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CYP2E1 genotype and transaminase level of tuberculosis patients receiving fixed dose combination of antituberculosis

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ABSTRACT

Introductions: Antituberculosis drug-induced liver injury (ATLI) had become a common serious side effect regarding anti-tuberculosis use. Isoniazid (INH) was believed as a significant factor related to ATLI incidence. A genetic factor related to INH metabolism (e.g., CYP2E1) was assumed as a major contributor of ATLI. This study aimed to investigate the genotype pattern of CYP2E1 and serum transaminase level on tuberculosis patients receiving a fixed-dose combination of anti-tuberculosis.

Methods: As many as 35 tuberculosis patients attending Pulmonary Outpatient Clinic of Sanglah Hospital were included in this cross-sectional study. Identification of CYP2E1 genotype was performed with a PCR-RFLP assay using *RsaI* and *DraI* restriction enzymes.

Results: This study revealed the proportion of c1/c1; c1/c2; and c2/c2 genotype of CYP2E1 on 5'-flanking region were 62.9%; 34.3%; and 2.8%, respectively; whereas the proportion of DD, CD and CC genotype of CYP2E1 on intron 6 were 60%; 28.6%; and 11.4%, respectively. The proportion of hepatotoxicity was 14.3%, while the average level of AST and ALT were 23.5±13.6 IU/L and 23.3±21.1 IU/L. There was no significant correlation between CYP2E1 genotypes and hepatotoxicity incidence.

Conclusions: The dominant proportion of CYP2E1 genotype on 5'-flanking region and intron 6 are c1/c1 and DD. However, we found no significant differences between CYP2E1 genotypes and hepatotoxicity.

Keyword: antituberculosis drug-induced liver injury, isoniazid, CYP2E1 genotype
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INTRODUCTIONS

World Health Organization (WHO) reported that about 9 millions of new tuberculosis cases were found worldwide in 2013 and 1.5 millions of death caused by tuberculosis in the same year. More than half tuberculosis cases were found in Asia, approximately 95% in developing countries. Indonesia had become the third country with the highest number of tuberculosis cases after India and China.[1,2]

Antituberculosis drug-induced liver injury (ATLI) had become the most frequent side effect of antituberculosis use, and might potentially cause the serious effect to patient's health.[3] The incidence of ATLI showed many variations in different populations: 15.6% in Brazil; 48.4% in China; 32.1% in Taiwan; and 18.8% in India.[3-6] Isoniazid was assumed as anti-tuberculosis that most responsible for ATLI incidence.[3,7-9]

Many factors could increase the risk of hepatotoxicity due to anti tuberculosis use, one of which is a genetic factor, primarily involved in antituberculosis metabolism. Cytochrome P450 2E1 (CYP2E1) gene is one of these important genetic factors which specifically involved in INH metabolism.[3,4,5]

Several studies about the association between CYP2E1 genotype and hepatotoxicity had been conducted, but the results remained contradictive. Studies in India and Brazil revealed that there was no significant correlation between CYP2E1*5B genotype and ATLI incidence.[3,6] Several studies carried out in China showed opposite result that CYP2E1*5B genotype c1/c1 significantly increased the incidence of ATLI.[4,5,9] A meta-analysis conducted by Sun *et al.* (2008) had revealed similar result that c1/c1 genotype of CYP2E1*5B together with GSTM1 null and NAT2 slow acetylator significantly increased ATLI incidence.[10] A study conducted in India showed that the proportion of *DraI* polymorphism on CYP2E1*6 together with slow acetylator of NAT2 genotype was higher in drug-induced hepatitis subjects compared to non-hepatitis subjects.[6]

To date, no adequate information has been found yet regarding the proportion of CYP2E1 5'-flanking region (CYP2E1*5B) and intron 6 (CYP2E1*6) genetic variation in tuberculosis patients in Indonesia and Bali. Since CYP2E1 genetic variation had a substantial effect on ATLI incidence, a study investigating CYP2E1 genotype and serum transaminase level in tuberculosis patients is necessary to conduct.

RESEARCH DESIGN AND METHODS

Patients Selection

This study was a cross-sectional design. The subjects of this study were 35 tuberculosis patients attended Pulmonary Outpatient Clinic of Sanglah Hospital between July 2014 to February 2015 and received a fixed-dose combination of anti-tuberculosis category 1. Subjects were selected using a consecutive sampling technique.

