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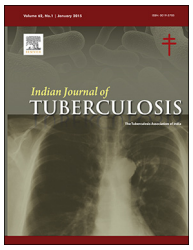

Original Article

CYP2E1 polymorphism, acetylator profiles and drug-induced liver injury incidence of Indonesian tuberculosis patients

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ABSTRACT

Objective: A polymorphism of *CYP2E1* may be directly associated with the development of INH hepatotoxicity. We conducted this study to evaluate the association between polymorphisms of *CYP2E1*, Isoniazid (INH) concentration and the acetylator status of INH in cases of Indonesian tuberculosis patients with drug-induced liver disease (DILI).

Methods: We conducted our study with a cohort design consisting of 55 Indonesian adult tuberculosis (TB) patients. Acetylating phenotypes were studied in using the metabolic ratio of plasma AcHZ/HZ. DILI was defined using CTCAV version 4.0. The allelic and genotypic frequency distributions of *CYP2E1* rs 3813867 were studied using the polymerase chain reaction – *amplification refractory mutation system* (ARMS) methodology.

Results: Patients with an INH concentration of more than 7 μg/mL showed a higher risk of developing DILI when compared with patients who showed a therapeutic range of 3–6 μg/mL INH (OR: 1.3, 95% CI: 0.2–8.2). Slow acetylators had a higher incidence of DILI when compared with rapid acetylators (OR: 4.6, 95% CI: 1.3–15.9). Meanwhile, subjects with GC had a higher risk of DILI incidence (OR: 4.3, 95% CI: 0.8–24.4).

Conclusion: Our study shows that polymorphisms of CYP2E1 and slow acetylator may have role in the DILI incidence.

1. Introduction

In 2015, the tuberculosis (TB) prevalence in the world reached 42% lower than in 1990. In 2014, of the 9.6 million of TB incidence, 58% were from South-East Asia and Western-Pacific region. Still, India, Indonesia and China had the largest number of cases from global total number (23%, 10% and 10%, respectively). The treatment success rate for newly diagnosed TB patients reached 86% in 2013 which was still sustained since 2015.[1] Treatment success is the key outcome of TB burden reduction. Some factors may influence the

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treatment success of TB, like hospital facilities, staffing education, population covered and centralized or decentralized health care system.[2]

The TB treatment regimen is a four-drug combination consisting of isoniazid (INH), rifampicin (R), pyrazinamide (Z), and ethambutol (E). This anti-tuberculosis therapy can cause adverse drug events. The most commonly reported adverse drug event of anti-tuberculosis drugs is hepatotoxicity.[3–5] Among these compounds, isoniazid (INH) strongly associated with anti-tuberculosis drug-induced hepatotoxicity (ATDH) or drug-induced liver injury (DILI). Previous reports showed INH induced liver injury or hepatotoxicity in approximately 5% of patients.[4–6] Other studies showed the development of ATDH in approximately 1 to 36% of patients.[7,8] INH is metabolized by *N-acetyltransferase-2* (NAT-2) into acetylisoniazid. Next, the metabolite is hydrolysed to *acetylhydrazine* (AcHZ) and *hydrazine* (HZ) by hepatic N-acetyltransferase-2 (NAT-2).[9] These compounds are then oxidized by cytochrome P450 2E1 (*CYP2E1*) to form intermediate hepatotoxins.[10,11]

There are large variations in the metabolism of the antituberculosis drug isoniazid, including polymorphisms of the *NAT-2* gene. This enzyme is markedly decreased in the livers of slow acetylators (SA). The elimination of INH follows a bimodal or trimodal distribution consisting of slow (SA), intermediate (IA) and rapid acetylators (RA). There is a strong correlation between these phenotypes and *NAT2* genotypes in Caucasians.[11–13] Previous studies have reported inconsistent results on whether slow or rapid acetylators are a risk factor for INH-induced hepatotoxicity.[9,13–15]

The objective of this study was to determine the association between *CYP2E1* polymorphisms and the development of DILI in Indonesians and determine the genotypes and phenotypes related to a change in the risk of DILI.

