



The Correlation of CD4+ T-Lymphocyte Count and Chemokine Ligand 13 Levels in Human Immunodeficiency Virus Patients Receiving Anti-retrovirus Therapy in Sanglah Central General Hospital

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Abstract

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BACKGROUND: Human immunodeficiency virus (HIV) infection is an infectious disease caused by the HIV-1 or HIV-2 virus. Cluster of differentiation 4+ (CD4+) T lymphocytes have an important role in the immune process. Chemokine Ligand 13 (CXCL13) is chemotactic for receptor-expressing cells (CXCR5), including B cells and follicular helper (Tfh) T cells. CXCL13 examination can be used to detect HIV antibodies and as a marker of disease progression and monitoring anti-retrovirus therapy (ART) in HIV-infected patients. Giving ART will reduce CXCL13 levels and show B cell activation, so CXCL13 can be used as a new marker of immune or antibody formation during acute or chronic HIV infection. CXCL13 examination can be used to detect HIV antibodies and as a marker of disease progression and monitoring ART in HIV-infected patients. Giving ART will reduce CXCL13 levels and show B cell activation, so CXCL13 can be used as a new marker of immune or antibody formation during acute or chronic HIV infection.

AIM: The aim of the study was to propose a study on the relationship between CD4+ T lymphocyte levels and CXCL13 levels in HIV patients who had received ART at the Sanglah Central General Hospital (RSUP), Denpasar. This study was start from March 2021 to August 2021 at the Sanglah Central General Hospital.

MATERIALS AND METHODS: This study used analytic observational study with a cross-sectional design, conducted at the Voluntary Counseling and Testing and the Clinical Pathology Laboratory at Sanglah Central General Hospital with 55 samples include in inclusion criteria.

RESULTS: In this study, the mean age of the research subjects was 42.18 ± 10.31 years with 58.2% male, 41.8% female having received ART with a mean of 63 months. The average number of CD4+ T lymphocytes was 451.53 ± 295.118 . Median CXCL13 level was 50,551. The correlation between CXCL13 levels and the number of CD4+ T lymphocytes was -0.209 . This correlation was not significant with $p = 0.127$ ($p < 0.05$), then partial correlation was performed. The partial correlation of CXCL13 levels and the number of CD4+ T lymphocytes was -0.308 , a negative direction indicating there was an inverse correlation. This correlation is a significant with $p = 0.023$ ($p < 0.05$) after partial correlation and is in the category of weak correlation.

CONCLUSIONS: There are a significant negative correlation after partial correlation of CXCL13 levels and the number of CD4+ T lymphocytes in HIV patients at Sanglah Hospital during the treatment phase.

Introduction

Human immunodeficiency virus (HIV) infection is an infectious disease caused by the HIV-1 or HIV-2 virus. The HIV virus mainly attacks the cluster of differentiation 4+ (CD4+) T lymphocyte cells which have an important role in the immune process, causing a progressive decrease in the immune response resulting in quite high mortality (Bowen *et al.*, 2016). According to the World Health Organization in 2018 reported, 37.9 million people were infected with HIV with 1.7 million new cases of infection. In Indonesia, an estimated 640,000 people suffer from HIV infection, with 46,000 new cases of infection, 0.4% aged between

15 and 49 years. The death rate from HIV/Acquired immune deficiency syndrome is around 38,000 [1].

In HIV-1 infection, there is a characteristic immunologic defect, namely, progressive humoral immune dysregulation, including changes in the frequency of specific B cells associated with viremia. Administration of therapy is important to improve B cell function in chronic infections. Chemokine Ligand 13 (CXCL13) is secreted by follicular dendritic cells (DCs) in response to the activation of lymphotoxin receptors, which are important chemokines in lymphoid tissue. CXCL13 is chemotactic for receptor-expressing cells (CXCR5), including B cells and follicular helper (Tfh) T cells. Elevated levels of CXCL13 were found in chronic HIV patients; however, with anti-retrovirus therapy

(ART), a decrease in CXCL13 levels was found due to inhibition of HIV virus replication [2], [3].

CXCL13 examination can be used to detect HIV antibodies and as a marker of disease progression and monitoring ART in HIV-infected patients. Giving ART will reduce CXCL13 levels and show B cell activation, so CXCL13 can be used as a new marker of immune or antibody formation during acute or chronic HIV infection [4], [5].

