Original Article

The Difference in Serum Levels of IP-10 in Pulmonary Tuberculosis Patients with Positive AFB and AFB Conversion

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Abstract

Background: Tuberculosis (TB) is caused by Mycobacterium tuberculosis, which most commonly infects the lungs. Diagnostic modalities are required in smear-negative TB. IP-10 is a potent chemokine for detecting the presence of TB infection. This study aimed to determine the difference in serum IP-10 levels in patients with smear-positive pulmonary TB and pulmonary TB patients with AFB conversion after two months of therapy.

Methods: This was an analytic observational study with a cross-sectional approach. Sampling was carried out by consecutive sampling methods. AFB examination was performed using Ziehl-Neelsen staining, and serum IP-10 was measured using ELISA.

Results: The study results obtained mean IP-10 levels in TB patients with smear positive and AFB conversion after two months of treatment of 459 pg/mL and 204.4 pg/mL, respectively. Statistical analysis using independent t-test received *P*<0.0001. The optimal cut-off value was 306,1 pg/ml (90% sensitivity; 95% specificity; area under the curve: 0.948, 95% CI=0.88-1; *P*=0.0001).

Conclusion: There was a significant difference between IP-10 levels in TB patients with smear positive and AFB conversion.

Keywords: AFB, IP-10, Pulmonary tuberculosis

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INTRODUCTION

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis.[1] As many as 1.5 million people die from TB every year. This makes TB an infectious disease with a very high mortality rate. The World Health Organization (WHO) 2019 reports concluded that Indonesia was in the third highest position in the world for the number of TB cases after India and China.[2]

Indonesia is a country with a large private health sector that is not very well connected to the NTP (National Tuberculosis-control Program) reporting network. In 2016, about only 360,565 TB cases were reported to national level authorities, while the estimated TB incidence was 1,020,000. The Minister of Health Decree, which came into effect in Indonesia in 2017, established TB notification a mandatory nationwide. A national TB inventory study was carried out in 2017 under the NTP and the Indonesian National Institute of Health Research and Development. The aim was to directly measure the rate of reported TB cases detected in the national TB surveillance system (SITT, Integrated Tuberculosis Information System) managed by the NTP. It was observed that the annual incidence rate for 2017 was estimated at 319 per 100,000 population per year.[3]

In clinical application, the TB diagnosis process encountered various difficulties and obstacles where the findings of TB cases with sputum smear examination were only 44% in adult cases and 15–20% in children. The gold standard examination for the diagnosis of TB is to detect the presence of Mycobacterium tuberculosis from culture examination, but it practically takes a longer time that prolongs the diagnosis process. The decision to diagnose and manage TB has become more difficult if the sputum AFB (acid fast bacilli) examination shows a negative result, especially for patients with suspected TB who are difficult or unable to expel

sputum so that sputum AFB examination cannot be performed.[4]

In such cases, a rapid diagnostic test modality is needed, such as tuberculin skin test (TST), chest X-ray, Mycobacterium tuberculosis nucleic acid amplification examination and/or pathological examination of biological specimens. However, this examination has a diagnostic significance level that is not better than the smear examination or sputum culture. Likewise, the TST has low specificity in individuals receiving the Bacille Calmette – Guérin (BCG) vaccination and in individuals living in countries with high TB prevalence.[4]

Currently, many experts have been developing new diagnostic modalities, especially in the field of TB immunodiagnostics. IFN-γ inducible protein 10 (IP-10) is one of the most studied alternative biomarkers for TB diagnostics. This protein is a pro-inflammatory chemokine secreted by monocytes, neutrophils, macrophages, and endothelial inflammatory cells of Th1 lymphocytes into the foci of inflammation.

IP-10 expression is stimulated by several T-cell signals, notably IFN γ and TNF α but also IL-2, type II IFN, IL-27, IL-17/IL-23 and IL-1β which favor pathogen-specific adaptive immune responses by positive feedback. In-vitro, IP-10 is released at a rate 100 times higher than IFN γ.[5] IP-10 can provide a dynamic response to any kind of inflammation, making it important for further clinical investigations in patients who have experienced a decrease of IP-10 due to treatment failure, TB relapse or co-occurring comorbidities.[6,7]

IP-10 has been found to be elevated in plasma in both children and adults with active TB, and has been evaluated by various methodologies. Interestingly, IP10 can also be detected in the urine of adults and children with active TB. IP-10 levels dropped after potent therapy. Compared to blood, urine biomarkers offer the advantage of non-invasive sample collection, especially in Indonesian children, and also pose a lower safety risk for healthcare workers.[8,9]

As an effort to reduce the burden and impact of TB globally and nationally, early identification and adequate management of TB are the main objectives of TB control programs. Rapid TB diagnostic modalities are still very much needed considering that the currently available diagnostic modalities are not effective enough to quickly diagnose cases with smear negative TB, therefore a new diagnostic modality is needed.[4] The purpose of this study was to determine the difference in serum IP-10 levels in patients with smear positive and negative TB at RSUDZA Banda Aceh.

