Research Article

The influence of interferon-gamma +874 T/A and interleukin-10 −1082 G/A polymorphisms on culture conversion in Javanese multidrug-resistant tuberculosis patients

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ABSTRACT

Aim: To determine the influence of gene polymorphisms, especially IFN-γ +874 T/A and IL-10 −1082 G/A to sputum culture conversion of Javanese MDR-TB patients in Indonesia. In this study, the influence of IFN-γ +874 T/A and IL-10 −1082 G/A gene polymorphisms to the sputum culture conversion of Javanese MDR-TB patients was investigated. **Method:** This study was conducted in patients suspected to have MDR-TB in Moewardi hospital from January 2011 to April 2014. Sputum conversion status, epidemiology, and clinical data of patients were collected. IFN-γ and IL-10 polymorphisms were obtained using Polymerase chain reaction methods. During the study period, 120 from 179 participants that met the inclusion criteria were willing to sign the informed consent. Seven patients dropped out, and six patients died. **Result and Discussion:** A total of 61 patients had MDR-TB, and 46 patients had TB resistant to rifampicin. Patients with mixed genotypes of +874 A/A IFN-γ and −1082 A/A IL-10 had the longest time of sputum culture conversion (Hazard ratio [HR] = 2.88; 95% confidence interval [CI]: 1.030–8.028; and *P* = 0.040). Patients with mixed genotypes of +874 T/T IFN-γ and −1082 G/A IL-10 have the fastest time of culture conversion (HR = 0.441; 95% CI: 0.223–0.870; and P = 0.018). Culture conversion of MDR-TB patients was influenced by −1082 A/A IL-10 gene polymorphism (deceleration) and +874 T/T IFN-γ and −1082 G/A IL-10 gene polymorphism (acceleration). **Conclusion:** The +874 T/T IFN-γ and -1082 A/A IL-10 gene polymorphisms influence culture conversion (deceleration) and the +874 T/T IFN-γ and −1082 G/A IL-10 gene polymorphisms accelerate the culture conversion of Javanese MDR-TB patients.

KEY WORDS: Culture conversion, Interferon-gamma, Interleukin-10, Multidrug-resistant tuberculosis, Polymorphisms

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is usually treated by administration of a time course of antibacterial drugs. However, in recent years, multidrug-resistant TB (MDR-TB) strains have emerged that are resistant to both isoniazid (INH) and rifampicin (R) with or without resistance to other anti-TB drugs.[1,2] In 2013, there were 480,000 MDR-TB cases and 210,000 fatalities due to MDR-TB worldwide.[3] Treatment of MDR-TB is very complicated, very lengthy (19–24 months), and expensive, and the cure rate is <70%.[2] A complete cure is shown by three consecutive negative sputum cultures over a minimum interval of 30 days apart in the intensive phase.[3]

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Some human genes and their genetic variants influence the likelihood of TB infection. INF-γ has the effect of controlling the intracellular growth of *M. tuberculosis*, while interleukin-10 (IL-10) promotes it. A single nucleotide polymorphism (SNP), +874 T/A, located

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in the first intron of the interferon-gamma (INF-γ) gene influences the secretion of cytokines due to TB infection.[4]

IL-10 is produced by T helper (Th2) cells.[5] The immune status of the host is reflected in the balanced and imbalanced condition of Th1 and Th2 cytokines, which can influence clinical manifestations.[6] IL-10 production is controlled at the transcription and translation level. IL-10 gene polymorphisms are generally found in two regions. In these regions, there are two microsatellites at the initiation site of transcription (1.2 kb and 4 kb) and three mutation sites (−1082 G/A, −819 C/T, and −592 C/A). The IL-10 1082 G/A gene polymorphism is correlated with increased production of IL-10 in T cells and monocytes. In TB patients in Cambodia, TB susceptibility was correlated with an IL-10 polymorphism at the 1082 position, but this was not seen in susceptible Spanish and Gambian patients.[7] The frequency of IL-10 1082 G/A polymorphisms is higher in TB patients, while the IL-10 −819 C/T and −592 C/A polymorphisms are correlated with diagnoses of asthma.[8]

These polymorphisms have a strong correlation with race/ethnicity. The objectives of this study were to determine the influence of gene polymorphisms, especially IFN-γ +874 T/A and IL-10 −1082 G/A to sputum culture conversion of Javanese MDR-TB patients in Indonesia. In this study, the majority of the MDR-TB patients were Javanese ethnicity admitted to Dr. Moewardi General Hospital, a referral hospital for MDR-TB patients in Central Java province.