CD4+ T lymphocytes in lymph nodes are the main targets of HIV-1 infection. In patients with acute HIV infection, a decrease in CD4+ T lymphocyte cells was found because they were damaged by the HIV virus or because they were differentiated into Tfh by CXCL13 [6]. Furthermore, Tfh will interact with B cells in the GC to produce more CXCL13, so that in acute HIV infection, there is a decrease in CD4+ T lymphocyte cells accompanied by an increase in CXCL13 levels [7].

Based on the explanation above, there is a relationship between levels of CD4+ T lymphocyte cells and levels of CXCL13 in acutely infected HIV-infected patients, while ART will show the opposite situation. Several studies have studied the relationship between CD4+ and CXCL13 T lymphocyte cells in HIV, but no studies have found a relationship between CD4+ T lymphocyte levels and CXCL13 levels in patients who have received ART. This prompted the researcher to propose a study on the relationship between CD4+ T lymphocyte levels and CXCL13 levels in HIV patients who had received ART at the Sanglah Central General Hospital (RSUP), Denpasar. The functionality that CXCL13 has is very important. In HIV-positive patients, the secretion of the CXCL13 molecule was higher than in non-HIV patients. In patients with chronic HIV infection, there is an association between CXCL13 levels and changes in chemotactic B cell potential due to loss of circulating Tfh cells [8].

Physiological concentrations of CXCL13 are facilitated by the migration of B cells and T helper follicular lymphocytes to B cell follicles and the germinal center thereby ensuring correct B cell responses; changes in chemokine secretion trigger a deregulatory B cell response. Studies by Cohen describe the mechanisms by which HIV modifies homeostasis. CXCL13 production. Stimulation of mononuclear cells in peripheral blood cells with replicating HIV virus results in increased secretion of CXCL13. Furthermore, monocytes are the main population responsible for the production of CXCL13, but myeloid cells or plasmacytoid DCs produce these chemokines in small amounts [9].

Starting from these findings, there are mechanisms that can occur. Initiated by activation of two Toll Like Receptors (TLR) 7 and 8, which are located in the endosome compartment, has the ability to recognize HIV RNA sequences, induces

CXCL13 secretion from peripheral blood mononuclear cells. After combining TLR7 and TLR8 with their ligands, they produce a hormone that stimulates antiretroviral activity, interferon-alfa (IFN- α). Furthermore, if there is an inhibition of IFN- α function with specific antibodies, it will inhibit the production of CXCL13 [10].

The relationship between CXCL13 levels and CD4+ T cells was demonstrated by a study by Cagigi *et al.*, namely, the decrease in the number of CD4+ T cells in HIV infection will increase non-specific immune activation which is the main factor in increasing CXCL13 levels due to polyclonal stimulation of B cells [10].

In acute HIV infection, there is a decrease in CD4+ T lymphocytes accompanied by an increase in CXCL13 levels. This is in accordance with a longitudinal study conducted by Mehraj *et al.*, 2019 in Canada which showed a significant inverse relationship between levels of CD4+ T lymphocytes and CD4/CD8 ratio with CXCL13 levels in HIV-infected patients [7].

Materials and Methods

In this research used, an analytic observational study with a cross-sectional design conducted at the Voluntary Counseling and Testing (VCT) and the Clinical Pathology laboratory at Sanglah Central General Hospital. The allocation of research time carried out during March 2021 to August 2021. The study sample was all patients with HIV infection who were selected consecutively and met the inclusion criteria and exclusion criteria.

Inclusion criteria in this study are: HIV positive, Age >19 years, have received ART for at least 6 months and the exclusion criteria in this study are: Acute infection, autoimmune disease, tuberculosis infection, chronic obstructive pulmonary disease, diabetes mellitus, cancer or malignancy, and chronic kidney failure with total of sample in this research is 55 patients.

Research method examination used was 3 mL of venous blood which was accommodated in an ethylene diamine tetraacetic acid tube. CXCL13 examination with Sandwich ELISA method by Elabscience. The ELISA microplate in this test has been coated with human B lymphocyte chemoattractant specific antibody. ELISA quality assurance with precision and accuracy tests. The precision test is measured by the within run method, namely, by checking the sample 3 times simultaneously. The accuracy test is carried out using the spike and recovery method [11]. Examination for CD4+ with BD Fasclytic and quality control BD Fasclitics examination was according to the Standard Operating Procedure (SOP) with the BD CS&T Bead used on the BD flowcytometer [12].