2. Materials and methods

2.1. Patients

This study used a cohort design. A total of 55 Indonesian adult patients with newly diagnosed TB at 20 Public Health Centres in the Provinces of Yogyakarta (10 Public Health Centres) and Lampung (10 Public Health Centres) were enrolled. The patient's recruitment was conducted from January until December 2013. The inclusion criteria included all adult patients (age >18 years) newly diagnosed as a pulmonary TB patient who were receiving category 1 TB medications and signed the informed consent for study participation. Exclusion criteria consisted of TB patients with HIV/AIDS, an AST and ALT two-fold higher than normal baseline concentrations, hepatitis or a history of hepatitis, a haemoglobin concentration <8 mg/dL, cessation of medication for more than 2 weeks, a history of kidney diseases, or refusal of blood sampling procedures and patients who refused to participate in this study.

All patients received a standard TB treatment for the first 2 months, including oral INH (300 mg), rifampicin (600 mg), pyrazinamide (20 mg/kg body weight), and ethambutol (800 mg). After 2 months of treatment, the patients were given INH and rifampicin for an additional 4 months. The total duration of antituberculosis treatment was 6 months. Serum alanine transaminase (ALT), aspartate transaminase (AST) and alkalyne phosphatase were measured before the antituberculosis therapy and then monthly until the end of treatment.

Genomic DNA was extracted from blood samples using a DNA Gene JET Genomic DNA Purification Kit (Thermo Scientific®, Nutrilab Pratama, Jakarta, Indonesia) according to the manufacturer's instructions. Analysis and Identification of SNP (−1055) C/G (Rs 3813867) was completed using an *amplification refractory mutation system* (ARMS) method. Two primer sets of *CYP2E1* (Rs 3813867) were used: forward I ''5-GTACAAAATTGCAACCTATG-3'' to detect the *CYP2E1* polymorphic gene (F-primer – 1), forward II ''5-GTAGAAAATTGCAACCTATG-3'' to confirm the normal gene fragment and (F-primer – 2) reverse ''5-ATCTTGTCTTTGTTGATCCC-3'' (Genebank accession number P05181). The cycling conditions involved preliminary denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final elongation step at 72 °C for 2 min. The PCR products of single nucleotide polymorphism (SNP) (−1055) C > G (rs3813867) were obtained for 55 subjects. The results showed a band at 230 bp. After electrophoresis using a 2% agarose gel, samples were observed under UV light and imaged. Samples with homozygote CC (cytosine-cytosine) appeared using primer set Forward 1-Reverse (F1-R), samples with homozygote GG (guanine-guanine) appeared using primer set Forward 2-Reverse (F2-R), and samples with homozygote CG (cytosine-guanine) appeared using both primer sets F1-R and F2-R.

Venous blood samples were collected 2 h after drug administration. Determination of acetylator status was made using the AcHZ/HZ ratio with a *cut-off point* of 15.00. A slow acetylator (SA) was defined as an AcHZ/HZ ratio ≤ 15.00, and a rapid acetylator (RA) was defined as an AcHZ/HZ ratio >15.[16] The AST and ALT levels were measured with an automatic chemical analyser. Isoniazid and its metabolites, HZ and AcHZ, were measured using HPLC. Statistical significance was analyzed using correlation tests. DILI incidence was defined as an ALT and/or AST level above the upper limit normal value (ULN) listed in Common Toxicity Criteria for Adverse Events version 4.0 (CTCAE v. 4). The normal values of ALT and AST are 0–50 mg/dL and 0–33 mg/dL, respectively, for males. The normal values for ALT and AST are 0–34 mg/dL and 0–27 mg/dL, respectively, for females. Increased ALT and/or AST or an increased ALP were categorized as grade 1+, (>1.0–2.5 × ULN), grade 2+ (>2.5–5.0 × ULN), grade 3+ (>5.0–20 × ULN), or grade 4+ (>20.0 × ULN).[17]

This study was approved by the National Ethics Committee of the National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, and written informed consent was obtained from all participants.

2.2. Statistical methods

Statistical analysis was conducted using the SPSS® statistical package, version 16.0. The odds ratio (OR) and confidence interval (CI) were calculated using binary logistic regression analysis. This analysis evaluated the risk and association of DILI, INH and acetylator status. Allele frequencies were

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