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# Difference and Factor Associated with Interferon Gamma Level in Pulmonary Tuberculosis Patients and Healthy Control

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## Abstract

**BACKGROUND:** Interferon-gamma (INF-γ) is an important cytokine in the immune response against Mycobacterium tuberculosis. Interferon-gamma activates macrophage to kill intracellular mycobacterium.

**AIM:** The aim of this study is to determine the difference of plasma INF-γ level in pulmonary tuberculosis (PTB) patients compared to a healthy control and factors associated with interferon-gamma level.

**METHODS:** This is a case–control study. Fifty subjects were selected, including 25 new PTB patients with positive sputum smear, and 25 healthy control (non TB patients) with no symptoms of tuberculosis, normal chest X-Ray and no history of previous tuberculosis. Interferon-gamma concentration was determined by an ELISA technique. Data were analyzed with independent t-test and the results were statistically significant at $p < 0.05$.

**RESULTS:** Interferon-gamma level was significantly higher in PTB patients compared to healthy control ($p = 0.024$). Mean ± SD interferon gamma level was 317.2 ± 201.97 pg/ml in PTB patients and 213.5 ± 86.43 pg/ml in healthy control. Acid fast bacilli (AFB) positivity was significantly associated with interferon gamma level ($p < 0.001$). Interferon gamma level in TB patients with AFB 1+ was 503.22 ± 146.15 pg/ml, AFB 2+ was 337 ± 81.61 pg/ml, and AFB 3+ was 88.27 ± 51.32 pg/ml. Sex, body mass index (BMI), and age were not associated with INF-γ level.

**CONCLUSIONS:** Interferon gamma level was significantly higher in PTB patients than healthy control. Sex, BMI, and age were not associated with INF-γ level. Interferon-gamma level was significantly associated with AFB positivity in pulmonary tuberculosis patients.

## Introduction

Tuberculosis (TB) is still an important global health problem in the world with estimated 10 million cases and 1.3 million deaths worldwide in 2020. Indonesia has the third highest number of TB cases in the world after India with 845,000 TB cases in 2020 [1].

Some factors affect the occurrence of pulmonary TB such as host, environment, and agent. Host factors that can be associated with tuberculosis are age, gender, malnutrition, diabetes, and immune status [2]. One of the most important factors for immunity to M. tuberculosis infection is INF-gamma cytokine.

Interferon-gamma is a major component in immunological cell signaling and is an important regulatory protein for overall immune system function. The main sources of IFN-γ are natural killer (NK) and natural killer T (NKT) cells, which are effectors of the innate immune response, and CD8 and CD4 Th-1 effector T cells of the adaptive immune system. IFN-γ also contributes to macrophage activation by increasing phagocytosis and production of potent pro-inflammatory and antimicrobial cytokines, including superoxide radicals, nitric oxide, hydrogen peroxide, and lysosome enzyme that can destroy DNA and cells walls of *Mycobacterium tuberculosis*. IFN-γ also controls the differentiation of naive CD4 T cells into Th-1 effectors, which mediates cellular immunity against infection [3].

Researches on interferon-gamma levels in pulmonary TB patients have given different results. Research by Widjaja *et al*. found that serum IFN-γ levels of patients with pulmonary tuberculosis were lower than healthy people in the community [4], while other studies found that interferon gamma levels in TB patients were higher than healthy people [5], [6], [7]. Knowledge of interferon-gamma in the body's defense against tuberculosis infection is important. This study aims to determine the differences in interferon-gamma levels in pulmonary TB patients compared to healthy control and factors associated with interferon-gamma level.