Data analysis

The analysis and presentation of the data used are as follows:

1. All data obtained in this study were analyzed descriptively and the results are presented in the form of mean ± standard deviation (SD)
2. Test the normality of the data using the Kolmogorov–Smirnov test
3. Correlation analysis between the number of CD4+ T lymphocytes and CXCL13 using the Spearman correlation test
4. Multivariable test with linear regression was performed to assess the relationship between CD4+ T lymphocytes and CXCL13 after controlling for confounding variables analytically. Statistical significance was assessed based on 95% confident interval and p-value
5. The whole process of data analysis above uses the help of SPSS software for Windows version 24.0.

Table 2 shows the results of the examination of the CD4+ T lymphocyte count and CXCL13 levels in 55 HIV patients receiving ART. The number of CD4+ T lymphocyte cells of the research subjects obtained an average of 451.53 ± 295.118.

Levels CXCL13 in 55 subjects who had received ART with median 50,551. The lowest level CXCL13 is 5,582 and the highest level CXCL13 is 714,706.

Relationship between chemokine ligand 13 levels and Cluster of differentiation 4+ T-lymphocyte count in human immunodeficiency virus patients on anti-retrovirus therapy

The results of the Spearman correlation analysis test to determine the relationship between CXCL13 levels and the number of CD4+ T lymphocytes in HIV patients on ART are shown in Table 3.

Table 3: Correlation test results for chemokine ligand 13 levels and the number of cluster of differentiation 4+ T lymphocytes

Variable	Number of CD4+ T lymphocytes+		
	n	r	p*
CXCL13	55	-0.209	0.127

*Significant (p < 0.05). CXCL13: Chemokine ligand 13, CD4+: Cluster of differentiation 4+.

Results

Characteristics of research participants

Based on Table 1, the average age of the research subjects was 42.18 ± 10.31 years, with the youngest age being 19 years and the oldest being 59 years. In the research subjects with gender variable, 32 samples (58.2%) were male and 23 samples (41.8%) were female. The study subjects had received ART with an average of 63 months, with the shortest duration of ART use 6 months and the longest duration of ART using 96 months.

Table 1: Characteristics of research subjects

Variable	Results
Age (years) mean ± SD	42.18 ± 10.31
Gender, n (%)	
Male	32 (58.2)
Female	23 (41.8)
Duration ART (months) (minimum–maximum)	63 (6–96)

SD: Standard deviation, ART: Anti-retroviral therapy.

Cluster of differentiation 4+ T-lymphocyte count and chemokine ligand 13 levels in human immunodeficiency virus patients receiving anti-retrovirus therapy

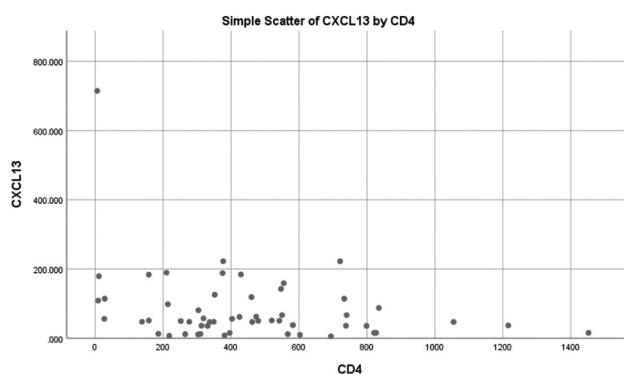
The CD4+ T lymphocyte count and CXCL13 levels in HIV patients receiving ART are shown in Table 2.

Table 2: Total cluster of differentiation 4+T lymphocytes and chemokine ligand 13 levels in research subjects result variable

Variable	Results
CD 4+ T lymphocytes (cell/mm ³) (mean ± SD)	451.53 ± 295.118
CXCL13 level (pg/mL) (median minimum–maximum)	50.551 (5.582–714.706)

SD: Standard deviation, CXCL13: Chemokine ligand 13, CD 4: Cluster of differentiation 4.

Table 3 shows the correlation between levels of CXCL13 and levels of CD4+ T lymphocytes of -0.209, with a correlation magnitude of 0.209 and a negative direction indicating that there is an inverse correlation where if there is an increase in levels of CD4+ T lymphocytes, then there is a decrease in levels of CXCL13 or vice versa. This relationship was not statistically significant with p = 0.127 (p < 0.05), then partial correlation was performed to control the control variance that caused it to be insignificant using a simple plot, to determine the distribution of the data.



