Association of Polymorphism +874 T/A Interferon Gamma Gene with Susceptibility to Pulmonary Tuberculosis in Medan, Indonesia

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Abstract

BACKGROUND: Pulmonary tuberculosis (TB) is still a disease that concerns in the world due to its high incidence, especially in developing countries, including Indonesia. Polymorphism +874 T/A interferon-gamma (IFN-γ) is one of the host genetic factors that have been widely studied and has been shown to be associated with susceptibility to pulmonary TB.

Aim: This study aims to determine the association polymorphism +874 T/A IFN-γ gene with susceptibility to pulmonary TB in Medan population.

METHODS: A total of 82 pulmonary TB patients and 85 healthy controls were examined in this case-control study. SPSS 25 is used to process and analyze all data where the Pearson Chi-square is used to analyze the association between genotype and susceptibility to pulmonary TB.

RESULTS: The polymorphism +874 T/A IFN-γ gene was not significantly associated with susceptibility to pulmonary TB in patients compared to controls (p = 0.395) and there was no association between allele frequency and susceptibility to pulmonary TB (p = 0.158).

CONCLUSION: Our study suggests no association of polymorphism +874 T/A IFN-γ gene with pulmonary TB in Medan, Sumatera Utara, Indonesia.

Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium TB, which is still cause morbidity rates for millions of the world’s population every year. In 2018, the total global TB incidence is 10 billion people, in which Southeast Asia accounts for around 4.37 billion cases. TB is also a major health problem in Indonesia and ranks third in the world [1]. Not everyone infected with TB develops TB. Only 10% of infected develop TB disease and have risk factors such as diabetes, the elderly, alcohol abuse, infection, or use of prolong corticosteroids. There is compelling evidence that host genetic factors are important in determining the outcome of infection in association of host genetic with TB susceptibility, which has also been widely studied such as case-control studies, candidate gene and family approaches, and genome-wide linkage analysis, including interferon gamma (IFN-γ) [2], [3].

The human IFN-γ gene is located on chromosome 12 (12q15) and stretches for about 6 kb consisting of 4 exons and 3 introns [4]. Human IFN-γ is a glycosylated protein with a length of 143 amino acids and has little sequence homology with the IFN-α and IFN-β classes. The natural role played by IFN-γ is as a modulator of the immune system [5]. The role of IFN-γ can be found in both innate and adaptive immunities. The innate immune response to intracellular bacteria consists of phagocytes and NK cells, the interactions of which are mediated by cytokines (interleukin [IL]-12 and IFN-γ). The typical adaptive immune response to these microbes is cell-mediated immunity, in which T-cells activate phagocytes to eliminate microbes. Innate immunity can control bacterial growth, but elimination of bacteria requires adaptive immunity [6].

The 874 T allele is known to be associated with high genotype production and the 874 A allele is associated with low genotype production. In addition, the polymorphism of +874 T/A on intron 1 of this IFN-γ gene is said to inhibit the binding site of the transcription factor nuclear factor kappa B (NF-kB) which induces gene expression. This polymorphism is located at
position +874 from the initial place of translation. The T to A polymorphism is associated with the putative NF-KB binding site, which may have functional implications for transcription of the human IFN-γ gene [7], [8].

This study was conducted to determine whether there was an association between polymorphism +874 T/A IFN-γ gene and susceptibility to pulmonary TB in Medan.

Materials and Methods

Study design
A total of 82 pulmonary TB patients and 85 healthy controls were examined in this case–control study. The diagnosis of pulmonary TB was confirmed by clinical, chest X-ray, and positive direct smear. The control subject was healthy people who do not have pulmonary TB based on anamnesis, normal chest X-ray and who do not have a history of pulmonary TB before. This research was conducted after obtaining ethical clearance from the Health Research Ethics Committee of the Universitas Sumatera Utara.

Genotyping
DNA was amplified in a 10 µl reaction as previously described [8]. The Amplification Refractory Mutation System polymerase chain reaction (PCR) method is used to process the sample of both groups and records the +874 T/A polymorphism on first intron of the IFN-γ gene. The primer sequencing was as follows: IFN-γ (intron 1) 5’- tcaacaaagctgatactcca-3; IFN-γ Primer A 5’-ttcttacaacacaaaatcaaatca-3; IFN-γ Primer T 5’-ttcttacaacacaaaatcaaatct-3; internal control primer (1) 5’-gccttccaaccattccctta-3; and internal control primer (2) 5’-tacqggatgctgtgttcttc-3.

The PCR reaction was performed using 10 cycles (95°C for 1 min, 95°C for 15 s, 62°C for 50 s, and 72°C for 40 s) followed by 20 cycles (95°C for 20 s, 56°C for 50 s, and 72°C for 50 s). PCR products were examined by electrophoresis with 2% agarose gel and ethidium bromide 0.5 µg.