## Methods

This is a case–control study with pulmonary TB patients as the case group and healthy people as the control group. Cases were found by consecutive sampling and controls were subjects from healthy

people. This study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

## Subjects

Cases were pulmonary tuberculosis patients recruited from several TB services in Medan city, Indonesia, from March to July 2017. The inclusion criteria in the case group were newly diagnosed pulmonary tuberculosis patients with positive sputum smear and chest radiography consistent with active disease, age 18−65 years. The exclusion criteria in case group were HIV positive, known to present diabetes mellitus and other severe diseases, and consuming immunosuppressive drugs such as corticosteroid and cancer chemotherapy. Control group was healthy subjects with no symptoms of tuberculosis, normal chest X-ray and no history of previous tuberculosis. All subjects were interviewed, and a written informed consent was obtained. An anticoagulated peripheral blood specimen was collected, and Interferon gamma level was analyzed using ELISA kit.

### *IFN-γ level examination procedure*

A 3 ml blood was taken from the median cubital vein in EDTA-contain tube and shaken back and forth. The tubes were then centrifuged at 5000 rpm for 30 min. Plasma extracted and saved in microtube, coated with paraffin and stored in the freezer at −80°C until being used. Blood plasma and ELISA kit were at room temperature. The standard solution was tested by Duplo, while the other was added to the sample that had been augmented with Assay Diluent. Every well added with rabbit anti-IFN-γ polyclonal antibody. The plates then were sealed with acetate plate sealer and washed with wash buffer. Goat anti-rabbit conjugated alkaline phosphatase was added to every well then sealed and incubated for 45 min at room temperature. Afterward, the sealer was opened, the fluid drained, then the plates were washed with wash buffer. Staining reagent was added and incubated for 6 min at room temperature. Finally, a stop solution was put in. The result of INF-γ level was read by ELISA reader [8]. Data were analyzed with independent t-test, and the results were statistically significant at $p < 0.05$.

## Results

Table 1 shows a total of 50 subjects consisting of 25 pulmonary TB patients as the case group and 25 healthy people as the control group. The number of male subjects was higher in both groups; the highest number was in the 16−45 age group, while the 56−65 age group was the lowest. As for the body mass index (BMI), underweight was found in 40% of the case group but not in control group. Overweight and obese were not found in the case group but was found in the control group.

**Table 1: Characteristics of case and control group**

| Characteristic | PTB case, n (%) | Healthy control, n (%) |
|---|---|---|
| Sex | | |
| Male | 15 (60.0) | 14 (56.0) |
| Female | 10 (40.0) | 11 (44.0) |
| Age | | |
| 16–25 | 6 (24.0) | 10 (40.0) |
| 26–35 | 3 (12.0) | 12 (48.0) |
| 36–45 | 8 (32.0) | 0 |
| 46–55 | 7 (28.0) | 2 (8.0) |
| 56–65 | 1 (4.0) | 1 (4.0) |
| BMI | | |
| Underweight | 10 (40.0) | 0 |
| Normal | 15 (60.0) | 14 (56.0) |
| Overweight | 0 | 4 (16.0) |
| Obese | 0 | 7 (28.0) |
| Total | 25 (100) | 25 (100) |

PTB: Pulmonary tuberculosis, BMI: Body mass index.

Table 2 shows that interferon-gamma level was significantly higher in PTB patients than the level in the healthy control group (317.2 ± 201.97 vs. 213.5 ± 86.43; $p = 0.024$).

**Table 2: Interferon-gamma level in case and control group**

| INF-γ | PTB case (n = 25) | Healthy control (n = 25) | p |
|---|---|---|---|
| Mean ± SD (pg/mL) | 317.2 ± 201.97 | 213.5 ± 86.43 | 0.024* |
| Median (pg/mL) | 348 | 204 | |
| Minimum (pg/mL) | 25.8 | 67 | |
| Maximum (pg/mL) | 849 | 393 | |

*Independent t-test. SD: Standard deviation, INF-γ: Interferon-gamma, PTB: Pulmonary tuberculosis.

Table 3 shows that interferon gamma level was significantly associated with AFB positivity ($p < 0.001$). The highest level of interferon gamma was discovered qin pulmonary TB patients with AFB 1 + (503.22 ± 146.15 pg/mL), followed by AFB 2+ (337 ± 81.61 pg/mL) and AFB 3+ (88.27 ± 51.32 pg/mL).