The figure shows a distribution where the number of CD4+ T lymphocytes and CXCL13 levels is low (uneven distribution to the right). Therefore, the variable control is then carried out by means of partial correlation. From the results of data processing with partial correlation, the results are shown in Table 4.

Table 4 shows the results of the partial correlation of CXCL13 levels and the number of CD4+ T lymphocyte cells with the control variable for the duration of ART use, which was found to be -0.308, with a large

Table 4: Partial correlation of cluster of differentiation 4+T lymphocyte cell count and chemokine ligand 13 levels with the control variable for the duration of anti-retrovirus therapy use variable control

Variable control	CD4+ T lymphocyte cell count and CXCL13 levels		
	n	r	p*
Duration of ART	55	-0.308	0.023

*Significant (p < 0.05). CXCL13: Chemokine ligand 13, CD4+: Cluster of differentiation 4+, ART: Anti-retrovirus therapy.

correlation of 0.308 and a negative direction indicating that there is an inverse correlation where if there is an increase in the level of CD4+ T lymphocytes, then there is a decrease in CXCL13 levels or vice versa. This relationship was statistically significant with $p = 0.023$ ($p < 0.05$) after partial correlation and was included in the category of weak correlation.

Discussion

Research subjects with gender variable obtained as many as 32 samples (58.2%) were male and 23 samples (41.8%) were female. The tendency of exposure to risk factors or behavior is quite high in urban areas such as the behavior of sex worker customers, injecting drug users, tattoos, and others and this is because men are more likely to have risky sexual relations than women and men are more likely to use illegal drugs (syringes) than women [13].

Similar results were also obtained by Celikbas *et al.*, 2008, 72% of the sample were male. The study at VCT BRSU Tabanan also found 55.6% more males [14], [15] Kauffman *et al.*, 2005 also found more males with a frequency of 73.7%. Based on the New South Wales HIV Strategy (NSW HIV strategy) in 2016–2021, data obtained about 22 of the 40 new cases (55%) diagnosed were heterosexual men and 45% heterosexual women. About 36% of men diagnosed were born in Australia, 27% in Sub-Saharan Africa, and 9% in South-east Asia, South and Central Asia, Oceania, and Northern and Western Europe, respectively. From October to December 2020, it was obtained around 71% [16], [17]. Different things were found by Deyno *et al.*, 2018, data on HIV patients on ART were 62.4% female [18]. According to the research by Ebonyi *et al.*, 2014, there were data on the existence of a significant relationship between female sex with low CD4 levels, logistic regression analysis that was not adjusted for female sex factors had an OR of 1.95 for having low CD4 levels [19].

In this study, subjects had received ART with an average duration of use of 63 months, with the shortest duration of ART use 6 months and the longest duration of ART use being 96 months. Most of the subjects in this study had received ART for more than 2–5 years. This is in accordance with the research of Nurmayati *et al.*, 2019 findings in 30 patients showed that most

had been on treatment for 2–5 years with a percentage of 90% [20]. According to Deyno *et al.*, 2018, response to therapy in the early 1st week and 1st month is a strong predictor of the success of ART and is an important factor in improving immune conditions seen at the beginning of ART administration (18). In this study, 55 samples were obtained with the results of examining the number of CD4+ T lymphocytes in HIV patients who received ART for at least 6 months. The number of CD4+ T lymphocyte cells of the research subjects obtained an average of 451.53 ± 295.118 . With the cutoff value recommended by the previous studies of 73 cells/mm³, this study found an increase in the number of CD4+ T lymphocytes by 50 people (90.9%). This happened because the study sample had already received ART. This is in accordance with research by Susila Utama, 2016; Yogani *et al.*, 2015, that patients who have received ART for the first 6 months will experience an increase in the number of CD4+ lymphocytes [21], [22].

In this study, the examination of CXCL13 levels was carried out in HIV patients who had received antiretroviral therapy for at least 6 months. With the cutoff value recommended by several previous researchers, namely, 137 pg/mL, this study found a decrease in CXCL13 levels in 45 people (81.8%) while 10 people did not experience a decrease, this could be due to the long duration of ART use. This result was also conveyed by Mehraj *et al.*, 2019 that there were two study participants who did not experience a decrease in CXCL13 levels without normalization after long ART use [5]. Another study, according to Muema *et al.*, 2020, stated that long-term use of ART will cause cross reactive anti-HIV neutralizing antibodies in the GC, so that virus replication still occurs in the GC, despite viral suppression in the extra germinal area [23].