Statistical analysis
All genotype frequencies were tested for Hardy–Weinberg using calculator Hardy–Weinberg equilibrium (HWE) [9]. Statistical analysis was done using SPSS 25 with $P < 0.05$ as a significant value. Demographic characteristics were tested using the $\chi^2$ test where gender and age were included in the study. The Kolmogorov–Smirnov test is used when the data are not normally distributed. Genotype frequencies between two groups were compared by Pearson Chi-square. Odds ratio (OR) and 95% confidence interval (CI) were used as estimates of risk for all samples.

Results

Subject characteristic
A total of 167 DNA sample from pulmonary TB patient and healthy control (82 PTB and 85 HC) were included in this study. The demographic characteristics were collected by secondary data. There is no statistical difference in age and gender in the two study groups (Table 1). Although in this study, there was no association between polymorphism 874 T/A with pulmonary TB, both population in this study were in a HWE which in the case group at $p = 1.31$ and in the control group $p = 0.699$.

Discussion
TB, caused by MTB, remains the leading cause of morbidity and mortality in the worldwide. It is estimated that one-third of the world’s population is infected with MTB [10]. As mentioned earlier, one of the important transcription factors for IFN-γ gene expression is NF-κB. NF-κB refers to a group of structurally related transcription factors that play a central role in inflammation, lymphocyte activation, cell survival, and secondary lymphoid organ formation [6].

In this study, it was found that there were more male than female in both case and control groups. Based on the distribution according to gender, in both groups, the most genotype that found in male was the

| Table 1: Genotype distribution by gender |
|----------------------------------------|
| Polymorphism +874 T/A | PTB | HC |   |
|                        | Male, n (%) | Female, n (%) | Total | Male, n (%) | Female, n (%) | Total |
| Genotype               |             |             |       |             |             |       |
| TA                     | 24 (29.27)  | 9 (10.98)   | 33 (40.25) | 31 (36.47)  | 7 (8.24)    | 38 (44.71) |
| TT                     | 12 (14.63)  | 1 (1.22)    | 13 (15.85) | 13 (15.30)  | 5 (5.88)    | 18 (21.18) |
| AA                     | 22 (26.83)  | 14 (17.07)  | 36 (43.9)  | 19 (22.30)  | 10 (11.76)  | 29 (34.11) |
| Total                  | 58 (70.73)  | 24 (29.27)  | 82 (100)   | 63 (74.12)  | 85 (100)    |       |
| Allele                 |             |             |       |             |             |       |
| T                      | 48 (29.27)  | 11 (6.71)   | 59 (35.98) | 57 (33.53)  | 17 (10)     | 74 (43.53) |
| A                      | 68 (41.46)  | 37 (22.56)  | 105 (64.02)| 69 (40.59)  | 27 (15.88)  | 96 (56.47) |
| Total                  | 116 (70.73) |             |       |             |             |       |

PTB: Pulmonary tuberculosis; HC: Healthy control.
Table 2: Genotype distribution by age group

| Polymorphism +874 T/A | PTB, n (%) | HC, n (%) | χ² | p-value |
|-----------------------|-----------|-----------|-----|---------|
| Gender                |           |           |     |         |
| Male                  | 58 (70.7) | 63 (74.1) | 0.100 | 0.752a |
| Female                | 24 (29.3) | 22 (25.9) |       |         |
| Total                 | 82 (100)  | 85 (100)  |       |         |
| Age                   |           |           |     |         |
| 16–25                 | 23 (28.1) | 28 (32.9) | 0.316 | 1.000b |
| 26–35                 | 30 (36.6) | 27 (31.8) |       |         |
| 36–45                 | 16 (19.5) | 15 (17.7) |       |         |
| 46–55                 | 12 (14.6) | 12 (14.1) |       |         |
| 56<                   | 1 (1.2)   | 3 (3.5)   |       |         |
| Total                 | 82 (100)  | 85 (100)  |       |         |

(a): Using Chi-square. (b): Using Kolmogorov–Smirnov. PTB: Pulmonary tuberculosis; HC: Healthy control.

TA genotype, while the AA genotype was mostly found in the female group (Table 1). However, in the statistical calculation, there was no statistically different between gender and susceptibility to pulmonary TB (p = 0.752).

Table 3: Subject characteristic

Based on genotype distribution by age group, AA genotype were mostly found in both Pulmonary Tuberculosis and Healthy control group. From Table 2, it can be seen that the AA genotype was most prevalent at the age of 26–35 in the pulmonary TB group and at the age of 16-25 years in the control group. Overall, in both groups, the AA genotype was mostly found in the 16-25 year age group. The most age group in this case and control group was the age range of 16–25 years old and the least age group was >56 years old. However, in the statistical calculation (Table 3), there was no statistically different between age and susceptibility to pulmonary TB (p = 1.000).

