



# Association of Urinary Interferon Gamma Protein-10 Levels and Low Levels of Cluster of Differentiation 4 Serum in Patients with Tuberculosis-Human Immunodeficiency Virus Coinfection

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## Abstract

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**BACKGROUND:** Tuberculosis (TB) infection caused by *Mycobacterium tuberculosis* was the most frequent opportunistic infection in human immunodeficiency virus (HIV) patients that lead to increasing of morbidity and mortality. Very low cluster of differentiation 4 (CD4) levels causing problem in TB/HIV diagnostic because unspecific clinical manifestation. Urine interferon gamma protein-10 (IP10) examination is a method that is relatively easier and safer to do, so it can be used as an alternative method in establishing the diagnosis TB/HIV.

**AIM:** The aim of this study was to investigate the association between urinary IP-10 and level of CD4 serum in patients with TB/HIV coinfection.

**MATERIALS AND METHODS:** This study was conducted at the Department of Internal Medicine, Faculty of Medicine, Dr. M. Djamil Hospital, Padang, Indonesia, involved 30 patients with active TB/HIV coinfection whose urine and blood serum were collected to evaluate IP-10 and CD4 level.

**RESULTS:** There is a significant relationship between urine IP10 levels as a biomarker for the diagnosis of active pulmonary TB in HIV patients and serum CD4 levels in TB-HIV coinfecting patients with moderate correlation strength.

**CONCLUSION:** Further research is needed with a larger sample size to see the effect of low serum CD4 levels on the sensitivity of urine IP10 diagnosis.

## Background

Human immunodeficiency virus (HIV) pandemic is increasing worldwide, including Indonesia. Tuberculosis (TB) infection caused by *Mycobacterium tuberculosis* (MTB) was the most frequent opportunistic infection in HIV patients that lead to increasing of morbidity and mortality. Based on the WHO Global TB Report 2019, there were 37% of TB cases in HIV positive in Indonesia which become the most common cause of death in HIV/acquired immunodeficiency syndrome (AIDS) patients [1], [2], [3].

People with HIV/AIDS often come with very low immune response, marked by very low cluster of differentiation 4 (CD4) levels, especially in TB/HIV coinfection. This condition causing problem in TB/HIV diagnostic because unspecific clinical presentation and examination compared to patient TB without HIV. Nowadays, various methods of examination including urine samples examination have been widely used in diagnosing TB because it is relatively easy to collect, fast, and safer among health workers [3], [4], [5].

Interferon gamma protein-10 (IP-10) or also known as CXC-motif chemokine-10 (CXCL-10) is a

chemokine secreted by monocytes, endothelial cells, and fibroblast through stimulation of interferon (IFN) types 1 and 2 due to HIV infection. Increased levels of IP-10 in serum or urine can be a marker of TB coinfection in HIV patients. Urine IP10 examination is a method that is relatively easier and safer to do, so it can be used as an alternative method in establishing the diagnosis of TB in HIV other than AFB examination and sputum culture [6], [7], [8]. Study by Petrone *et al.* suggests that IP-10 blood and urine allow to assess the state of immune activation in TB/HIV patients in adult and children [9], [10], however, studies that shown how the relationship between urine IP10 levels and blood CD4 levels of HIV patients have not been found, therefore, this becomes novelty of this study. This study is expected to evaluate the association between urine IP-10 levels and serum CD4 levels of TB patients with HIV (TB-HIV coinfection).

## Methods

### Subject criteria

This study is a cross-sectional study involved 30 patients diagnosed as active TB with HIV who

underwent diagnostic and therapeutic measures in outpatient treatment in voluntary counseling and testing (VCT) Outpatient Department, Dr. M. Djamil Hospital, Padang. The inclusion criteria are all HIV patients with active TB coinfection, aged between 18 and 65 years old, and approved to participate in research by signing informed consent. Exclusion criteria were patients with chronic hepatitis and kidney disease, patients with malignancy, and patients with acute infectious diseases. The ethics committee approved this study of the Faculty of Medicine of Universitas Andalas with registration number 194/KEP/FK/2016.