MATERIALS AND METHODS

A cohort study was performed in suspected MDR-TB patients admitted to Dr. Moewardi General Hospital from January 2011 to April 2014. A total of 120 of 179 participants who met the inclusion criteria were willing to participate in the study. Approval for the study was obtained from the Institutional Review Boards of the Faculty of Medicine of Sebelas Maret University and Dr. Moewardi General Hospital, Surakarta, Indonesia. Written informed consent was obtained from all participants. All of the procedures were conducted according to the principles of the declaration of Helsinki.

Seven patients later dropped out and six patients passed away during the observation period. A total of 61 TB patients were confirmed as having MDR-TB and 46 TB patients were confirmed as resistant to R alone. The patients were followed from January 2011 to April 2014 to assess their clinical characteristics, including body mass index (BMI) and diabetes mellitus comorbid. All of the MDR-TB patients were bacteriologically confirmed as having TB (all with pulmonary TB) and were HIV-negative. The inclusion criteria were Javanese ethnicity MDR-TB participants. While the exclusion criteria were pregnancy, HIV/AIDS participants, diabetes mellitus, and participants who received immunosuppressant drugs, discontinuing criteria were dropped out participants, a participant who had an adverse event that caused discontinuation of therapy, a participant who died during the study, and participants who retreated from this study. A total of 61 MDR-TB participants were subjected to genotypic analysis of the SNP IFN-γ +874 T/A and IL-10 −1082 G/A.

The 61 MDR-TB patients were subjected to genotypic analysis of the SNPs in IFN-γ at +874 (i.e. T/A) and IL-10 at −1082 (G/A). To determine the association between IFN-γ +874T/A and IL-10 −1082 G/A and disease susceptibility, the genotypic analysis was also performed on the 46 R-monoresistance patients. Thus, these non-MDR-TB participants were used as a control group.

DNA was extracted from ethylenediaminetetraacetic acid blood samples using the high pure polymerase chain reaction (PCR) template preparation kit (Roche Applied Science, Mannheim, Germany). The DNA samples were then used to examine the +874 T/A INF-γ polymorphism using a specific PCR sequence with the primer set: Antisense 5'-TCA ACA AAG CTG ATA CTC CA-3', sense +874T 5'-TTC TTA CAA CAC AAA ATC AAA TCT-3', and sense +874A: 5'-TTC TTA CAA CAC AAA ATC AAA TCA-3'.[9] To examine the −1082 G/A IL-10 SNP, a specific PCR sequence with a primer set was used: Antisense 5'- TGT AAG CTT CTG TGG CTG GA-3', sense −1082G: 5'- CTA CTA AGG CTT CTT TGG GAG-3', and sense -1082A: 5'- ACT ACT AAG GCT TCT TTG GGA A-3'.[10]

The DNA was amplified using the fast start HiFi PCR system dNTPack (Roche Applied Science) with initiation denaturation conditions of 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. At the end of the cycle, the last elongation step was done at 72°C for 7 min. PCR products were verified through electrophoresis using an agarose 1.5% gel containing 0.5 µg/ml ethidium bromide (100 V for 30 min) and visualized using a ultraviolet transilluminator with the GelDoc™XR (Biorad) tool. All samples were verified twice.

The morning/spot sputa of MDR-TB patients were collected and packed in styrofoam with cool packs and sent to Balai Laboratorium Kesehatan Semarang through a 1-day delivery service. Sputum cultures were examined by mycobacteria growth indicator tube method and Lowenstein Jensen culture. A negative result was defined as no *M. tuberculosis* growth within 8 weeks.

