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The Role of Chemokine Family, CXCL-10 Urine as a Marker Diagnosis of Active Lung Tuberculosis in HIV/AIDS Patients

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***Abstract*—**Human Immunodeficiency Virus (HIV) pandemic increased significantly worldwide. The rise in cases of HIV/AIDS was also followed by an increase in the incidence of opportunistic infection, with tuberculosis being the most opportunistic infection found in HIV/AIDS and the main cause of mortality in HIV/AIDS patients. Diagnosis of tuberculosis in HIV/AIDS patients is often difficult because of the uncommon symptom in HIV/AIDS patients compared to those without the disease. Thus, diagnostic tools are required that are more effective and efficient to diagnose tuberculosis in HIV/AIDS. CXCL-10/IP-10 is a chemokine that binds to the CXCR3 receptor found in HIV/AIDS patients with a weakened immune system. Tuberculosis infection in HIV/AIDS activates chemokine IP-10 in urine, which is used as a marker for diagnosis of infection. The aim of this study was to prove whether IP-10 urine can be a biomarker diagnosis of active lung tuberculosis in HIV-AIDS patients. Design of this study is a cross sectional study involving HIV/AIDS patients with lung tuberculosis as the subject of this study. Forty-seven HIV/AIDS patients with tuberculosis based on clinical and biochemical laboratory were asked to collect urine samples and IP-10/CXCL-10 urine being measured using ELISA method with 18 healthy human urine samples as control. Forty-seven patients diagnosed as HIV/AIDS were included as a subject of this study. HIV/AIDS were more common in male than in women with the percentage in male 85.1% vs. 14.5% of women. In this study, most diagnosed patients were aged 31-40 years old, followed by those 21-30 years, and > 40 years old, with one case diagnosed at age less than 20 years of age. From the result of the urine IP-10 using ELISA method, there was significant increase of the mean value of IP-10 urine in patients with TB-HIV/AIDS co-infection compared to the healthy control with mean 61.05 pg/mL ± 78.01 pg/mL vs. mean 17.2 pg/mL. Based on this research, there was significant increase of urine IP-10/CXCL-10 in active lung tuberculosis with HIV/AIDS compared to the healthy control. From this finding, it is necessary to conduct further research into whether urine IP-10/CXCL-10 plays a significant role in TB-HIV/AIDS co-infection, which can also be used as a biomarker in the early diagnosis of TB-HIV.

***Keywords*—**Chemokine, IP-10 urine, HIV/AIDS, Tuberculosis.

I. Introduction

HIV is a major health problem that affects millions of people worldwide. The Joint United Nations Program on HIV/AIDS reports that approximately 78 million people around the world have been infected by HIV, although the number of newly diagnosed HIV/AIDS continues to fall [1]; but in some countries, such as Indonesia, it continues to grow rapidly [2]. The rapid epidemic growth of HIV is also followed by the rise of untreatable and undiagnosed tuberculosis co-infection. Tuberculosis (TB) is the most frequent opportunistic infection found in HIV/AIDS patients. World Health Organization data in 2012, shown that there were 8.6 million newly recorded TB patients, with 13% being HIV/AIDS patients [3]. Indonesia is among the 10 largest countries with highest number of TB-HIV co-infection cases.

In individuals with HIV, the presence of opportunistic infections such as tuberculosis can make the virus growth more rapidly, so that disease progression become more severe, marked by the decline of CD4 (Cluster of Differentiation) serum [4]. The weakening of the immune system makes diagnosis of tuberculosis co-infection in HIV patients, a great challenge. Radiological TB in patients with HIV/AIDS is not typical as it is in those who are not suffering from HIV. Similarly, sputum examination was greatly influenced by the degree of immunodeficiency, which is often negative in HIV/AIDS patients, while sputum culture (gold standard of TB) took about three months to determine a TB infection [5]. New modalities that are more efficient and easy to collect are required to diagnose TB-HIV.

Interferon-γ-inducible Protein 10 (IP10), also known as CXCL10 (Chemokine CXC-10), is a member of family CXC-chemokine that is present in patients with co-infection TB-HIV. IP10 was thought to be involved in the activation of monocytes and Th1 cells into inflamed foci tuberculosis [6]. Serum and pleural IP10 (CXCL10) were already in use as a biomarker in TB treatment evaluation and disease progression. The IP10 (CXCL10) urine examinations method has several benefits, such as being easier to collect by health-care workers and minimal bacterial contamination, in diagnostic TB-HIV co-infection [7]. The aim of this study is to evaluate if IP10 (CXCL10) urine can be a useful biomarker for diagnosing active tuberculosis in HIV/AIDS patients.

II. Material and Methods

A. Subjects

Forty-four (44) HIV patients with suspected tuberculosis were recruited from VCT (Voluntary Counseling and Testing) Polyclinic in M. Djamil Hospital, Padang, Indonesia based on

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clinical and laboratory diagnosis of HIV. Age and gender were noted as the baseline characteristics of this study.

