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Research Article

GENOTYPE POLYMORPHISMS OF *NAT2* AND *CYP2E1* GENES ASSOCIATED WITH DRUG INDUCED LIVER INJURY (DILI) IN INDONESIAN TUBERCULOSIS PATIENTS

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ABSTRACT

Currently, Indonesia is in the fifth rank of highest TB prevalence over the world. One of the TB problem is low patients' adherence due to the oral antituberculosis induced hepatotoxicity. Polymorphisms of *NAT2* and *CYP2E1* genes had important role in the isoniazid (INH)-induced hepatotoxicity. The aim of this study was to evaluate the polymorphisms profile of *NAT2* and *CYP2E1* genes associated with hepatotoxicity induced by INH. We used cohort design in Public Health Centers and Lung Clinics of Yogyakarta and Lampung. The inclusion criteria were adult subjects (> 18 yo), newly diagnosed TB and treated by oral antituberculosis, normal function of renal and live and willingness to participate in this study. Subjects were excluded when having positive reaction of HbsAg test, history of HIV and abnormality of renal and liver function. The SNPs of *NAT2* and *CYP2E1* were designed using IPlex method of DNA sequenom. Among 57 TB patients, we found 14 patients with higher INH serum concentration and experienced increase of ALT-AST. Subjects with SNPs of rs 2070676, rs 1329149, rs 3813867, rs 6413432, rs 8192772, rs 2031920, rs 2515641, rs 8192775, rs 915908 of *CYP2E1* experienced increase of ALT and AST. Subjects with SNPs of rs 1799930, rs1799931, rs1801279, rs1801280, rs1799929 , rs1208, rs1041983 of *NAT2* are associated with the increase of ALT and AST. The polymorphisms of *CYP2E1* and *NAT2* may have a role in the mechanisms of INH induced DILI.

Key words: CYP2E1, NAT2, tuberculosis, isoniazid, Indonesia

INTRODUCTION

Tuberculosis (TB) is still become the high burden in Indonesia with the incidence of around 300.000 in 2014. Patients adherence to TB treatment is important to get the effective treatment and to prevent the occurence of multidrug resistent TB (MDR). Some factors which could influence patients' adherence are forgetfullness, lack of knowledge, herbal medicine use, feeling better and drug side effects (Tesfahuneygn *et al*., 2015).

Hepatotoxicity is the most frequent of drug side effects during the TB treatment. This side effect may cause the decrease of adherence and patients' quality of life (Babalik *et al*., 2012). Previous study in India showed that Drug-induced Liver Disease (DILI) was appeared in 3.8% and patients' characteristics such as older age and alcohol intake could predict the development of DILI (Gaude *et al*., 2015). Moreover, our previous study showed that around 7.5% TB patients were considered as experiencing early DILI (Atthobari *et al*., 2013). Other previous study showed that gender, ethnic and acetylator status of *NAT2* gene could predict the development of hepatotoxicity (Chamorro *et al*., 2013).

Currently, many pharmacogenetic studies in ethnicities around the world show some evidences about the association between polymorphisms of *NAT2, CYP2E1* and *GST1* genes with INH-induced hepatotoxicity (Perwitasari *et al*., 2014; Santos *et al*., 2012; Guaoua *et al*., 2014; An *et al*., 2101). The study in Moroccans population revealed that the most prevalent phenotype of *NAT2* and *CYP2E1* was slow acetylators (72.39%) which had hepatotoxicity risk (Guaoua *et al*., 2014).

In Brazilian population, the hepatotoxicity was experienced by 6.7% patients and there was significant association between slow acetylators of *NAT2* and *CYP2E1* genes and the hepatotoxicity risk (Santos *et al*., 2012).

NAT2 haplotypes which have decrease of enzyme function due to the slow acetylator status are NAT2*5B, NAT2*6A, dan NAT2*7B (Higuchi *et al.,* 2007). Some SNPs (Single Nucleotide of Polymorphisms) of *CYP2E1* which are supposed to have correlation with antituberculosis-induced hepatotoxicity are rs 2070676, rs 1329149, rs 3813867, rs 6413432, rs 8192772, rs 2031920, rs 2515641, rs 8192775, rs 915908 (Krishnakumar *et al.,* 2010). In Indian population, the subjects with GST M1 null and combined GST M1 and GST T1 had significant association with the hepatotoxicity risk (Gupta *et al*., 2013). Moreover, some genes, such as HLA, UGT, NOS, BACH and MAFK were also predicted as genes associated with anti-tuberculosis induced hepatotoxicity due to the expression of antioxidant enzymes (Perwitasari *et al*., 2014).