RESULTS

This study was conducted in the programmatic management drug resistance of TB ward in Dr. Moewardi Hospital from January 2011 until April

2014. The 120 research participants were examined for the +874 T/A INF-γ and −1082 G/A IL-10 gene polymorphisms, monthly sputum cultures were taken, and patients were observed until the culture conversion appeared. As noted above, seven patients dropped out and six patients died during the observation period. The mean age of the 61 MDR-TB patients was slightly older than the 46 R-resistance patients (42.0 ± 10.9 vs. 40.0 ± 12.6). A total of 36 out of 61 MDR-TB patients (59.0%) had previous anti-TB treatments, especially category two, higher than the R-resistance patients, 27 of 46 patients (58.7%). The frequency of BMI <18.5 results in this study was higher than R-resistance group (72.1% vs. 67.4%). The differences based on gender, mean age, and previous history of anti-TB drugs between MDR-TB patients and R resistance patients can be seen in Figures 1 and 2.

The A/A genotype for +874 INF-γ gene polymorphism examination was the most frequent genotype found in MDR-TB and R-resistance patients, followed by the T/A and T/T genotype [Table 1]. A total of 31 patients of the 61 MDR-TB patients (50.8%) with BMI <18.5 had the A allele (odd ratio [OR] = 0.76; 95% confidence interval [CI]: 0.295–1.956; and $P = 0.569$). In the R-resistance group, 29 of 46 patients (63.0%) with BMI <18.5 had the A allele (OR = 2.30; 95% CI: 0.978–5.421; and $P = 0.054$).

The G/A genotype for −1082 G/A IL-10 gene polymorphism examination was the most frequently found in MDR-TB and R-resistance patients, followed by A/A and G/G genotype [Table 1]. A total of 39 patients of the 61 MDR-TB patients (50.8%) with BMI <18.5 had the A allele (OR = 1.60; 95% CI: 0.713–3.610; and $P = 0.252$). While in R-resistance patients, 28 of 46 patients (60.9%) with BMI <18.5 had the A allele (OR = 1.62; 95% CI: 0.529–4.979; and $P = 0.396$).

The frequency of +874 A/A INF-γ gene polymorphisms was higher in MDR-TB patients than R-resistance patients (OR = 2.0; 95% CI: 0.661–6.086; and $P = 0.219$). Allele A from +874 INF-γ was higher than allele T (OR = 1.51; 95% CI: 0.846–2.693; and $P = 0.163$) [Table 1]. Genotype A/A of −1082 IL-10 was more frequently found in MDR-TB patients than in R-resistance patients (OR = 10.0, 95% CI: 0.584–171.202, and $P = 0.112$). The combination of genotype A of +874 INF-γ and genotype A of −1082 IL-10 was the most frequent combination found (OR = 5.88; 95% CI: 0.692–49.200; and $P = 0.070$). Frequency of allele −1082A IL-10 was higher than allele −1082G IL-10 (OR = 1.29; 95% CI: 0.748–2.220; and $P = 0.360$). The allele combination of +874A INF-γ and genotype A of −1082G IL-10 was higher in MDR-TB patients than in R-resistance patients (OR = 2.0; 95% CI: 0.682–5.865; and $P = 0.207$).