METHODS

This was an analytic observational study with a cross-sectional design. The subjects in this study were taken at the Integrated Tuberculosis Service (PTT) clinic of RSUDZA Banda Aceh. This study was conducted from July 2019 to May 2020. Data collection for this study was performed from November to December 2019. The target population of this study were pulmonary TB patients in Aceh province, while the affordable population were pulmonary TB patients who came to the PTT clinic of RSUDZA from November to December 2019.

The inclusion criteria of this study were patients aged 18–60 years who were diagnosed as new TB cases with positive smear, positive sputum culture, and positive molecular rapid test (GeneXpert), as well as new cases of pulmonary TB patients with AFB conversion after two months of therapy. Patients with sepsis, immunocompromised, drug resistant TB, and extrapulmonary TB were excluded.

We used a quota sampling technique where a sample size of 40 people had previously been determined. All patients who met the study criteria would be included as study subjects until the required number of subjects is met. The number of samples in this study were 40 people with 20 people in each group. The data were analyzed using the IBM SPSS Statistics 21 application using independent t-test and the cut-offs were analyzed using the ROC (receiver operating characteristic) curve to maximize sensitivity and specificity.

RESULTS

Data collection in this study was carried out from November to December 2019. The samples in the study were patients diagnosed with TB at PTT clinic of RSUDZA Banda Aceh. There were 41 patients enrolled, while 1 patient was excluded because of HIV positive.

Table 1. Demographic Characteristics and General Characteristics of Subjects

Characteristics	TB Smear Positive		TB AFB Conversion	
	n	%	n	%
Gender				
Male	13	65	13	65
Female	7	35	7	35
Age				
18–45 years old	12	60	15	75
46–65 years old	8	40	5	25
Smoking Status				
Yes	9	45	9	45
No	11	55	11	55
Body Mass Index				
Low (<18.5)	1	5	20	100
Normal (18.5–24.9)	17	85	0	0
Overweight (25–29.9)	2	10	0	0

The study subjects were divided into two groups, namely TB patients with smear positive and TB patients with AFB conversion after two months of treatment with 20 subjects each.

Table 2. Differences in IP-10 Levels on Subject Demographic Characteristics

Characteristics	IP-10 (Mean±SD)	*P*
Gender		
Male	330.1±157.9	0.163
Female	334.6±230.3	
Age		
18–45 years old	324.3±179.2	0.793
46–65 years old	347.2±198.6	
Smoking Status		
Yes	325.1±151	0.174
No	336±209.8	
Body Mass Index,		
Low (<18.5)	-	
Normal (18.5–24.9)	316.7±175.4	0.107
Overweight (25–29.9)	596.5±237.3	
AFB examination at the beginning of the diagnosis (Group of Smear Positive)		
1+	486.7±196.9	
2+	325.1±10.8	0.482
3+	448.2±129.6	

Characteristics of the subjects in this study were grouped based on gender, age, smoking status, body mass index (BMI), blood glucose levels and the AFB value of TB patients at the time of initial diagnosis. Table 1 shows the frequency distribution of study subjects.

This study obtained no differences in IP-10 levels between gender, age, smoking status, BMI, and AFB values with *P*-values of 0.163, 0.793, 0.174, 0.107, 0.482, respectively. There was no significant difference between IP-10 levels and AFB values in smear positive patients (*P*=0.482). The IP-10 examination was performed at the beginning of the diagnosis of TB in the smear positive group and after the patients took antituberculosis drugs (ATD) in the AFB conversion group, therefore the AFB value data in the conversion group could not be analyzed because the time for AFB examination and the time for taking IP-10 serum was different. There was no statistically significant difference between IP-10 levels and blood glucose levels. There was no difference in IP-10 levels in TB patients with positive and negative BTA

Table 3. Differences in IP-10 Levels on Blood Glucose Levels

Indicator	Mean±SD	*P*
Levels of IP-10	331.7±183.5	0.258
Blood Glucose Levels	109.7±10.6	

In this study, the optimal cut-off was determined by ROC analysis of serum IP-10 levels in pulmonary TB patients with smear positive and AFB conversion after two months of therapy. The cut-off value was selected to maximize sensitivity and specificity.