### IP-10 urine and CD4 serum measurement

Urinary and blood serum of patients with TB/HIV were collected using to measure IP-10 urine and CD4 serum of samples. IP-10 urine were measured using Human CXCL10/IP10 ELISA Cat.#RAB0119-1KT (Sigma-Aldrich) for urinary examination. Low number of CD4 (<50%) was carried out using BD fluorescent-activated cell sorter (FACS) count system using FACS method. Bivariate analysis of urine IP-10 levels and CD4 levels was analysis using computerized statistical analysis program, SPSS 21 for Mac.

## Results

From 30 samples taken from the VCT Polyclinic of Dr. M. Djamil Padang Hospital, 26 people with HIV were male (86.7%) and 4 women (13.3%). Age range between 21 and 30 years of the many suffering from HIV with a percentage of 36.7% with a mean age of  $35.4 \pm 8.3$  years. Characteristics of the study are shown in Table 1.

**Table 1: Baseline characteristic of study**

Characteristic	n = 30	%
Age (years old), Mean $\pm$ SD		
21–30	11	36.7
31–40	9	30
>40	10	33.3
Sex		
Men	26	86.7
Women	4	13.3

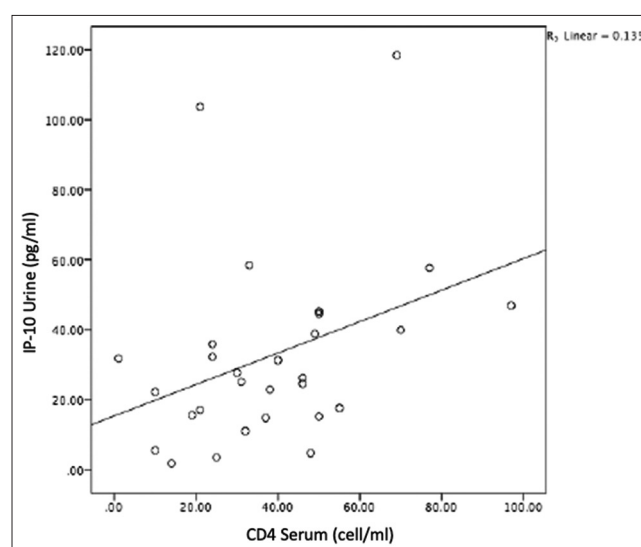
The mean serum levels of CD4 coinfecting patients with TB-HIV research are obtained of  $38.9 \pm 21.47$  cells/ $\mu$ L, which show that endurance is very low, with 83% of patients had <50 CD4 cells/ $\mu$ L. The mean level of IP10 urine obtained  $32.9 \pm 26.2$  pg/mL higher than normal levels in healthy controls is 17 pg/mL. Age is still proportional and there is no significant difference. This is in accordance with the 2014 report of the Directorate General of Communicable Disease Eradication and Environmental Health, Ministry of Health of the Republic of Indonesia, which stated that the highest number of HIV/AIDS sufferers was in the productive age group 54.7% (age group of

**Table 2: Mean of urinary IP-10 levels and CD4 serum in TB/HIV coinfection**

Variable	n = 30	Median (Min-Max)
IP-10 urine (pg/ml)		
Mean $\pm$ SD	$32.9 \pm 26.2$	27 (18–118.4)
CD4 (sel/ml), Mean $\pm$ SD	$38.9 \pm 21.5$	
<50	25 (83.3%)	
50–100	5 (16.7%)	

CD4: Cluster of differentiation 4, IP-10: Interferon gamma protein-10, TB: Tuberculosis, HIV: Human immunodeficiency virus.