CYP2E1 Genotyping

DNA was isolated using guanidine isothiocyanate method. The PCR-RFLP assay was used to detect mutation on 5'-flanking region and intron 6 of CYP2E1 gene. The sequences of CYP2E1*5B forward and reverse primer was 5'-CCAGTCGAGTCTACATTGTCA-3' and 5'-TTCATTCTGTCTTCTAACTGG-3', respectively; whereas the sequences for CYP2E1*6 forward and reverse primer was 5'-TCGTCAGTTCCTGAAAGCAGG-3' and 5'-GAGCTCTGATGGAAGTATCG CA-3'. For CYP2E1*5B and CYP2E1`*6, DNA chains were denatured at 94°C for 5 minutes, followed by 35 cycles of reaction (94°C denaturation for 45 seconds, 57°C annealing for 45 seconds, 72°C elongation for 45 seconds); ended by a final extension at 72°C for 5 minutes. PCR product was digested using *RsaI* and *DraI* restriction enzyme (*New England Biolabs*). The mixture was incubated at 37°C for 90 minutes. Electrophoresis of PCR-RFLP product use 2% of agarose gel. Wild-type c1/c1; heterozygote mutant c1/c2; and homozygote mutant c2/c2 showed two bands (352 and 61 bp); 3 bands (413, 352 and 61 bp); and one band (413 bp) on electrophoresis visualization, respectively. W*ild-type* (DD), heterozygote mutant (CD); and homozygote mutant (CC) showed three bands (600, 300 and 100 bp); 4 bands (900, 600, 300 and 100 bp), and two bands (900 and 100 bp), respectively.

Serum Transaminase Level Measurement

The measurement of serum transaminase level measurement was carried out at Clinical Pathology Laboratory of Sanglah Hospital using spectrophotometry method. Serum transaminase level above ULN of AST and/or ALT was considered to be hepatotoxic.

Statistical Analysis

Chi-square test was used to detect a significant association between patients' characteristics or CYP2E1 genotypes and hepatotoxicity incidence. The analysis was performed using statistical software. The p-value below 0.05 was considered to be statistically significant.

RESULTS

Subject Characteristics

As many as 35 tuberculosis patients were included in this study. Subject characteristics were shown in Table 1.

Genetic pattern of CYP2E1 on 5'-flanking region was determined by electrophoresis visualization (Figure 1). Based on electrophoresis result, the proportion of c1/c1; c1/c2 and c2/c2 genotype were 62.9%; 34.3%; and 2.8%, respectively.

Genetic pattern of CYP2E1 on intron six was determined by the electrophoresis visualization (Figure 2). Based on electrophoresis result, the proportion of DD; CD and CC genotype were 60%; 28.6%; and 11.4%, respectively.

The average level of AST and ALT of subjects were 23.5±13.6 IU/L and 23.3±21.1 IU/L. Around 14.3% subjects showed the elevation of AST and/or ALT level. From that number, only one subject (2.9%) showed AST and/or ALT elevation 3 to 5 times ULN. Hepatotoxicity incidence based on subject characteristics and CYP2E1 genotypes were presented in table 2. There was no significant correlation between hepatotoxicity incidence and subject characteristics or CYP2E1 genotypes.

Table 1 Subject Characteristics

No	Subject Characteristics	n (%)
1	Sex	
	Male	20 (57.1)
	Female	15 (42.9)
2	Age	
	< 30 y.o	16 (45.7)
	≥ 30 y.o	19 (54.3)
3	Initial BTA status	
	Positive	21 (60)
	Negative	14 (40)
4	Alcohol consumption	
	Yes	2 (5.7)
	No	33 (94.3)
5	Comorbid disease	
	Yes	0 (0)
	No	35 (100)
6	Other medication	
	Yes	7 (20)
	No	28 (80)

Table 2 Hepatotoxicity incidence based on subject characteristics and CYP2E1 genotype

