**CCR2 V64I polymorphism in rifampicin resistant tuberculosis patients in Moewardi General Hospital Surakarta, Indonesia**

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**Abstract.** CC chemokine receptor-2 (CCR2) play important roles in inflammation. The CCR2 V64I polymorphism already reported associated with many diseases; however, the association of CCR2 V64I polymorphism with tuberculosis is still unknown. Also, there is no report about the presentation of CCR2 V64I polymorphisms in Indonesian tuberculosis patients with rifampicin-mono resistant status has ever been published, to the best of our knowledge. This study evaluated the presence of CCR2 V64I polymorphisms in Javanese rifampicin-mono resistant tuberculosis patients. In an ongoing molecular epidemiology study of human genomic polymorphisms and infections, 51 Javanese rifampicin-mono resistant tuberculosis patients in Dr. Moewardi General Hospital in Surakarta were enrolled in the study. The blood samples were aliquoted and fractionated. The nucleic acids were extracted from all blood samples and subjected to the CCR2 V64I polymorphisms detection by a polymerase chain reaction-sequence-specific primer (PCR-SSP) technique. PCR products were analyzed in 3% agarose. CCR2 64V and 64I homozygote were found in 23.5% (12/51) and 23.5% (12/51) blood samples, respectively. The CCR2 VI genotype was found predominant in Javanese rifampicin-mono resistant tuberculosis patients and may have an association with the clinical progression.

**1. Introduction**

Tuberculosis is a major threat to human health [1]. End tuberculosis strategy aims at ending the tuberculosis epidemic by 2030 [2]. However, treatment failure and resistance amplification are common among patients with rifampicin-resistant tuberculosis [3]. The emergence of drug-resistant tuberculosis, including that of the rifampicin-resistant, compromises global tuberculosis control [4].

Chemokines are low-molecular-weight proteins that play a key role in inflammatory processes [5]. With its receptors, both are crucial for an extensive immune response against infectious diseases such as tuberculosis [6]. Chemokines and its receptors are critical for initiating and coordinating the organized and sequential recruitment and activation of immune cells into Mycobacterium tuberculosis.
infected lungs [7]. CC chemokine receptor 2 (CCR2), a monocyte chemo attractant protein-1 (MCP-1) receptor, is important in the recruitment of immune cells as well as non-immune cells under pathological condition [8,9]. The genetic polymorphisms of CCR2 V64I may influence the susceptibility of some disease in Asian countries, however, there are no data on the CCR2 genotypes in rifampicin-mono resistant tuberculosis, for the best of our knowledge. We, therefore, aim to investigate the association of CCR2 V64I genetic polymorphism with tuberculosis, focusing on Javanese rifampicin-mono resistant tuberculosis patients in Dr. Moewardi General Hospital in Surakarta.

2. Materials and Methods

In this ongoing study 51 Indonesian Javanese tuberculosis patients with rifampicin-mono resistant status detected by the Gene Xpert system and confirmed by Drug Susceptibility Test at Dr. Moewardi General Hospital, Central Java province, Indonesia were enrolled. The clinical characteristics of these patients were assessed. Peripheral blood samples were obtained from all patients to examine their hematologic profiles. Since the study was a part of our research group (A-IGIC/A-Infection, Genomics, Immunology, & Cancer research group) molecular epidemiology study for blood-borne pathogens, all blood samples collected from the patients also subjected for immunological and molecular assays to find out the HIV, HBV, HCV, HDV, GBV-C, HTLV-1/2, and Toxoplasma gondii infection status as described previously [10-29]. Approval was obtained from the institutional ethical committee review boards of the Faculty of Medicine of Universitas Sebelas Maret and Dr. Moewardi General Hospital, Central Java province, Indonesia. Written informed consent was obtained from all study participants. All procedures were conducted according to the principles of the Declaration of Helsinki.

Genomic DNA was isolated from whole blood using the High Pure PCR Template Preparation Kit (Roche Applied Science, Mannheim, Germany). Then, the genomic DNA was used as a template for amplifications that were conducted using a common primer (5'-TGGAAAATAGGGCCACAGAC-3') and allele-specific reverse primers (5'-GGGCAACATGCTGGTCG-3' or 5'-TGGGCAACATGCTGGTCA-3') [30]. Fast Start HiFi PCR System dNTPack (Roche Applied Science) polymerase was used in the polymerase chain reaction-sequence-specific primer (PCR-SSP)reaction. For data interpretation, we conducted electrophoresis using a 2% agarose gel (100 V for 30 minutes), which was visualized using a UV trans-illuminator. All samples were tested at least twice.

3. Results and Discussion

A total of 51 Javanese tuberculosis patients with rifampicin-mono resistant status were subjected to genotypic analysis of CCR2 V64I polymorphisms status. The samples were derived from 25 (49.0%) men and 26 (51.0%) women aged 18–65 years old (mean: 41.6 ± 11.6 years). All patient bacterial cultures indicated the presence of Mycobacterium tuberculosis. Data for the patient body mass index (BMI) were obtained from 47 patients, with a mean BMI of 16.5 ± 3.4 kg/m². Most patients (34/47, 72.3%) were found underweight (BMI <18.5 kg/m²), whereas 27.7% (13/47) patients were normal weight (BMI = 18.5–24.9 kg/m²). Smoking history was found in 25.5% (13/51) patients. None of the patients had extrapolmonary tuberculosis. Relapse history was found in 21.6% (11/51) patients. Having a history of treatment failure of I and II category were found in 21.6% (11/51) and 47.1% (24/51) patients, respectively. Most patients had normal CD4+ T cell counts (>500 cells/μL, 36/51, 70.6%) and percentages (≥29%, 37/51, 72.5%). CD4+ T cell counts of 200–500 cells/μL was observed in 29.4% (15/51), and none had CD4+ T cell counts <200 cells/μL. CD4+ T cell percentages of 14–28% was found in 27.5% (14/51) patients, and none had <14%.