21–30 years old). The mean serum CD4 level of the patient HIV with TB was  $38.9 \pm 21.47$  cells/ $\mu$ L, which shown very low levels of CD4 (<50 cells/mL), as shown in Table 2. Results of bivariate analysis using Spearman correlation obtained  $p = 0.015$  ( $p < 0.05$ ) with Spearman's  $r = 0.440$ , which shown a significant positive association between urinary IP-10 levels and serum CD4 levels with moderate correlation strength. The correlation between urine IP10 levels with CD4 levels is shown in Figure 1 below



**Figure 1: Correlation of urinary interferon gamma protein-10 and cluster of differentiation 4 serum in tuberculosis/human immunodeficiency virus coinfection**

## Discussion

This study obtained data that men suffer from HIV more than women. This is in accordance with the WHO epidemiological data in 2010 which estimated that 80% of the 47,500 new HIV cases were men (approximately 38,000 cases). The most common age who experienced HIV in the study was the age range between 21 and 30 years, but the difference between the ranges is a patient with HIV/AIDS Stage 4 who has a very low immune system. Cahyadi's 2014 study in Aceh showed that 66% of TB-HIV coinfecting patients had CD4 cells below 49 cells/mL. The condition of decreased CD4 levels is one indication of adequate

anti-TB drugs, but the difficulty of diagnosing TB in HIV patients is a challenge in the treatment of TB coinfection. Clinical symptoms that are no longer specific and sputum examinations that often show negative results are obstacles in starting anti-TB therapy, in addition to the possibility of drug allergies, worsening prognosis, and interactions of TB drugs with antiretrovirals (ARVs) [11], [12].

The urine IP-10 level in this study was obtained to increase. Once HIV invades human body, it affects IFN regulatory factor that enhanced stimulation of chemokine factor including CXCL-10. IP-10 reported to be produced at high levels in HIV with coinfection such as TB, hepatitis C virus, or cryptosporidiosis compared to patient with HIV monoinfection and TB without HIV. The exact mechanisms in TB with HIV, increased of IP-10, can be explained by synergic effect of IFN- $\gamma$  that primarily induced production of IP-10 that occurs in HIV infection. As in HIV infection, production of IFN was higher compared to non-HIV, thus regulation of innate and adaptive immune response through IFN-induced-T cell a chemoattractant was also increased. IFN- $\gamma$  also interacts with other cytokines such as TNF- $\alpha$  and platelet-derived growth factor regulated by activation of STAT-1 and NF $\kappa$ B. Examination of urine IP10 as one of the markers of inflammation has been widely used in determining the evaluation of treatment and prognosis of patients with TB. Elevated urine IP10 levels were found in pulmonary TB from other lung infections, compared to other inflammatory markers such as IL-8, RANTES, MIG, and MCP-1 [13], [14], [15].

Correlation analysis between serum CD4 levels and urine IP10 using Spearman correlation found a significant relationship with moderate correlation strength ( $p < 0.05$ ,  $r = 0.44$ ). Low level of CD4 serum may also cause by other factor such as low adherence of ARV treatment or asymptomatic infection in patient caused by already low immune system. This shows that the low level of serum CD4 cell count in TB-HIV coinfecting patients is associated with increased urine IP10 levels. Contreas *et al.* study, 2014, also showed that elevated IP10 levels in HIV patients were associated with disease progression, activation of other chronic inflammations, and HIV infectious replication which further aggravates the activation of MTB bacteria [15], [16].

The limitation in this study was that extrapulmonary TB was not excluded in this study because the symptoms were not specific to HIV patients and often resembled the clinical symptoms of HIV itself.

## Conclusion

There is a significant relationship between urine IP10 levels as a biomarker for the diagnosis of

active pulmonary TB in HIV patients and serum CD4 levels in TB-HIV coinfecting patients with moderate correlation strength. Further research is needed with a larger sample size to see the effect of low serum CD4 levels on the sensitivity of urine IP10 diagnosis.

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