No.	Subject Characteristic	Hepatotoxic n (%)	Non hepatotoxic n (%)
1	Age		
	< 30 y.o	2 (5.7)	14 (40)
	≥ 30 y.o	3 (8.6)	16 (45.7)
2	Sex		
	Male	2 (5.7)	18 (51.4)
	Female	3 (8.6)	12 (34.3)
3	Initial BTA status		
	Positive	3 (8.6)	18 (51.4)
	Negative	2 (5.7)	12 (34.3)
4	Alcohol consumption		
	Yes	1 (2.9)	1 (2.9)
	No	4 (11.4)	29 (82.9)
5	Comorbid disease		
	Yes	0 (0)	0 (0)
	No	5 (14.3)	30 (85.7)
6	Other medication		
	Yes	2 (5.7)	5 (14.3)
	No	3 (8.6)	25 (71.4)
7	Genotip CYP2E1*5B		
	Wild type	3 (8.6)	19 (54.3)
	Mutant	2 (5.7)	11 (31.4)
8	Genotip CYP2E1*6		
	Wild type	1 (2.9)	13 (37.1)
	Mutant	4 (11.4)	17 (48.6)

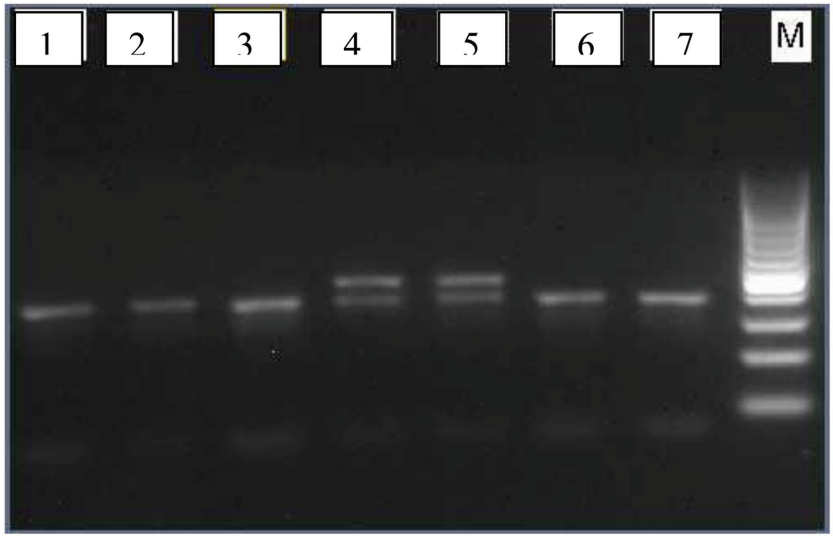

Figure 1 Electrophoresis visualization of PCR-RFLP product of CYP2E1*5B
M: marker; Sample no. 1, 2, 3, 6 and 7 showed two bands 352 and 61 bp (wild type c1/c1);
Sample no. 4 and 5 showed 3 bands 413, 352 and 61 bp (heterozygote c1/c2)

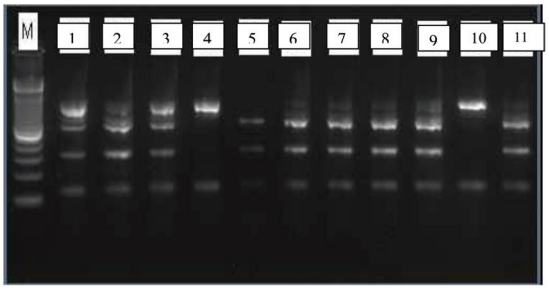

Figure 2 Electrophoresis visualization of PCR-RFLP product of CYP2E1*6
M: marker; Sample no. 5, 6, 7, 8, 9 and 11 showed 3 bands 600, 300 and 100 bp (wild type DD);
Sample no. 1, 2, and 3 showed 4 bands 900, 600, 300 and 100 bp (heterozygote mutant CD);
Sample no. 4 and 10 showed 2 bands 900 and 100 bp (homozygote mutant CC)

DISCUSSIONS

Isoniazid (INH) is the most important factor for causing hepatotoxic effect after anti-tuberculosis medication. On INH metabolism, CYP2E1 enzyme together with NAT2, GSTM1, and GSTT1 functioned to catalyze phase I metabolism of INH in the liver. INH is a prodrug that required further biotransformation into the active form acetyl-INH (catalyzed by an NAT2 enzyme) and hydrazine. Thus hydrazine and acetyl-INH would be converted into acetyl hydrazine and furthermore into diacetylhydrazine (by NAT2). CYP2E1 also converted acetyl hydrazine into a toxic metabolite that required detoxification first (by GST enzyme) before excreted.[11,12,13]