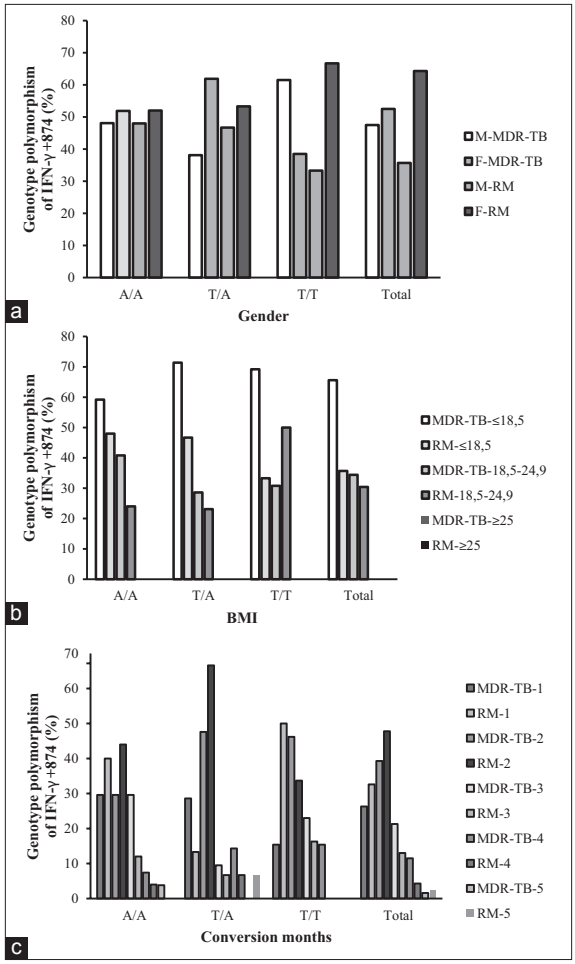

Figure 1: Distribution of f subject multidrug-resistant tuberculosis (MDR-TB) and R monoresistant (RM) genotypepolymorphism INF-γ +874 based on: (a) Gender, (b) body mass index, and (c) conversion months. M-MDR-TB: Male MDR-TB and F-MDR-TB: Female MDR-TB, RM

Survival analysis was done using Cox regression and presented in Kaplan–Meier survival plots and proportional hazards to determine compare conversion acceleration between the groups. Patients with the −1082 A/A IL-10 genotype took a longer time for sputum culture conversion than the G/A and G/G genotype (HR = 1.61; 95% CI: 0.785–3.281; and $P = 0.195$). Patients with mixed genotype +874 A/A INF-γ and −1082 A/A IL-10 took the longest time for sputum culture conversion (HR = 2.88; 95% CI: 1.030–8.028; and $P = 0.040$), followed by +874 T/A INF-γ and −1082 G/A IL-10 (HR = 1.069; 95% CI: 1.069–4,950; and $P = 0.030$). Patients with mixed genotype +874 T/T INF-γ and −1082 G/A IL-10 had the shortest culture conversion time (HR = 0.441; 95% CI: 0.223–0.870; and $P = 0.018$). Patients with allele +874T INF-γ and −1082A IL-10 had the longest culture conversion time (HR = 1.11; 95% CI: 0.694–1.876; and $P = 0.704$), meanwhile patients with allele +874A INF-γ and −1082G IL-10 had the shortest culture conversion time (HR = 0.81; 95% CI: 0.505–1.302; and $P = 0.387$) [Table 1].

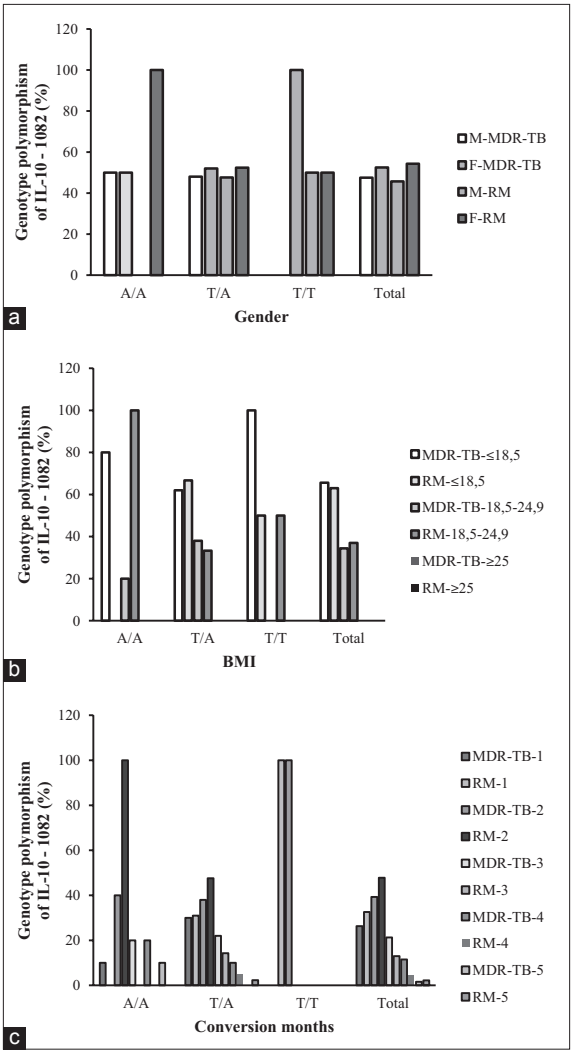

Figure 2: Distribution of subject multidrug-resistant tuberculosis (MDR-TB) and R monoresistant (RM) genotype polymorphism Interleukin-10 −1082 based on: (a) Gender, (b) body mass index, and (c) conversion months. M-MDR-TB: Male MDR-TB, F-MDR-TB: Female MDR-TB, and RM: R monoresistant

