ORIGINAL PAPER

Correlation between CD4 serum and interleukin-10 expression in placenta of HIV-positive pregnant women receiving combined antiretroviral therapy

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Abstract

Introduction: Human immunodeficiency virus (HIV) in pregnant women affects mothers' immune responses, with an increase in pro-inflammatory cytokines. Cluster of differentiation 4 (CD4) is one of the specific factors that can describe the immunity of HIV-infected patients. Low levels of interleukin (IL)-10 in placental serum disrupt prostaglandin balance, causing a massive inflammatory response. The aim of this study was to assess the correlation between CD4 serum and IL-10 expression in the placenta of HIV-infected pregnant women receiving combined antiretroviral therapy (ART).

Material and methods: This cross-sectional study used a quantitative analytic approach, and was conducted at Prof. Dr. I.G.N.G. Ngoerah General Hospital Denpasar from May 2022 to September 2022. In this study, full-term pregnant women (≥ 37-42 weeks of gestation) with HIV infection, who have been receiving ART for at least 6 months were enrolled. IL-10 level in placenta and CD4 count in plasma were assessed. Pearson's correlation test was applied, with a significant p-value < 0.01.

Results: The Pearson's correlation test results, CD4 levels, and H-score IL-10 were not influenced by the control variable. Mother's age with p = 0.000 (< 0.01) and r_{xy} value of 0.723 was classified as a strong correlation category, with coefficient of determination $r_{xy}^2 = 0.523$. Analysis with controls for age, duration of treatment, and HIV stage showed significant results (p = 0.000) in correlation between CD4 count and IL-10 expression, with $r_{xy} = 0.702$ and coefficient of determination $r_{xy}^2 = 0.493$.

Conclusions: CD4 serum levels and IL-10 expression in the placenta of HIV-positive pregnant women on combined antiretroviral therapy, demonstrated a significant relationship.

HIV AIDS Rev 2025; 24, 4: 297-301
DOI: https://doi.org/10.5114/hivar/177394

Key words: CD4, pregnant women, antiretroviral therapy, HIV infection, IL-10 expression.

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Article history:
Received: 31.08.2023
Revised: 27.11.2023
Accepted: 20.12.2023
Available online: 20.11.2025

International Journal of HIV-Related Problems
HIV & AIDS Review

Introduction

Human immunodeficiency virus (HIV) is a pandemic infection, which can be found in almost all countries in the world. In 2018, the Joint United Nations Program on HIV/AIDS (UNAIDS) reported that around 37.9 million people were infected with HIV worldwide, out of which, 18.8 million were women [1].

Especially in pregnancy, HIV infection not only impacts the health of mother, but also the health of fetus. HIV in pregnant women also affects the mother's immune response, with a decrease in pro-inflammatory and anti-inflammatory cytokines. If there is an excessive inflammatory response in the placenta, poor outcomes may occur in the fetus. HIV infection during pregnancy can have severe consequences for the fetus, including prematurity, low birth weight, low Apgar scores, and neuro-developmental difficulties [2, 3].

During pregnancy, an average patient experiences a decrease in immunoglobulin and complement levels (in early pregnancy), along with a significant reduction in immunity, especially T lymphocyte cells. HIV has a tropism for T lymphocyte cells with cluster of differentiation 4 (CD4). CD4 is a protein on the cell surface, the binding site for HIV to enter into T lymphocyte cells. Hence, CD4 T lymphocytes can be one of the specific factors, which can describe the immunity in HIV-infected patients [4, 5].

In Indonesia, various efforts have been made to reduce the rate of HIV transmission from mother to baby, one of which is administering antiretroviral therapy (ART) [6, 7]. Prescribing ART to women before and during pregnancy as well as throughout breastfeeding, can help prevent transmission of the disease from mother to baby, primarily due to ART treatment capability to reduce viral loads and increase mother's CD4 level [8]. However, the prescription of ART may not be able to eliminate the inflammatory response that occurs as a form of resistance to HIV infection in pregnant women [9, 10].