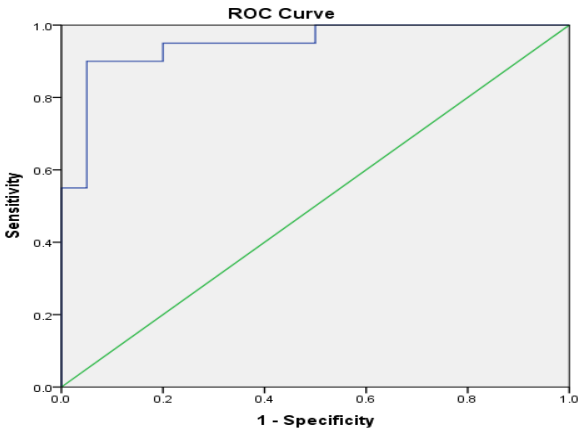

Figure 1. ROC Curve

The optimal cut-off value in this study was

306.1 pg/ml with a sensitivity of 90% and a specificity of 95% (area under curve 0.948; 95% CI=0.88-1; *P*=0.0001). This cut-off value indicates that TB patients with AFB conversion had an IP-10 level lower than 306.1 pg/ml.

Table 4. Difference in IP-10 levels in TB patients with smear positive and smear negative

	TB AFB Positive (Mean±SD)	TB AFB Negative (Mean±SD)	*P*
IP-10	459±171.1	204,4±75.69	0.0001

DISCUSSION

The data obtained pointed out that from a total of 40 subjects who met the inclusion criteria, the majority were male of 26 subjects (65%), while the rest were female of as many as 14 subjects (35%). Subjects with AFB positive and AFB conversion were 20 subjects each. The proportion of men and women in smear-positive TB patients were 13 subjects (65%) and 7 subjects (35%), respectively. The same proportion was also calculated in the number of men and women in TB patients with AFB conversion.

This study observed that the highest number of TB patients in RSUDZA occurred in the age range of 45–64 years (14 subjects), followed by ages 25–34 years (11 subjects), then ages 35–44 years (10 subjects). Subjects who had a history of smoking were 18 people or 45% of the total number of subjects, where the number was the same in TB patients with AFB positive and AFB conversion, namely 9 people each.

All TB patients with AFB conversion after two months of therapy had normal BMI with a total of 20 subjects (100%). Meanwhile, most TB patients with AFB positive had normal BMI, namely 37 subjects (92.5%). There was 1 subject (2.5%) with a low BMI and 2 subjects (10%) with overweight BMI.

Comparative analysis of IP-10 levels on the demographic characteristics of the subjects mentioned that none of the demographic characteristics had a significant correlation with IP-10 levels. The *P*-values of IP-10 for gender, age, smoking status and BMI were 0.163, 0.098, 0.174, 0.107, respectively.

Comparative analysis of IP-10 levels in TB patients with AFB positive and AFB conversion received a *P*-value of 0.00, which implied that there was a significant difference in IP-10 values among TB patients with smear positive and AFB conversion. This study stated that IP-10 is an immunodiagnostic tool which can be used to determine Mycobacterium tuberculosis infection.

IP-10 is one of the CC chemokines that escalates in polymorphonuclear monocytes and granulocytes after Mycobacterium tuberculosis infection.[10] Elevated levels of IP-10 have also been found in autoimmune diseases such as systemic lupus erythematosus,[11] and in occupational asthma.[12] So far, IP-10 was considered to only indicate the presence of inflammation and might not be very specific for use in diagnosing TB, however, Ruhwald et al. reported that IP-10 response was specific for particular antigens possessed by Mycobacterium tuberculosis and could be used to diagnose TB infection.[10]

IP-10 or CXCL-10 is encoded by chromosome number 4 in the q21 band. CXCL10 is induced by IFN-γ. CXCL10 will activate the CXCR3 receptor located on T lymphocytes, plasma cells or B cells, natural killer cells, dendritic cells and macrophage cells. When macrophages are infected by Mycobacterium tuberculosis, Th1 cells produce IFN-γ, which induces the production of various cell types that produce CXCL10. Thus, the IP-10 level is higher in tuberculosis infection. IP-10 will continue to attract and recruit Th1 cells, resulting in more CXCL10 production. Chemotaxis of immune cells continuously generates tissue damage, leaving permanent scars.[13]

The optimal cut-off value in this study was 306.1 pg/ml (90% sensitivity, 95% specificity, area under curve 0.948, 95% CI 0.88-1; *P*-value = 0.000). This cut-off value indicated that TB patients with AFB conversion had an IP-10 level lower than 306.1 pg/ml.

A study by Hong et al. in South Korea mentioned that the cut-off value of IP-10 levels in non-HIV pulmonary tuberculosis adults patients with patients without pulmonary tuberculosis or in other words adults suffering from diseases other than TB was 119 pq / mL.[14] Meanwhile, Petrone et al.

conducted a study in Uganda, reported the cut-off value for pulmonary TB patients in children compared to children without pulmonary TB was 109.1 pq/mL.[9]

LIMITATIONS

Several limitations were found in this study such as the limited number of samples, the cost and time of the study which limited the researchers using a prospective cohort study design.

CONCLUSION

There was a significant difference between IP-10 levels in TB patients with smear positive and AFB conversion after two months of treatment.

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CONFLICT OF INTEREST

None.

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