DISCUSSION

This was the first study of the influence of +874 T/A INF-γ and −1082G/A IL-10 gene polymorphisms on sputum culture conversion in Javanese MDR-TB patients. The problems in MDR-TB treatments are very lengthy treatment times (19–24 months), a <70% cure rate, and the 4–12 weeks it takes to determine the sputum culture conversion.[11,12]

The median age of the patients in this study was older than in the study of gene polymorphisms in TB susceptibility in Indonesia by Pakasi *et al*.,[13] with a median age of 27.5 years in TB case group and 33 years in the control group. A TB study in Papua by Kenangalem *et al*.[14] showed the average age of 26 years (21–35 years) while in non-Papua areas, it was 30 years (24–38 years). The study in Iran found the average age of their MDR-TB sample to be 44.2 ± 17.4 years.[15] At the same time, in other MDR-TB studies in other countries, the average age results were almost the same as those in this study, above 40 years old.[16]

The BMI results of the patients were generally in the underweight category (BMI <18.5), according to the WHO classification. In normal adults, protein catabolism and anabolism are equal, but in TB patients, protein catabolism is higher than anabolism.[17] There is a disturbance in the proliferation of lymphocytes during malnutrition as well as cytokine Th1 production including INF-γ, such that there is a decrease in gene expression and INF-γ.[18,19]

The results in this study were nearly the same as a +874 A/A polymorphism INF-γ study in Hongkong, China (45.7%), but lower than seen in a study in South Africa (47%). However, it was higher than in Spain (28%) and Sicily (26%). In the +874 A/A IFN-γ genotype, production by peripheral blood mononuclear cells decreased compared with T/A and T/T during the early diagnosis and after treatment was completed. The +874 IFN-γ A/A genotype increased the TB risk and was related to the severity level and TB reactivation.[20] A study in Egypt showed that the +874 A/A INF-γ genotype frequency was higher than the T/A and T/T genotypes in TB patients compared with healthy control participants.[4] In Shanghai, China, a higher frequency of patients with latent TB and pulmonary TB had the +874A INF-γ genotype.[21]

In this study of Javanese patients, we only found 1.64% (1 patient) with the IL-10 G/G genotype in the MDR-TB group and 4.3% (two patients) in the R-resistance patients; thus, allele A was higher than allele G. These results were similar to the results in other Asian countries which showed that the −1082 G/G IL-10 genotype was less frequent compared to European and African populations.

A study of the Han ethnic population in China showed the IL-10 −1082 G/A and G/G genotypes showed an increased protective effect against TB. A meta-analysis stratifying according to ethnicity showed the −1082 G/A IL-10 gene polymorphism correlated with an increased TB risk in the homozygote recessive group (G/G vs. G/A and A/A: OR = 1.69 and 95% CI: 1.19–2.39) in European populations but not in American, Asian, and African populations. Analyses sampling the risk of TB noted an increase ina homozygote model (G/G vs. A/A: OR = 2.00 and 95% CI: 1.16–3.45).[22] The −1082A IL-10 allele correlated with pulmonary TB severity and extrapulmonary TB susceptibility as well.[23]

The +874 T/A INF-γ gene polymorphism did not correlate with sputum culture time. Each genotype nearly reached point one while the −1082 A/A IL-10 gene polymorphism showed that −1082 A/A IL-10 needed 1.61 times more culture conversion time than the −1082 G/A and G/G

Table 1: Hazard ratio genotype polymorphism interferon–γ +874 and interleukin-10 −1082 with alella interferon–γ +874 and alella interleukin-10 −1082 to sputum conversion MDR-TB patients java ethnic