**Table 3: Association of interferon gamma level and AFB positivity in tuberculosis patients**

| INF-γ | AFB 1+(n = 9) | AFB 2+(n = 8) | AFB 3+(n = 8) | p |
|---|---|---|---|---|
| Mean ± SD (pg/mL) | 503.22 ± 146.15 | 337 ± 81.61 | 88.27 ± 51.32 | <0.001* |
| Median (pg/mL) | 471 | 330.5 | 81.05 | |
| Minimum (pg/mL) | 351 | 232 | 25.8 | |
| Maximum (pg/mL) | 849 | 442 | 187 | |

*Independent *t*-test. SD: Standard deviation, AFB: Acid fast bacilli, INF-γ: Interferon-gamma, PTB: Pulmonary tuberculosis.

Table 4 shows the association of INF-γ level with age, sex, and BMI in case and control group. Interferon-gamma level was higher in male than in female in both groups, but not statistically significant. In PTB group, INF-γ level was lower in underweight patients than in those with normal BMI. In healthy control group, INF-γ level was higher in overweight BMI than in normal and obese BMI. Howevere, the difference in INF-γ level was not significantly associated with BMI in both groups. Age was also not associated with INF-γ level in both groups.

**Table 4: Association of interferon-gamma level with age, sex, and body mass index in pulmonary tuberculosis and non-pulmonary tuberculosis group**

| Characteristic | PTB case (n = 25) | | p | Healthy control (n = 25) | | p |
|---|---|---|---|---|---|---|
| | n (%) | IFN-γ level (mean ± SD) | | n (%) | IFN-γ level (mean ± SD) | |
| Sex | | | | | | |
| Male | 15 (60.0) | 335.453 ± 227.65 | 0.78* | 14 (56.0) | 226.6 ± 99.74 | 0.277 |
| Female | 10 (40.0) | 289.94 ± 163.65 | | 11 (44.0) | 196.91 ± 66.71 | |
| Age | | | | | | |
| 16–25 | 6 (24.0) | 409.36 ± 269.22 | 0.773* | 10 (40.0) | 168.14 ± 68.62 | 0.516 |
| 26–35 | 3 (12.0) | 106.1 ± 89.79 | | 12 (48.0) | 246.91 ± 88.54 | |
| 36–45 | 8 (32.0) | 310.81 ± 168.37 | | 0 | 0 | |
| 46–55 | 7 (28.0) | 329.7 ± 146.78 | | 2 (8.0) | 215 ± 118.79 | |
| 56–65 | 1 (4.0) | 442 ± 0 | | 1 (4.0) | 264 | |
| BMI | | | | | | |
| Underweight | 10 (40.0) | 233.52 ± 218.76 | 0.12** | 0 | 0 | 0.078 |
| Normal | 15 (60.0) | 373.067 ± 175.56 | | 14 (56.0) | 177.88 ± 72.16 | |
| Overweight | 0 | 0 | | 4 (16.0) | 255.25 ± 118.98 | |
| Obese | 0 | 0 | | 7 (28.0) | 177.88 ± 72.16 | |

*One-way Anova, **Kruskal–Wallis test. SD: Standard deviation, BMI: Body mass index, INF-γ: Interferon-gamma, PTB: Pulmonary tuberculosis.