Disturbances in cytokine patterns and immunity levels during pregnancy are reflected in pathological processes of pregnancy. Low levels of interleukin (IL)-10 in placental serum interrupt the balance of prostaglandins, followed by an increase in IL-6, IL-8, tumor necrosis factor (TNF-α), and prostaglandin E2 (PGE-2), causing a massive inflammatory response. Consequently, the progressive inflammatory response affects the occurrence of spontaneous abortion and possibility of preterm delivery [11]. Previous research sought to link the effect of IL-10 expression, CD4 count, and HIV titer, showing a positive relationship between blood plasma IL-10 expression and HIV titer (viral load) [12].

Therefore, the aim of the present study was to determine the correlation between CD4 serum and IL-10 expression in the placenta of HIV-positive pregnant women receiving combined ART.

Material and methods

Study design

In this cross-sectional study, quantitative analytic approach was used. The research was conducted at Prof. Dr. I.G.N.G. Ngoerah General Hospital Denpasar, Bali, Indonesia, from May 2022 to September 2022. All pregnant women enrolled provided signed written informed consent. The study was approved by the Ethics Commission of the Faculty of Medicine, Udayana University, with registry number of 891/UN14.2.2.VII.14/LT/2022.

Sample population and data collection methods

Full-term HIV-positive pregnant women on ART were included in the study, and were examined for IL-10 level in the placenta and CD4 count in the plasma [13]. Inclusion criteria were pregnant women diagnosed with HIV, single fetuses alive intrauterine, having labor at gestational age of ≥ 37-42 weeks, and have been receiving ART according to national guidelines for at least 6 months. Exclusion criteria were pregnant women with intrauterine infections, pregnancy complications, such as gestational diabetes, preeclampsia, hypertension in pregnancy, intrauterine bleeding, and pregnant women with chronic or autoimmune diseases, including diabetes mellitus, hypertension, systemic lupus erythematosus, hyperthyroid/hypothyroid, heart disease, kidney failure, and malignancy. All women included in the study were selected as per a consecutive sampling method until the desired number was reached.

Pregnant women living with HIV are women with HIV infection, confirmed by clinical examination and standard serological tests, such as three serial strategy, using three methods with different sensitivity, specificity, and antigen preparation. Anti-HIV examination methods include a rapid method or rapid diagnostic test (RDT), enzyme-linked immunosorbent assay (ELISA) test, and Western blot analysis [7].

Gestational age had to meet the criteria for good dating, i.e., gestational age in weeks and days calculated as the difference between the first day of last menstrual period and the day of examination, and confirmed by ultrasound during the first trimester. A single fetus was also determined on the basis of ultrasound examination. Treatment of HIV infection with nucleoside reverse transcriptase inhibitor (NRTI) class ART included combination of three intraoral drugs, such as tenofovir (1 × 300 mg), lamivudine (1 × 300 mg), and efavirenz (1 × 600 mg) in the last 6 months. HIV infection stage was determined based on WHO criteria (stages, I-IV). IL-10 expression was measured using the immunohistochemistry test, where trophoblast cells expressing IL-10 were counted quantitatively with a semi-quantitative histology method (*H*-score). CD4 counts data were categorized into low (< 350 cells/mm^3) and high (≥ 350 cells/mm^3) levels.

An informed consent sheet to read and sign was provided to all pregnant women selected as the first research sample. On the anamnesis, physical and obstetric examinations were carried out. After the clinical examination, a sample of patient's blood from a peripheral vein of at least 3 ml was obtained. The aspirated blood was stored in a blood tube containing EDTA for CD4 hematological examination using a flow cytometry test, or CD4 data from the third trimester were employed. After delivery, either by spontaneous parturition or cesarean section, a sample of placental tissue was taken by making an incision in the central part of maternal pars, with a size of 3 cm × 3 cm. Samples were packed in special bottles and soaked in 10% formaldehyde liquid. Placenta samples was then sent to the Histology Laboratory of the Faculty of Medicine, Udayana University for IL-10 immunohistochemical examination.