Our study objectives was to evaluate the polymorphisms profile of *NAT2* and *CYP2E1* genes associated with INH- induced DILI.

MATERIAL AND METHODS

We used cohort design with adult-newly diagnosed TB patients treated with oral antituberculosis as Fixed-Dose Combination (Rifampicin, Isoniazid, Pyrazynamide and Ethambutol) at Public Health Centers and Lung Hospitals of Yogyakarta and Lampung. Patients' characteristics data and laboratory results, including INH serum concentration were taken from the medical record.

The SNPs of *NAT 2* and *CYP2E1* genes were designed using Sequenom IPlex SNP Genotyping and also according to the previous studies (Krishnakumar *et al.,* 2010; Guaoua *et al.,* 2014; Mishra *et al*., 2013; Sheng *et al*., 2014; Xiang *et al*., 2014; Rana *et al*., 2014; Gupta *et al*., 2013; Santos *et al*., 2013; Lv *et al*., 2012; Yamada *et al*., 2009) . SNPs of *CYP2E1* were rs 2070676, rs 1329149, rs 1410897, rs 1961456, rs 2070675, rs 2070677, rs 2408258, rs 2515642, rs 3813867, rs 6413432, rs 7092584, rs743535, rs 8192772, rs 915906, rs 2031920, rs 2515641, rs 8192775, rs 2249694, rs 2249695, rs 2480259, rs 743534 and rs 915907. SNPs of *NAT2* were rs 6984200, rs1208, rs1799929, rs1799931, rs 1799930, rs1801279 and rs1801280.

Inclusion criteria of this study were adult-newly diagnosed patients treated with oral antituberculosis, normal function of renal and liver at baseline measurement. Subjects were excluded when having HIV and diabetes mellitus history, liver abnormality history, abnormality of renal and liver function, reactive results of HBsAg test. DILI was defined as the level of ALT and AST was above the upper limited number of ALT and AST or ALT or AST.

This study has been approved by National of Ethics Committee, National Health Institute, Jakarta. All subjects received the information about the study and signed the consent form.

Data was analyzed descriptively and linear regression was performed to understand the association of between AST-ALT and INH.

RESULTS AND DISCUSSION

We recruited 57 adult-newly TB patients with the age average is 38.11 (SD= 14.57) and body weight avergae is 48.96 (SD=1.2). Most of the patients are male (63.16%). The average of AST and ALT measured in the end of the intensive treatment are 27.23 U/L (SD=13.36 U/L) and 21.25 U/L (SD=13.14). However, the average of ALT and AST increased at the end of the intensive treatment are 51.42 (SD=7.43) and 90.47 (SD=41.7), respectively. The average of INH serum concentration was 14.22 μg/ml (SD=7.08) μg/mL.

The linear regression test revealed the significant association between ALT-AST level and INH serum concentration ($p<0.05$; data not shown). The association shows that the higher ALT-AST, the higher INH serum concentration. This finding is in line with previous study which stated that there is significant correlation between ALT-AST level and INH serum concentration (Nelwan *et al*., 2014). In contrast, study of Jeong *et al* (2015), informed that there were no significant differences of antituberculosis serum levels between groups with and without hepatotoxicity.

Table I. Most frequent genotype in each SNPs of *NAT2* and *CYPP2E1* associated with DILI

SNPs	Genotype	%
NAT2		
rs1208	AA	70.0
rs1799929	CC	74.0
rs1799931	GG	54.0
rs1801279	GG	92.0
rs1801280	TT	66.0
rs 6984200	AT	34.0
rs 11996129	CT	38.0
CYP2E1		
rs 2031920	CC	72.0
rs 2515641	CC	44.0
rs 8192775	GG	60.0
rs 6413432	TT	52.0
rs 8192772	TT	46.0
rs 915908	AA	10.8

Table II. Variants of SNPs in *NAT2* gene in Indonesia and Moroccan population associated with DILI

SNP	Genotype	
	Indonesian population	Moroccan population*
rs1208, NAT*12A	AA	A > G
rs1799929, NAT2*11	CC	C > T
rs1799931, NAT2*7	GG	G > A
rs1801279 , NAT2*14	GG	G > A
rs1801280 , NAT2*5	TT	T > C

*(Guaoua *et al.,* 2014)

In the other hand, there were significant differences of metabolic ratio of acetyl INH and INH. The metabolic ratio of acetyl INH and INH was lower in the hepatotoxicity group than in the non-hepatotoxicity group.