Table 4: Association between the genotype and susceptibility to pulmonary TB

A study in Pakistani population also found no association between the +874 T/A polymorphism with susceptibility to pulmonary TB. The TT genotype frequency was significantly low in TB patients (p = 0.02), but it was not proved that the TT genotype had a protective association with the pulmonary TB group (p = 0.07). This may be because the sample size is too small in this Pakistani population [15]. However, the AA genotype was found to have association with the risk of TB incidence (p = 0.035). The AA genotype was shown to be significantly associated with susceptibility to TB among active TB (pulmonary and extra TB) while the AT genotype was shown to be significantly associated with susceptibility to TB (p = 0.0006) [16]. The AT genotype was shown to be significantly associated with susceptibility to TB compared to the AA genotype (TT genotype was considered baseline) [14].

In contrast to the results of this study, study in South African population and Warao indigenous subject found an association between polymorphism +874 T/A with susceptibility to TB with p = 0.017 and p < 0.0001, respectively [17]. A similar study in a Brazilian population also concluded the same result with p < 0.0001 and also found that plasma IFN-γ concentrations were higher in controls (mean = 0.949 IU/ml) in compare to TB patients (mean = 1.348 IU/ml). In this study, it was also found that the plasma IFN-γ levels in pulmonary TB patients with genotype AA were less than other genotypes. This implies strong evidence that this polymorphism decreases the production of IFN-γ and decreases the
activation of cellular immunity in pulmonary TB infection [18]. Study in Sicilian patient also concluded that the TT genotype was significantly decreased in TB patients (p = 0.02431) [19]. Although there was no association of alleles with susceptibility to pulmonary TB (p = 0.23), research in Iran population found that there was an association between polymorphism +874 T/A with susceptibility to TB (p = 0.007) [20]. Not all the same studies that examining the association of the +874 T/A interferon gamma polymorphism with susceptibility to pulmonary tuberculosis have the same results. The reason for this difference in results in each population may be that other factors, such as malnutrition, can reduce genotypic effects that were not investigated in this study. Undiagnosed TB cases in the control group as well as the influence of the length of time taking anti-TB drugs in TB cases may also have influenced the outcome of the study.

After statistical test was done, there was also no association between frequency allele of the IFN-γ gene and susceptibility to pulmonary TB in Medan. In this study, the A allele was the most prevalent in both groups of both cases and controls and was not proven to be statistically different in relation to susceptibility to pulmonary TB in Medan (Table 5) (p = 0.159). In a study in Brazil, there was an association between allele A with susceptibility to pulmonary TB [18]. T alleles were more prevalent in controls and possibly act as a protective factor against TB (18). Allele A was shown to be significantly associated with susceptibility to active TB among Brazilians subject (14). The A allele was shown to be significantly associated with susceptibility to TB in South African and Southeast Chinese population with p = 0.0055 and 0.0023, respectively [21]. The T allele was significantly low in TB patients in Turkish population (p = 0.024) [22]. However, a study in Sicilian patient found more T alleles in controls but not significantly different [19]. This vulnerability is influenced by polygenic factors [18]. Several studies observing genetic heterogeneity and population stratification but not carried out in this study.

**HWE**

In principle, genotyping research using the Hardy–Weinberg calculator to assess whether the study population is in Hardy–Weinberg’s balance, which is indicated by p > 0.05. The requirements of the HWE theory include random population mapping, the population is large enough for random allele and genotype fluctuations, no mutations, no migration, and no differences in compatibility between genotypes [23]. In this study, both groups were in HWE (p-case = 1.31 and p-control = 0.699). This means that there is no genotype change from generation to generation in this study population.

This study only examined one polymorphism while many polymorphisms in IFN-γ genes affect the expression of this gene and this IFN-γ +874 T/A have been studied in children [24]. This study also did not examine the levels of IFN-γ which was associated with the polymorphism +874 T/A of the IFN-γ gene. In addition, there is many gene that related to the occurrence of Pulmonary TB for example Tumor Necrosis Factor-alpha -308G / A and Vitamin D Receptor Gene that have been previously studied in Medan [25,26]. Further research is expected to investigate the relationship between other IFN-γ gene polymorphisms in relation to the occurrence of pulmonary TB in Medan, Sumatera Utara, Indonesia.

**Conclusion**

There is no association of polymorphism +874 T/A IFN-γ with susceptibility to pulmonary TB in Medan. Further, investigations are needed to clarify the roles of these polymorphisms in the progression of pulmonary TB.

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