Statistical analysis

Data from clinical examination and laboratory tests were collected and analyzed using SPSS software version 26 for Windows. Data were displayed using a scatter plot with a regression line (trend line) to graphically visualize the distribution of data and relationships. Monte Carlo method was applied for data normality, while a linearity test was also used. Statistical method of partial correlation (Pearson's) test was performed if between two variables was a normal distribution, whereas if one of them was not normally distributed, partial correlation (Spearman's rank) was applied. *P*-value < 0.01 was defined as a significant difference value. Independent and control variables, which were statistically correlated with a dependent variable in the bivariate correlation analysis were collectively analyzed further with multivariate analysis and logistic regression method, to assess variables that were statistically related after the adjusted control variables. Correlation strength was defined as weak if the interval coefficient was 0.00-0.29, moderate if it was 0.30-0.69, and strong if it was 0.70-1.00.

Results

The demographic data of the study subjects, including mother's age, HIV stage, and duration of treatment are listed in Table 1. Most of the study subjects were aged between 20 and 34 years (94%), and majority were classified as HIV I stage (65%), with the most treatment duration of more than 12 months (84%).

Based on the results from the Pearson's correlation test, CD4 levels and *H*-score IL-10 were not influenced by the control variable. Maternal age, with $p = 0.000$ (< 0.01) and $r_{xy} = 0.723$, was classified as a strong correlation (Figure 1). The coefficient of determination r_{xy}^2 was 0.523. If converted to a percentage, the effect of CD4 levels on the IL-10 *H*-score was 52.3%. A positive r_{xy} value indicated a raise in CD4 count, increasing the IL-10 *H*-score value. The relationship between CD4 and IL-10 level at the time of controlled pregnancy showed similar results ($p = 0.000$, $r_{xy} = 0.723$, $r_{xy}^2 = 0.523$), as demonstrated in Figure 2. The Pearson's correlation test for the correlation of CD4 and IL-10 levels with the duration of treatment control yielded $p = 0.000$ (< 0.01)

Table 1. Demographic data of subjects

Variable	*n* (%)
Age (years)	
< 20	1 (2)
20-34	46 (94)
> 34	2 (4)
HIV stage	
I	32 (65)
II	12 (24)
III	1 (2)
IV	4 (8)
Duration of treatment (months)	
6-12	8 (16)
> 12	41 (84)