Data for the thorax radiographical examination were obtained for 90.2% (46/51) of rifampicin-mono resistant tuberculosis patients. Lung cavity, infiltration, fibrosis, fibrothorax, pleural effusion, atelectasis, and consolidation were observed in 39.1% (18/46), 89.1% (41/46), 52.2% (24/46), 2.2%
After two months, 65% (3/46), 2.2% (1/46), and 2.2% (1/46) patients, respectively. None of the patients suffered nodule, miliary TB, bulla, pneumothorax, or mass appearance.

During follow-up of MDR-TB patients, sputum conversion was observed mostly after two months of treatment (19/51, 37.3%). Other patients showed sputum conversion after one month (12/51, 23.5%), three months (6/51, 11.8%), and four months (2/51, 3.9%) of treatment. Four (7.8%) patients died before sputum conversion. CCR2 V64I and 64I homozygote were found in 23.5% (12/51) and 23.5% (12/51) patients, respectively. The CCR2 VI genotype was found in 52.9% (27/51) patients (Table 1).

Table 1. CCR2 V64I Genetic Polymorphism in Javanese Rifampicin-Mono Resistant Tuberculosis Patients

| BMI*                      | V/V (n= 12) | V/I (n= 27) | I/I (n= 12) |
|---------------------------|------------|------------|------------|
| Underweight (< 18.5 kg/m²) (n= 34) | 58.3 (7)   | 70.4 (19)  | 66.7 (8)   |
| Normal weight (18.5–24.9 kg/m²) (n= 13) | 33.3 (4)   | 22.2 (6)   | 25.0 (3)   |
| CD4+ T cell counts        |            |            |            |
| >500 cells/μL (n= 36)     | 75.0 (9)   | 70.4 (19)  | 66.7 (8)   |
| 200–500 cells/μL (n= 15)  | 25.0 (3)   | 29.6 (8)   | 33.3 (4)   |
| CD4+ T cell percentages   |            |            |            |
| ≥29% (n= 37)              | 75.0 (9)   | 74.1 (20)  | 66.7 (8)   |
| 14%–28% (n= 14)           | 25.0 (3)   | 25.9 (7)   | 33.3 (4)   |
| Relapse (n= 11)           | 0.0 (0)    | 33.3 (9)   | 16.7 (2)   |
| Treatment failure of I category (n= 11) | 33.3 (4) | 14.8 (4) | 25.0 (3) |
| Treatment failure of II category (n= 24) | 58.3 (7) | 40.7 (11) | 50.0 (6) |
| Thorax radiographically imaging |          |            |            |
| Cavity (n= 18)            | 25.0 (3)   | 37.0 (10)  | 41.7 (5)   |
| Infiltration (n= 41)      | 91.7 (11)  | 81.5 (22)  | 66.7 (8)   |
| Fibrosis (n= 24)          | 58.3(7)    | 44.4 (12)  | 41.7 (5)   |
| Fibrothorax (n= 1)        | 0.0 (0)    | 3.7 (1)    | 0.0 (0)    |
| Pleural effusion (n= 3)   | 0.0 (0)    | 7.4 (2)    | 8.3 (1)    |
| Atelectasis (n=1)         | 0.0 (0)    | 0.0 (0)    | 8.3 (1)    |
| Consolidation (n= 3)      | 0.0 (0)    | 3.7 (1)    | 0.0 (0)    |
| Sputum conversion (month)* |          |            |            |
| 1 (n= 12)                 | 25.0 (3)   | 7.4 (2)    | 58.3 (7)   |
| 2 (n= 19)                 | 50.0 (6)   | 44.4 (12)  | 8.3 (1)    |
| 3 (n= 6)                  | 8.3 (1)    | 18.5 (5)   | 0.0 (0)    |
| 4 (n= 2)                  | 8.3 (1)    | 0.0 (0)    | 8.3 (1)    |
| Died before sputum conversion (n= 4) | 0.0 (0) | 14.8 (4) | 0.0 (0) |

* missing data

Albeit the number patients involved in the present study was inadequate to perform statistical analysis (due to the difficult nature of the study condition), relapse was profound in patients with CCR2 I allele (Odds ratio [OR]=1.702, 95% CI:0.623-4.655, p= 0.299). Sputum conversion more than one month was also more frequent in patients with V allele (OR=1.684, 95% CI:0.685-4.144, p= 0.256). Taking all data together, CCR2 V64I genetic polymorphism may influence the clinical status of Javanese rifampicin-mono resistant tuberculosis patients; need further study to confirm the present finding.

The outcome of the disease is determined by a complex interaction among pathogen, host genetic variability, and surrounding milieu. Variation in expression or function of chemokines caused by
genetic polymorphisms could be associated with attenuated immune responses. Exploration of chemokine genetic polymorphisms in therapeutic response, gene regulation, and disease outcome is important [6]. Correct mononuclear cellular recruitment and localization are essential to ensure control of Mycobacterium tuberculosis growth without the development of diffuse and damaging granulocytic inflammation. Determination of how the key chemokines/cytokines and their receptors are balanced and how the loss of that balance can promote disease is vital to understanding tuberculosis pathogenesis and to identify novel therapies for effective eradication of this disease [7].

4. Conclusions
CCR2 V64I genetic polymorphism may influence the clinical status of Javanese rifampicin-mono resistant tuberculosis patients.

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