CORRELATION BETWEEN IFN-ɣ LEVELS, CHEST RADIOGRAPHY AND THE POSITIVITY OF SMEAR SPUTUM IN NEW TB CASES AT THE DR.SOETOMO HOSPITAL

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ABSTRACT

Tuberculosis is an infectious disease attacking lungs, triggering damage, and pulmonary dysfunction. Host cytokine responses will influence tuberculosis manifestations. The main host immune response is cellular immunity, and Delayed-Type Hypersensitivity (DTH). IFN-γ produced by Th-1, is a major cytokine acting to eliminate TB bacteria through macrophage activation. Chest radiography has an important value for the diagnosis of TB, especially in negative sputum smears. Radiological manifestations depend on several factors including host prior to TB exposure, age, and immune status. Sputum smear is also important in diagnosing, and assessing response to treatment of adult pulmonary TB. A cross-sectional study, comprising a total of 36 new pulmonary TB cases at the Dr.Soetomo Hospital who met the inclusion, and exclusion criteria, were establish. Plasma IFN-γ was examined by ELISA. Chest radiography was divided into three categories based on the National Tuberculosis Association of USA. Sputum smear data were taken from medical records. There were significant correlations between IFN-γ plasma levels with chest radiography (r= 0.365; ρ=0.029), IFN-γ with positive sputum smear (r= 0.447; ρ=0.006), positive sputum smear with chest radiography (r=0.674; ρ=0.001) IFN-γ plasma levels could reflect lesion area on chest radiography, and sputum smear positivity. IFN-γ plasma levels, chest radigraphy, and sputum positivity may reflect a Th-1immune response, so the more severe level of diseases, an immune response Th-1 become more activated.

Key words: IFN-γ, new TB cases, *Mycobacterium tuberculosis*, cellular immunity, delayed type hypersensitivity

INTRODUCTION

Tuberculosis infections are a major health problem in Indonesia. Indonesia still rank as forth in the world based on the WHO report, after India, China, and South Africa. Estimation for TB prevalence is 730,000 cases every year, 460,000 new cases per year. The death rate is approximately 67,000 per year.[1]

After it inhaled, and deposited in distal alveoli, *M.tuberculosis* is ingested by alveolar macrophages and neutrophils, monocytes derived macrophages, and dendritic cells. Whereas virulent strain called *M.tuberculosis* could survive within the resting macrophage, specific cytokins has an important role to control *M.tuberculosis,* and mediated by Th1, such as IFN-γ, tumor necrosis factor, and vitamin D.[2] Th2 cytokines are typically involved in humoral immunity mediated by antibody. Th2 has limited protection againts intracellular mycobacterial infection.[3]

Tuberculosis is an example of an infection with an intraceluler bacterium in which protective immunity and pathologic hypersensitive coexist, and the lession are mainly caused by the host response. More then 90% infected patients remain asymptomatic, but bacteria survive in lung, and can be reactivated. By 6-8 weeks after infection, regional lymhph nodes are involved, and CD^+4 T cells are activated. These T cell produce IFN-γ which activate macrophage, and also enhance their ability to kill phagocytosed bacilli.[4]

The importance of IFN-γ in response to *M.tuberculosis* has been widely investigated in experimental models and in humans. Knockout mice for IL-12, IFN-γ or T-bet are highly susceptible to TB. Furthermore, result of from other studies demonstrated that peripheral blood mononuclear cells from patients with active disease secreted lower IFN-γ in vitro, both in unstimulated culture or after stimulated mitogen or mycobacterial antigen.[5] Despite the important role of IFN-γ to control *M.tuberculosis*, some studies have shown that IFN-γ is insuficient to prevent

active disease. It has been shown that patients whose T cells produce higher amounts of IFN-γ are more likely to progress in to active disease than patients with weaker responses.[5] A study stated that although variation of IFN-ɤ production among various subjects, levels of IFN-ɤ are increased, and help in differenting diagnosis between pulmonary TB, and latent tuberculosis infections (using Mtb-Hag-10k in the PBMC).[6]

In cases negative smear and even positive smear, radiographic changes are helpful in disease diagnosis. Although some chest X-ray findings, including nodular or patchy infiltration in the posterior parts of the superior lobes or anterior segments of the inferior lobes particularly in bilateral involvement, and associated with cavity, are not specific for pulmonary tuberculosis, clinical manifestations and epidemiologic findings are very diagnostic.[7] The clinical course of tuberculosis, and appropriate radiological patterns depend on the interaction between organisms, and host responses.[8] Radiological manifestations of pulmonary tuberculosis depend on several factors from the host, including previous exposure to TB, age, and immunity status.[9] Host immune response has an important role to determine the result of interaction between host, and pathogens.

Sputum culture should be performed to confirm the diagnosis of pulmonary tuberculosis because Mycobacterium culture requires expensive tools, and time. Acid-fast staining that is simple, and easy has been widely used for diagnosing TB, and treatment evaluation.[10] Acid fast staining microscopically from patients sputum who have pulmonary infiltrates, and cavities are consistent with tuberculosis.[11]

This research was conducted to assess the correlation between cellular immune response, especially IFN-γ expression in new TB cases with chest radiography, and the positivity of sputum.

METHODS

A cross-sectional study was conducted consisting 36 adult new TB patients at the Dr.Soetomo Hospital that fulfilled requirement. IFN-γ plasma levels were examined by ucytechELISA.12 Chest radiography taken from medical records was divided into three categories based on the National Association of USA 1961. Acid fast bacilli staining data were taken from the medical records..

