decrease.<sup>12,13</sup>

When the CD4 lymphocyte count is <200 cells/l or less, the opportunistic infection is often occur in HIV patients. Infection will trigger inflammatory signaling pathways.<sup>8,14</sup> In addition, when the reverse transcriptase enzyme of HIV converts viral RNA into DNA, IFT16 recognizes changes in body DNA so that it secretes IL-1 $\beta$  to activate the

inflammatory cascade and cause CD4 cells undergo pyroptosis which will lead to a decrease in CD4 cell count. In this inflammatory state, there is an increase in nitric oxide synthesis, which then results in hyperhomocysteinemia through binding to vitamin B12. In addition, hyperhomocysteinemia does not only result from inflammation, but vice versa, oxidative stress caused by hyperhomocysteinemia will again increase inflammation.<sup>15,16</sup>

## 5. Conclusion

This study shows that a homocystein level is increased in HIV patients. This study also shows that there is significant difference in homocysteine levels between HIV patients with CD4<200 cells/μl and HIV patients with CD4 ≥200 cells/μl.

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