## Discussion

The characteristic data in the pulmonary TB patient group in Table 1 show that there were more men than women; most of them were in the productive age < 55 years (96%) and 40% of the subjects had an underweight body mass index. This is in accordance with the WHO data which states that the majority of tuberculosis are found in men for about 56%; 5.6 million in men, 3.3 million in women, and 1.1 million children and in all ages but mostly in productive age [1]. This could be due to the fact that more men work outside the home so that they are more likely to be infected than women; more men smoke and drink alcohol [9]; and fewer men visit health facilities [10]. Another study linked the possibility of biological differences in the hormonal system in men and women that could cause differences in the risk of developing tuberculosis by gender [11]. Low body mass index is also often found in tuberculosis patients. Malnutrition causes a decrease in immune function by direct effect on T cells, decrease of phagocytic function, cytokines, and complement which are important in fighting tuberculosis infection [12]. Another study found that a high BMI was a protective factor against the incidence of tuberculosis, but not a very high BMI (>30 kg/m$^2$) and a high BMI with diabetes mellitus [13].

Interferon-gamma levels of pulmonary TB patients compared to healthy people were found to vary in many studies. In this study, there was a significant difference of IFN-γ levels where the levels in pulmonary TB patients were higher than that in healthy people. These results are in line with the studies in Kenya [5] and Pakistan [6]. Increased IFN-γ level suggests that an upregulation in the pro-inflammatory response in the newly infected TB cases indicates innate protective response during early phase of *Mycobacterium tuberculosis infection* [5]. On the other hand, several other studies have found different things. Widjaja *et al*. from Indonesia found that serum IFN-γ levels of pulmonary tuberculosis patients were found to be lower than healthy people in the community. The assumption of this researcher was that the low level of serum IFN-γ in pulmonary tuberculosis patients was influenced by host factors such as genetic polymorphism of the gene +874 T/A IFN-γ gene, nutritional status of the host, age of the host, and gender [4]. Another study assessed IFN-γ levels after stimulation with ESAT-6 antigen to get IFN-γ levels in healthy people ranging from 98.84–135.47 pg/mL, in latent TB 74.6–142.2 pg/mL, and active TB 72.67–154.21 pg/mL. This difference in IFN-γ was not statistically significant, but these results showed that IFN-γ levels were higher in healthy people than in pulmonary TB patients [14].

This study also found an association between IFN-γ level and AFB positivity, with the highest IFN-γ level in TB patients with AFB 1+ followed by AFB2+ and AFB 3+. This result is consistent with other studies conducted in Indonesia [8], [15], [16]. Interferon gamma level and sputum positivity may reflect a Th-1 immune response, the more severe level of disease, and immune response Th-1 may become more activated [16].

In this study, there was no association between IFN-γ level with BMI. Study of Shavia *et al*. in Kenya found an association of body mass index with interferon-gamma level, that is, IFN-γ level was inversely correlated with BMI [5]. Another study also found higher level of IFN-γ in TB cases present with lower body weight [17]. The correlation/link/relationship between them might be that IFN-γ promotes weight loss in newly infected TB patients. Increased IFN-γ levels induce anorexia and upregulates IL-12, hence, promoting weight loss through epithelial cell damage [18]. This study also found no association between IFN-γ level and age in Pulmonary TB and healthy group. Bandrés *et al*. found the increased of IFN-γ production through aging in healthy people [19]. Study on nocturnal mouse lemur also found increased of INF-γ level with aging [20]. This study found no association between age and INF-γ level. There is evidence that sex is one variable that influences innate and adaptive immune response resulting in sex-specific outcomes of infectious and autoimmune disease, malignancies, and vaccines. Further study is needed to find a precise association between sex with immune response and IFN-γ, knowing that this will probably reflects complex interactions among genes, hormones, and environment [21], [22].

The limitations of this study were the small sample size and no examination of other cytokines and genetic factors. Larger sample size, examination of other cytokines and genetic factors can provide better insight into factors affecting interferon-gamma levels.

## Conclusion

Interferon-gamma is a cytokine that is important in the immune response to tuberculosis. This study found that interferon-gamma levels were higher in tuberculosis patients than healthy controls. Age, sex, and BMI were not associated with interferon-gamma levels, but AFB positivity rate correlated with the interferon-gamma level in tuberculosis patients. Further study is needed to know the factors associated with interferon-gamma level in PTB patients and healthy subjects.

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