Based on a study by Feyissa *et al.*, 2021, CXCL13 levels decreased in HIV patients who had received ART starting from the first 6 months of the treatment after the first 24 months of treatment [7].

Relationship between CD4+ T-lymphocyte count and CXCL13 levels in human immunodeficiency virus patients receiving ART

CD4+ T lymphocytes are the main regulators of the immune response in the body. In patients with acute HIV infection, a decrease in CD4+ T lymphocyte cells was found because they were damaged by the HIV virus or because they were differentiated into Tfh by CXCL13. When the HIV virus infects CD4+ T-lymphocytes, there is a drastic decrease in the number of CD4+ T lymphocytes.

In HIV disease, an inverse correlation will be found between CXCL13 levels and the number of CD4+ T lymphocytes. In progressive HIV disease, a decrease in the number of CD4+ T lymphocytes occurs due to cell damage accompanied by an increase in CXCL13 levels

due to increased B lymphocyte cell activity. However, in HIV patients on ART, there will be a decrease in CXCL13 levels and an increase in the number of CD4+ T lymphocytes. A decrease in the number of CD4+ T cells in HIV infection will increase non-specific immune activation which is the main factor in increasing CXCL13 levels due to polyclonal stimulation of B cells. The opposite event will occur in patients receiving ART [4], [23].

The Spearman correlation test between CXCL13 levels and the number of CD4+ T lymphocytes is -0.209 , with a large correlation of 0.209 and a negative direction indicating that there is an inverse correlation where if there is an increase in the level of CD4+ T lymphocytes, then there is a decrease in CXCL13 levels or vice versa. This relationship was not statistically significant with $p = 0.127$ ($p < 0.05$), then a partial correlation with the control variable for the duration of ART use was found to be -0.308 , with a correlation magnitude of 0.308 and a negative direction indicating that there is an inverse correlation where there is an increase in lymphocyte cell levels. T CD4+, then there is a decrease in CXCL13 levels or vice versa. This relationship was statistically significant with $p = 0.023$ ($p < 0.05$) after partial correlation and was included in the category of weak correlation.

This is in accordance with a study conducted by Cohen *et al*, 2015, which found a significant inverse correlation in a study of 50 patients [9].

According to Fiyessa *et al*, 2021, CXCL13 levels will increase at the start of ART, but after more than 24 months of ART, CXCL13 levels do not increase or return to normal (7) According to Muema *et al*, 2020 stated that the use of long-term antiretroviral therapy will cause cross reactive anti-HIV neutralizing antibodies in the GC, so that virus replication still occurs in the GC, despite viral suppression in the extra germinal area [23]. This is consistent with the study, where the average study sample had received antiretroviral therapy for more than 24 months, so that the levels of CXCL13 obtained were low.

Research according to Anderson *et al*, 2020 stated that the anti-inflammatory effect during untreated HIV infection may be related to the defense of the immune response that controls later viral replication. CXCL13 had an inverse correlation with the number of CD4+ T lymphocytes, possibly due to increased migration of CD4+ T lymphocytes to lymphoid tissue and sites of inflammation ($r = -0.66$ and $p = 0.038$) [24]. This statement is also in accordance with the results of this study.

The advantage of this study is that several studies have studied the relationship between CD4+ and CXCL13 T lymphocyte cells in HIV, but no studies have found a relationship between CD4+ T lymphocyte levels and CXCL13 levels in patients who have received ART. Disadvantage of this study is that there is no

stratification long-term use ART for analysis increase CXCL13 level.

Conclusions and Suggestion

There is a significant negative correlation after partial correlation of CXCL13 levels and the number of CD4+ T lymphocytes in HIV patients at Sanglah Hospital during the treatment phase, as suggestion for the future studies should use the CXCL13 examination with stratification of the type of antiretroviral drug used in HIV patients.

Ethical clearance

This study has been agreed with ethical clearance number 1360/UN/14.2.2.VII.14/LT/2021 by Ethic Commission Medical Faculty, Udayana University, Indonesia.

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