Of the 57 patients, there are 14 patients (24.56%) who had INH serum level above the MTC and the increase of ALT-AST. Table I presents the most frequent genotypes in each SNPs of *NAT2* and *CYP2E1* genes.

According to the previous studies, the SNPs of the NAT2 and CYP2E1 genes are mostly associated with DILI in multiethnic studies (Guaoua *et al*., 2014; Mishra *et al*., 2013; Sheng *et al*., 2014; Xiang *et al*., 2014; Rana *et al*., 2014; Gupta *et al*., 2013; Santos *et al*., 2013; Lv *et al*., 2012; Yamada *et al*., 2009)

Table II lists the different variants of SNPs in *NAT2* gene in Indonesia and Moroccan population which associated with DILI.

Among the SNPs rs 1208, rs 1799929, rs 1799931, rs 1801279 and rs 1801280, the genotypes in Indonesia population which associated with DILI are different from genotypes of Moroccan. In NAT2*12A, the subjects with A>G had risk to experience DILI. The pattern presents in NAT2*11, *7, *14 and *5 in Moroccan population. In our study, we did not find variants of rs 1041983, (NAT2*13A) which had higher risk of DILI in Moroccan population (Guaoua *et al*., 2014). NAT2*5 and *7 were known as the slow acetylator in Asian Population (Xiaozhen *et al*., 2012). However the NAT2*11 and *12 A were assigned as rapid acetylator (Sharma *et al*., 2010). According to the phenotypes of NAT2*12A, *11, *7, *5 and *14, most of our patients in this study are slow acetylator (54%).

Table III. Genotype variants of CYP2E1 gene in Indonesia and China population which associated with DILI

SNP	Genotype	
	Indonesian population	China population*
rs2031920	CC	CC
rs2515641	CC	CC
rs8192775	GG	AG

*(Tang *et al.,* 2013)

There are two SNPs of *NAT2* in our study, rs 6984200 and rs 11996129, which are associated with high level of ALT-AST and the high INH serum concentration. To the best of our knowledge, we cannot find previous studies which discussed about these two SNPs.

Table III presents the differences of genotype variants between Indonesia and China population which associated with DILI.

Some of our study results have similar results to China population. For SNPs rs2031920 and rs2515641 of *CYP2E1*, we found that subject with CC genotype developed DILI. Also in China, around 63.2% of CC experienced the increase of ALT and AST. Eventhough there are no significant differences of hepatotoxicity and between the variants, however, this findings support the evidence about the genotype which could cause DILI (Tang *et al.,* 2013).

The SNPs of rs 8192775, rs1329149 and rs3813867 show different genotype variants which have role in DILI which are GG, CT and GG, respectively between Yogyakarta and Lampung. The study findings of Tang *et al.* (2013), Yang *et al.* (2009) and Costa *et al.* (2012) show different genotype of DILI, which are AG, TT and CC, respectively.

The TT genotype of rs 6413432 in Indonesia and India population has a role in DILI. According to the rs 8192772, in Indonesia population, the TT genotype may cause DILI, however in India population, there were no genotype of this SNP which caused DILI . According to rs 207076, this SNP was not associated with DILI in Indonesia population, however in India, the genotype CC may cause DILI (Krishnakumar *et al,* 2010).

We cannot find the genotype of rs15908 in Indonesia which could induce DILI. But we found in China population, the GG genotype may cause DILI.

Our study has limitation due to the small sample sizes. Future studies with bigger sample size should be conducted. However, we can confirm that our study findings show that polymorphisms of *NAT2* and *CYP2E1* genes in Indonesia population may cause DILI during the INH treatment. There are 24.5% TB patients experienced increased of ALT-AST with high INH serum concentration.

Comparing our results with previous study in China and Morroccan, some genotypes of SNPs in *CYP2E1* and *NAT2* which can cause DILI in Indonesian population are similar. The prevalence of drug induced hepatotoxicity in India is quite high. Thus, health proffesionals should be aware with this explanation to educate the patients about hepatotoxicity symptoms and to monitor patients' condition during TB treatment.

CONCLUSION

In this study, we found significant association between INH serum concentration and increased evel of ALT-AST. The SNPs of NAT2 gene which associated with DILI are rs1799931, rs1801279, rs1801280, rs1799929, rs1208. Moreover, the rs2070676, rs1329149, rs3813867, rs6413432, rs8192772, rs2031920, rs2515641, rs8192775 of CYP2E1 gene are associated with DILI.

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