Human CYP2E1 gene is located on chromosome 10 specifically at region 10q24.3. This gene consists of 11,413 bp with nine exons and a specific TATA box. Many polymorphisms have been detected from many studies, including those which affecting protein expression on transcription, splicing, translation, or post-translational modification. The common genetic variations found on CYP2E1 were SNP at 5'-flanking region and intron 6 of CYP2E1 gene (CYP2E1*5B -1293G>C, -1053C>T and CYP2E1*6 7632T>A). These mutations were assumed to be very important in the ATLI incidence.[14]

Compared to the proportion of CYP2E1 genotype on tuberculosis patients in other populations, this study showed a similar result to those conducted in China and Japan. The percentage of

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wild-type c1/c1 genotype and mutant genotype in Chinese tuberculosis patients were 61-71% and 28-35%, respectively.[4,10,15] Similar to that result, the proportion of wild-type CYP2E1*5B in Japan was 62%, whereas the remains showed mutant genotype.[13] Slightly different from the proportion in Asia, the percentage of c1/c1 genotype in Brazil was much higher (90.4%).[3]

For CYP2E1*6 genotype proportion, this study result was similar to a study conducted by Sun et al. (2008) in China.[10] A Higher proportion of *DraI* polymorphism was found in Japan whereas lower proportion was found in Turkey.[13,14]

Regarding the AST and/or ALT elevation on tuberculosis patients receiving antituberculosis medication, this study revealed similar result to those conducted in other countries especially in Asia. The proportion of ATLI incidence on some studies in China showed variable results. Studies carried out by Lv *et al.* (2012); Huang *et al.* (2002); Wang *et al.* (2010); Lee *et al.* (2010) showed the proportion of ATLI were 20%; 14.7%; 48.4%; and 32.1%, respectively.[4,5,7,8,16] The percentage of ATLI incidence in India was 18.8% whereas in Brazil was 15.6%.[3,6] It was also stated that as many as 5% patients receiving a fixed-dose combination of anti-tuberculosis HRZ would show an increase in serum transaminase level 3-5 times ULN without clinical manifestation, and this commonly occurs in the first two months of treatment.[17,18] This study revealed that only one subject (2.9%) showed an increase in the serum transaminase level 3 to 5 times of ULN.

CYP2E1 gene had also been considered to correlate with the risk of cancer. Allele c2 (mutant) of CYP2E1 was reported to decrease the risk of colon adenoma in Japan.[19] Contrary to this result, study by Aydin-Sayitoglu *et al.* (2006) indicated that CYP2E1*5B polymorphism increased the risk of acute leukemia (ALL and AML) 3.6 and 3.9 times compared to healthy subjects.[20]

Previous studies regarding CYP2E1 gene and its association to ATLI had been conducted in many populations. A study in India revealed no significant correlation between *RsaI* polymorphism and drug-induced hepatitis.[6] Contrary to that result, a study by Teixeira *et al.* in Brazil (2011) reported that if CYP2E1 activity increased, the risk of hepatotoxicity would be higher. Genotype 1A/1A (wild type) had been considered to be a genetic marker for ATLI in this study. CYP2E1 genotype c1/c1; c1/c2; c2/c2 in hepatotoxic Brazilian subject was found 88.5%; 7.7%; and 3.8%, respectively.[3] This result was also confirmed by Cai *et al.* and Lee *et al.* (2010).[5,9] CYP2E1 genotype c1/c1 was reported to cause an increase in AST or ALT more than three times ULN following two-month-anti tuberculosis treatment and was considered to cause more serious manifestation of ATLI.[5] Similar to those result, study in China by Wang *et al.* revealed that CYP2E1 genotype was a potential risk factor for the occurrence of ATLI.[4] Those results were also supported by Sun *et al.* A meta-analysis conducted by Sun *et al.* revealed that c1/c1 genotype together with GSTM1 null and NAT2 slow acetylator significantly increased ATLI incidence.[10] Study conducted by Bose *et al.* (2011) revealed that *DraI* polymorphism in drug-induced hepatitis (DIH) subjects in India were significantly higher than that in non-hepatitis subjects (85.4% vs. 64.4%).[6] Slow acetylator genotype of NAT2 together with CD and CC genotype of CYP2E1*6 showed higher proportion of DIH subjects compared to non-DIH subjects (65.85% vs. 28.81%).[6]

CONCLUSIONS

The dominant proportion of CYP2E1 genotype on 5'-flanking region and intron 6 of tuberculosis patients receiving antituberculosis are c1/c1 and DD. There is no significant correlation between hepatotoxicity incidence and subject characteristics or CYP2E1 genotypes.

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