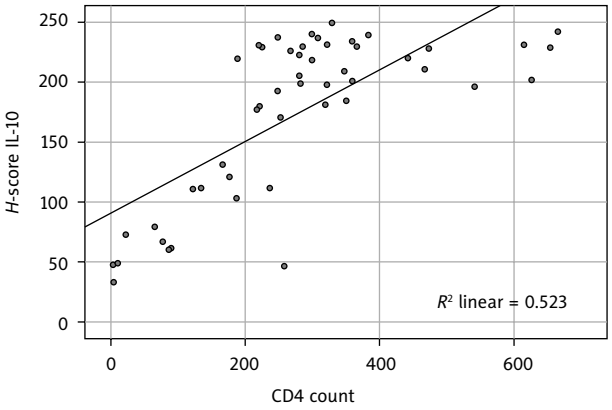

Figure 1. Correlation between CD4 count and IL-10

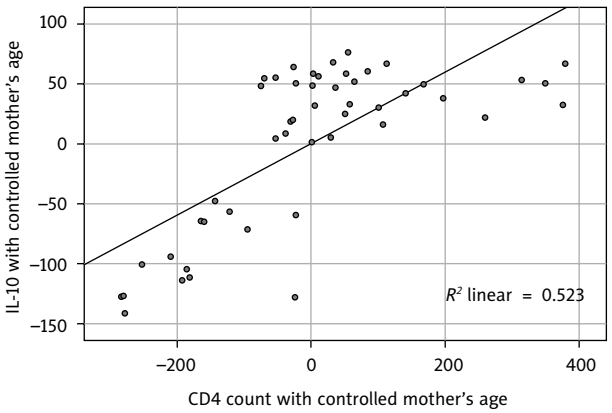

Figure 2. Correlation between CD4 and IL-10 with controlled age

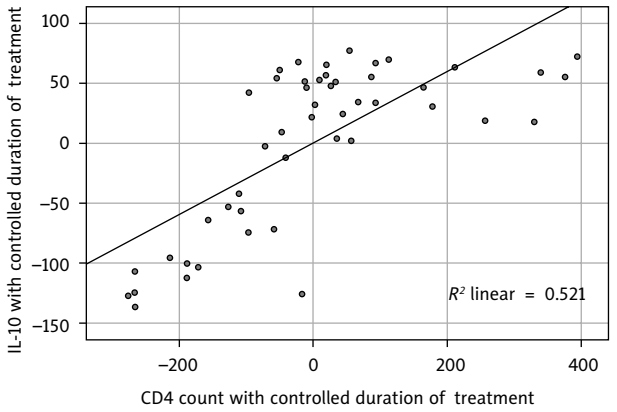

Figure 3. Correlation between CD4 and IL-10 with controlled duration of treatment

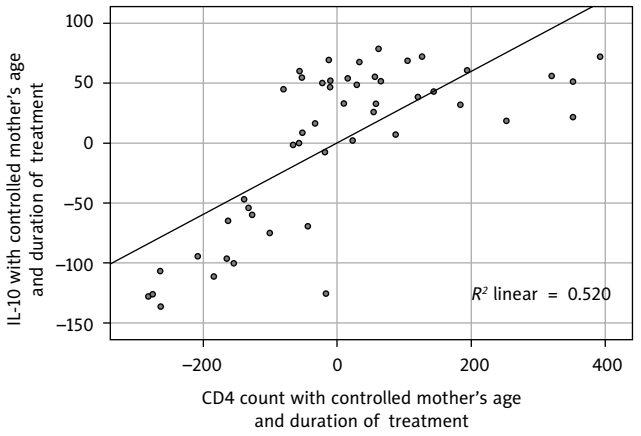

Figure 4. Correlation between CD4 and IL-10 with controlled age and duration of treatment

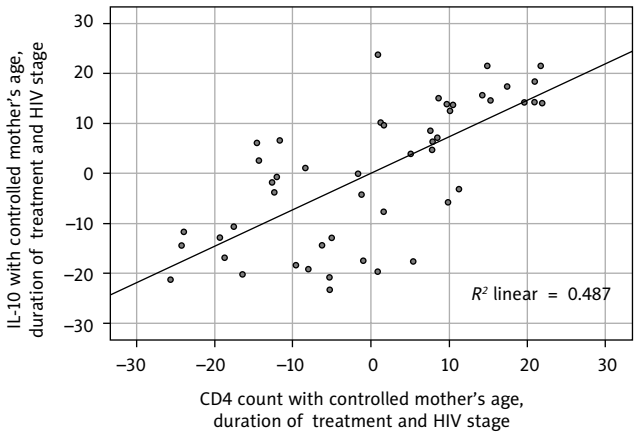

Figure 5. Correlation between CD4 and IL-10 with controlled age, duration of treatment, and HIV stage

and r_{xy} = 0.722. The coefficient of determination (r_{xy}^2) was 0.521, as presented in Figure 3. When controlling for maternal age and length of treatment simultaneously, the results of correlation between CD4 count and IL-10 remained significant (p = 0.000) (Figure 4). The final analysis using controls for maternal age, duration of treatment, and HIV stage revealed significant results (p = 0.000) in the correlation between CD4 count and IL-10 expression, with r_{xy} = 0.702 and coefficient of determination r_{xy}^2 = 0.493 (Figure 5).