B. IP10 (CXCL10) ELISA and Sputum Culture

Urine and sputum samples were taken from subject of study to examine IP10 urine using ELISA method and sputum were cultured using Lowenstein-Jensen medium in the Microbiology Department in Medical Faculty of Andalas University. IP10 urine analysis using Quantikinine Bio-Rad IP10 (CXCL10) with urine samples (for research only). Eighteen (18) healthy controls were determined to measure normal levels of IP10 urine (cut-off IP10 urine: 17 pg/mL).

C. Statistical Analysis

All data were analyzed using statistical package for social science (SPSS 21.0) for Windows program on the computer. All data were given as mean ± standard deviation (SD). Diagnostic test analysis assessed by determined sensitivity, specificity, positive and negative predictive value, and accuration of IP10 urine compared to the gold standard (sputum culture).

III. Result

Tuberculosis infection in HIV patients could worsen disease prognosis; therefore, efficient and non-invasive diagnostic tools are required in TB-HIV co-infection. In this research, CXCL10 (IP10) urine, a chemokine produced by chemokine CXC receptor 3 and CXC receptor 4 by stimulation of interferon gamma, were evaluated to see whether it can be used as a biomarker diagnostic in TB-HIV co-infection.

This study involved 44 HIV patients with suspected active lung tuberculosis in M. Djamil Hospital, Padang, West Sumatra, Indonesia. In this study, patients with co-infection TB-HIV are more common in men (84.1%) than women (15.9%). Patients aged 31-40 years old were found to have the highest rate of TB-HIV co-infection, followed by those aged 21-30 years and 41-50 years old at 38.6% and 29.5%, respectively; and only one case was recorded in a patient aged less than 20 years old. Cluster of differentiation (CD4) on this research were varied between 1 – 664 cell/µl, with CD4 less than 50 cell/µl found mostly (70%); which shown that TB-HIV co-infection patients comes with extremely low CD4. The baseline characteristic of this study can be seen in Table I.

IP10 urine levels of TB-HIV patients were obtained between 2.26 pg/mL until 420,11pg/mL, with mean of IP10 urine 65.23 ± 11.92 pg/mL. The mean of IP10 urine in TB-HIV patients increased from the normal levels of IP10 urine found in the 18 healthy controls (mean of healthy controls 17 pg/mL), which is used as the cut-off level on this study. As a gold standard examination in this study, we are using sputum culture examination with Lowenstein-Jensen medium. Based on sputum culture, we found 45% positive culture and 55% negative culture (Fig. 1). IP10 urine shown that 80% subjects were positive results and 20% were negative (Fig. 2).

TABLE I
Baseline Characteristic of Study

Characteristic	Sample (n = 44)
Age (years old)	34.70 ± 7.98
Minimum	18
Maximum	49
Median	34
Gender	
Men	37 (84.1%)
Women	7 (15.9%)
CD4 (cell/µl)	
< 50	30 (68.2%)
50 -100	5 (11.4%)
100-150	1 (2.3%)
150-200	2 (4.5%)
> 200	6 (13.6%)

TABLE II
Result of IP10 Urine in TB-HIV Patients

Variable	Mean	SD	Min	Max	Med	Healthy control Mean
IP-10 (pg/mL)	65.23	11.92	2.26	420.11	37.39	17 pg/mL

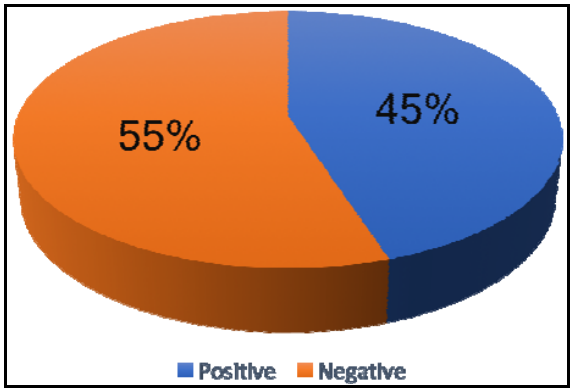

Fig. 1 Proportion of Sputum Culture in Detecting *M. tuberculosis*

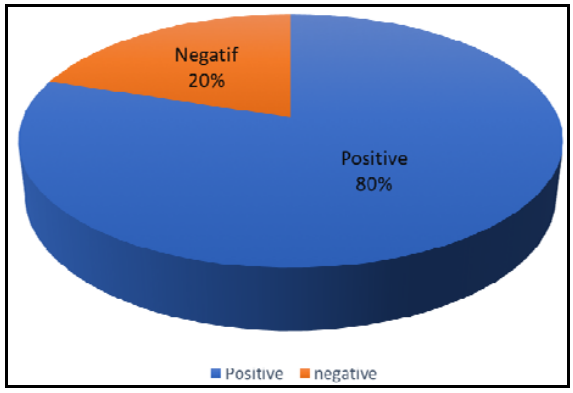

Fig. 2 Proportion of IP10 Urine in Detecting M. tuberculosis

Diagnostic test analysis conducted by assessing sensitivity, specificity, positive and negative predictive value, and accuration of IP10 urine compare to gold standard. In this study, the IP10 urine sensitivity was 95%, specificity 32%, and the positive and negative predictive value were 51% and 89%, respectively, and accuration was 60%. IP10 urine was shown to have higher sensitivity than the gold standard (95%) but lower specificity (32%). Diagnostic test results of IP10

urine and culture sputum with Lowenstein-Jensen can be seen in Table III.