RESULT AND DISCUSSION

Following characteristics data from 36 new TB cases samples during the period January 2015 until March 2015 are presented in Table 1.

Table 1. Characteristics of research subjects

Cases	36
Gender : Males/females	26 (72.2)/10 (27.8)
Mean age	35.33 ± 14.36
Minimal age	15
Maximal	69
Chest radiography:	
Minimal lesion	17 (47.2)
Moderate lesion	9 (25)
Extensive lesion	10 (27.8)
BTA sputum	
Negative	15(41.7)
+1	8 (22.2)
+2	8 (22.2)
+3	5 (13.9)

As many as 36 patients with new TB cases fulfilled the inclusion, and exclusion criteria. There were twenty six males (72.7%), and 10 females (27.8%) according to sex. Based on the age, new pulmonary TB cases were obtained with a mean ± SD of 35.33 ± 14.36 years. A total of 36 patients who had minimal lesions on chest ragiography were 17 (47.2%), total patients with moderate lesions were 9 patients (25%), extensive lesions were 10 patients (27.8%). (Table 1).

Table 2 could explain that the average plasma level of IFN- γ in pulmonary TB patients with minimal lesions was 4.5 ± 4.02 pg/mL, the mean plasma level of IFN-γ in pulmonary TB patients with moderate lesion was 14.16 ± 13.51 pg/mL whereas, the mean plasma levels of IFN-γ in patients with pulmonary tuberculosis with extensive lesions was 13.31 ± 13.31. A similar study was conducted by Hussein et al.,[14] on 54 active TB patients, and 37 healthy controls measuring IFN-γ levels in the blood, showed there was a significant difference in IFN-γ levels in active TB patients compared to 19 healthy controls.[14] Moderate lesions had higher IFN-γ level than extensive lesions which could not be explained completely but there was a significant correlation between IFN-γ, and chest radiography. Classification of chest radiography into small, moderate, and extensive lesion could be differed by the experts.

Table 2. Mean plasma levels of IFN-ɣ in patients with pulmonary TB new cases

Overview chest radiography	Mean ± SD	95% CI for mean		Spearman test
	IFN- γ (pg/mL)	Lower bound	Upper bound	
Minimal lesion	4.5 ± 4.02	2.43	6.57	
Moderate lesion	14.16 ± 13.51	3.78	24.55	
Extensive lesion	13.31 ± 13.31	3.78	22.83	
IFN-γ VS chest radiography				r = 0.365 (p=0.029)

Table 3. Mean IFN-ɣ levels in new pulmonary Tb patients based on the positivity of Acid Fast Bacilli Staining

AFB Positivity	mean±SD IFN-ɣ (pg/mL)	95% CI for mean		Spearman test
		Lower bound	Upper bound	
Negative	3.65 ± 2.49	2.27	5.04	
+1	12.31 ±10.71	3.35	21.27	
+2	8.96 ± 6.95	3.15	14.78	
+3	22.4 ± 18.99	-1.18	45.99	
IFN-γ VS sputum positivity				r = 0.447 (p = 0.006)

The highest level of IFN-γ in pulmonary TB patients could be found at positive of sputum positivity +3, and the lowest level of IFN-γ could be found at positive of sputum positivity +1(Table 3). There was a significant correlation between IFN-γ, and sputum positivity.

A strong, and significant correlation was found in the relationship between chest radiograpy and sputum positivity (r = 0674; p=0.0001). Another study showed a correlation between Chest radiography, and sputum smear such as Ebrahimzadeh *et al*.,[7] although radiographic findings were not diagnostic in pulmonary tuberculosis, they were helpful if the assessment associated with the view of clinical manifestations, and sputum smears.[7] Saidu *et al*.,[15] showed that patchy or streaking opacities, and cavity lesions were the commonest radiographic features in their study. Chest radiography remained an useful instrument to diagnosis, and subsequent by in the management of pulmonary TB.[15] It could be explained that the clinical course of tuberculosis, and appropriate radiological patterns depended on the interaction between organisms, and host responses.[8] Radiological manifestations of pulmonary tuberculosis depended on several factors from the host including previous exposure to TB, age, and immunity status.[9] Host immune response had an important role to determine result of interaction between host, and pathogen.

Table 4. Chest radiography vs sputum positivity

Correlation	Coeficient correlation Spearman's rho	P-value	Result
Chest radiography VS Sputum positivity	0.674	0.0001 (significant if<0.01)	significant

CONCLUSION AND SUGGESTION

This study result showed a significant correlation between IFN- γ plasma levels with chest radiography(r = 0.365; ρ=0.029), and a significant correlation between IFN- γ plasma level with sputum positivity (r = 0.447; ρ=0.006). Results of level IFN-γ between the severity level of the disease can be varied. It can be dependent on the interaction between the host cells, and CD+ 4 cells. These results showed that Th1 immune respons had a correlation with both lesion area, and sputum positivity. IFN-ɣ plasma levels, and sputum positivity may reflect a Th-1immune response, so at the more severe level of disease, an immune response Th-1 became more activated. This study had limitations, so more research using intracellular cytokines or PBMC culture/Peripheral blood mononuclear cells with approximately 96 samples is needed. Radiography and sputum smear findings in this study depended on the interaction of the host immune response to pathogens that were not included in this study.

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