Discussion

In the study, it was found that there was a strong relationship between CD4 and IL-10 levels, with a Pearson's correlation value of 0.723, of which 52.3% was caused by a direct relationship, while 47.7% was triggered by other influencing factors. Basically, CD4 count plays an important role in immune regulation, and in HIV infection, there is a decrease in CD4 T cells, expansion of CD8 T cells, and a shift from Th1 to Th2 immune responses. ART aims to suppress viral replication and restore the CD4/CD8 ratio as well as to improve Th1/Th2 balance. However, ART given during pregnancy can interfere with the pregnancy's natural immune program. A study conducted by Akoto *et al.* [14] reported that HIV-positive pregnant women had a higher IL-10 rates compared with pregnant women without HIV. This indicates a shift in the immune system in HIV-infected pregnant women compared with those without HIV. In a study conducted by Hygino *et al.* [15], a higher IL-10 production in HIV-1 infected pregnant women was mainly associated with CD4+ FOXP3 T cells. A genetic polymorphism in the promoter of the IL-10 gene that leads to decreased IL-10 expression has been related to a more rapid disease progression in late stages of HIV infection, suggesting that the anti-inflammatory effects of IL-10 may be protective in the setting of chronic immune activation.

In the current study, it was found that there was a significant positive correlation between CD4 levels and IL-10 expression in the placenta of HIV-infected women receiving ART, after controlling for age, length of treatment, and HIV stage, with a correlation value of 0.698. This shows that 48.7% had a direct relationship between CD4 levels and IL-10 expression in the placenta, while 51.3% was caused by the influence of other factors in HIV-positive pregnant women, who have been receiving ART for more than 6 months. This is contrary with a research by Mulyantari *et al.* [4], who assessed the expression of IL-10 in blood of HIV-positive non-pregnant women, and found that the higher the severity (stage) of HIV disease, the lower the CD4 count, followed by the increase in blood IL-10 levels. This may be due to differences in immunological characteristics between serum and placental IL-10 levels. However, the positive correlation in this study can be explained by the findings of Pornprasert *et al.* [16], where maternal CD4 serum levels were inversely related to placental TNF-α mRNA levels.

TNF-α is a pleiotropic cytokine that plays a role in inflammatory processes, initiates polymorphonuclear (PMN), and activates it, so that PMN can reach the site of infection. Blockade of TNF-α can increase IL-10 expression in human CD4+ count. An addition of TNF inhibitor drug, adalimu-

mab, to an anti-CD3-stimulated coculture of human CD4+ T cells/monocytes, led to an increase in the percentage of IL-10+ cells in the pro-inflammatory IL-17+, IFN-γ+, TNF-α+, GM-CSF+, and IL-4+ CD4+ T-cell sub-populations. In contrast, exogenous TNF-α greatly reduced the frequency of IL-10+ cells [17].

IL-10 is an important cytokine in the placenta in restraining the inflammatory process. Low IL-10 cytokine level causes many adverse pregnancy complications, such as spontaneous abortion, premature birth, and preeclampsia [11]. A study by Faye *et al*. [10] showed that IL-10 mRNA expression was found to be higher in the placenta of a group of HIV-infected women, who did not transmit the disease, compared with mothers, who were not infected with HIV. The expression level of IL-10 was also higher in HIV-1-infected women, who were not transmitting than in HIV-infected women, who could transmit the infection. This suggests that high levels of IL-10 in the placenta may contribute to a protective cytokine pattern in the placental environment. Therefore, a positive correlation between CD4 and IL-10 may have diagnostic value for predicting infant outcomes, but further research is needed to conform this finding.

Nonetheless, further statistical analysis has yet to be conducted to evaluate the relationship between research parameters. Considering the nuances included in the data, a more thorough investigation of statistical techniques is required. We suggest further research for evaluating confounding factors and relationships between variables.

Conclusions

Serum CD4 levels and expression of IL-10 in the placenta of HIV-positive pregnant women receiving combined ART had a significant relationship, and were categorized as having a strong correlation with a positive correlation when maternal age, duration of treatment, and HIV stage were controlled. Further research is needed to examine the cut-off relationship between IL-10 expression in the placenta and CD4 count in infant outcome, to ensure the diagnostic value of CD4 count as a predictor of infant outcome.

Disclosures

1. Institutional review board statement: The study was approved by the Ethics Commission of the Faculty of Medicine, Udayana University, with registry number of 891/UN14.2.2.VII.14/LT/2022.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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