TABLE III
DIAGNOSTIC TEST ANALYSIS OF IP10 URINE

		Culture M.tb		Total
		Positive	Negative	
IP-10 Urine	Positive	18 (94.7%)	17 (68%)	35 (100%)
	Negative	1 (5.3%)	8 (32%)	9 (100%)
Total		19 (100%)	25 (100%)	44 (100%)

IV. DISCUSSION

Human Immunodeficiency Virus (HIV) is an immunodeficiency disease which the incidence tends to increase annually worldwide. The increasing incidence rate of HIV was also followed by opportunistic infection such as tuberculosis [8]. In developing countries, tuberculosis remains a major challenge, especially tuberculosis with HIV, where symptoms, laboratory results and radiographic examination are often not specific, thus, making diagnosis more difficult than in common tuberculosis [9].

Sputum examination through Acid-fast bacilli (AFB) sputum and Lowenstein-Jensen method was one of method to diagnose tuberculosis in general populations, while patients with TB-HIV often shown negative results. Other method such as pleura fluid or gastric fluid lavage examination was difficult to do because patients with TB-HIV co-infection often comes to healthcare facilities with extremely low immune system. The latest research through urine examination has become one of the easier options to examine, because it is non-invasive, and safer for the healthcare professional [10].

Interferon-gamma-protein-10 (IP-10) is a family of CXC chemokines that are stimulated by the formation of interferon in HIV infection. The decline of immunity in HIV patients activated Mycobacterium tuberculosis (M.tb), which then stimulated the release of chemokine CXC receptor-3 (CXCR3) and chemokine CXC receptor-4 (CXCR4), and the stimulation of interferon gamma interferon to form protein-10 (IP-10) or called also CXCL-10 (Chemokines CXC-10) [11]. The discovery of IP-10 urine levels exceeding normal levels can be a biomarker for the occurrence of tuberculosis infection in people diagnosed with HIV.

In this study, the tuberculosis diagnostic gold standard, sputum culture is also a routine examination in the diagnosis of HIV with suspected pulmonary TB, and IP-10 urine as a new diagnostic tool. Samples with positive TB in sputum culture were obtained for 45%, and with IP-10 urine, it was 80%. From both diagnostic tools, the sputum culture has fewer positive results than IP-10 urine. Reference [12] also shows that sputum cultures have lower positive results, even though the sputum culture is the gold standard for the diagnosis of pulmonary tuberculosis in HIV, for which examinations take longer, generally about 3 months, and thus, is a weak examination method.

IP-10 urine has sensitivity and specificity higher than sputum culture examination in this study, where IP-10 had a sensitivity of 95% and specificity of 32%. Reference [13] shows that IP-10 urine has a higher sensitivity in patients with tuberculosis with HIV, so the IP-10 urine can be used as a biomarker of potential inflammatory, even more easily applied in the clinical comparison examination of blood samples such as C-reactive protein (CRP) and erythrocyte sediment rate (ESR). IP-10 urine examination also detected active pulmonary tuberculosis patients compared to healthy controls [14]. These results differ from the results of examination of other inflammatory markers such as IFN-gamma, TNF-a, IL-2, IL-8, MIP-1a, MIP-1b and RANTES, which is undetectable in urine tuberculosis-HIV patients. However, low specificity of the IP-10 urine in this study, indicating that IP-10 urine is not specific only to tuberculosis, but also can be found in the condition of chronic inflammation such as infection with the hepatitis C virus (HCV), which also recognized as an opportunistic infection in HIV. IP10 urine also shows effective results not only in adults but also in children; therefore, the early diagnosis of tuberculosis is possible. As many as 70% of the subjects in this study had a CD4 count below 50 cell/μl, which shows that the immunity of patients co-infected TB-HIV is extremely low, and thus, it is important to obtain early diagnosis and treatment in TB-HIV co-infection to improve the prognosis and survival rate of patients with HIV/AIDS.

V. CONCLUSION

IP-10 urine examination has a higher sensitivity compared to the gold standard examination, sputum culture, so that the IP-10 urine examination can be used as alternative diagnostic biomarkers in pulmonary tuberculosis patients with HIV, especially for people with very low immunity. Further research is required to look at the effect of IP-10 urine on the effectiveness of treatment and prognosis of TB-HIV co-infection patients.

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