Variable	Hazard ratio	95% confidence interval	*P*
Interferon–γ +874 A/A	1.05	0.626–1.746	0.865
Interferon–γ +874 T/A	0.86	0.513–1.491	0.623
Interferon-γ +874 T/T	0.93	0.600–0.454	0.761
Interleukin-10 −1082 A/A	1.61	0.785–3.281	0.195
Interleukin-10 −1082 G/A	0.66	0.331–1.301	0.228
Interleukin-10 −1082 G/G	0.79	0.109−1.301	0.817
Interferon–γ +874 A/A+Interleukin-10 −1082A/A	2.88	1.030−8.028	0.040
Interferon–γ +874A/A+Interleukin-10 −1082G/A	1.92	0.907−4.081	0.088
Interferon–γ +874T/A+Interleukin-10 −1082A/A	5.11	0.634−41.128	0.125
Interferon–γ +874T/A+Interleukin-10 −1082G/A	2.30	1.069−4.950	0.033
Interferon–γ +874T/A+Interleukin-10 −1082G/G	2.94	0.374−23.154	0.305
Interferon–γ +874T/T+Interleukin-10 −1082A/A	2.94	0.693−13.559	0.166
Interferon–γ +874T/T+Interleukin-10 −1082G/A	0.44	0.223−0.870	0.018
Alella Interferon–γ +874A to AlellaInterferon–γ +874T	1.08	0.704−1.468	0.930
Alella Interleukin-10−1082A to Interleukin-10 −1082G	1.16	0.809−1.669	0.417
Alella			
Alella Interferon–γ +874A+Alella Interleukin-10 −1082A	1.10	0.768−1.582	0.598
Alella Interferon–γ +874T+Alella Interleukin-10 −1082A	1.11	0.654−1.876	0.704
Alella Interferon–γ +874A+Alella Interleukin-10 −1082G	0.81	0.505−1.302	0.387
AlellaInterferon–γ +874T+Alella Interleukin-10 −1082G	0.96	0.636−1.441	0.833

IL-10 genotypes. The survival analysis results showed the combination +874 A/A INF-γ and −1082 A/A IL-10 had a culture conversion time of 2.88 times, followed by +874T/A INF-γ and −1082G/A IL-10 of 2.3 times. A survival analysis showed that the combination of +874T/T INF-γ and −1082 G/A IL-10 the culture conversion time was the shortest (0.44 times). This results supported previous study results that showed that +874 T/T INF-γ and −1082 G/A IL-10 were protective genotypes against TB such that these MDR-TB patients needed shorter times for culture conversion. IL-10 −1082 A/A genotypes needed longer time to culture conversion than +874 A/A INF-γ genotypes. The results of this study could not be compared to other studies because, based on our knowledge, this was the first study in correlating polymorphisms and sputum culture conversion in MDR-TB patients. However, a study done in South Africa showed the progressivity of MDR-TB in IL-10 detection in the blood of patients.[24]

The results showing that the +874 A/A INF-γ and the −1082 A/A IL-10 genotypes had a long time to culture conversion were supported by a previous study that showed +874 A/A INF-γ and −1082 A/A IL-10 were susceptible genotypes to *M. tuberculosis*. *M. tuberculosis* decreases host cellular immune response to survive inside the host. The incremental production of IL-10 suppresses the immune response and increases TB progressivity.[25]

CONCLUSION

The +874 T/T IFN-γ and −1082 A/A IL-10 gene polymorphisms influence culture conversion (deceleration) and the +874 T/T IFN-γ and −1082 G/A IL-10 gene polymorphisms accelerate the culture conversion of Javanese MDR-TB patients.

What is known about this topic

- TB to be caused by *M. tuberculosis*
- In recent years, MDR-TB strains have emerged that are resistant to both INH and R with or without resistance to other anti-TB drugs
- There were 480,000 MDR-TB cases and 210,000 fatalities due to MDR-TB worldwide (2013).

What this study adds

- This study was conducted to MDR-TB suspected and generated through the status sputum conversion, epidemiological, and clinical data of patients
- This study may be additional information about the polymorphisms data of MDR-TB that can be used in other studies, in the else area.

AUTHORS' CONTRIBUTIONS

All the authors have read and agreed to the final manuscript.

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