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

Dyah Aryani Perwitasari1*, Malinda Noverliyanti2, Endang Darmawan1, Ully Adhi Mulyani2, Jarir Atthobari3, Bob Wilffert4

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 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 rs2070676, rs1329149, rs3813867, rs6413432, rs8192772, rs2031920, rs2515641, rs8192775, rs915908 of CYP2E1 experienced increase of ALT and AST. Subjects with SNPs of rs1799930, 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 occurrence of multidrug resistant TB (MDR). Some factors which could influence patients’ adherence are forgetfulness, 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., 2011). 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 N-AT2 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 rs2070676, rs1329149, rs3813867, rs6414342, rs8192772, rs2031920, rs2515641, rs8192775, rs915908 (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 MAPK were also predicted as genes associated with antituberculosis induced hepatotoxicity due to the expression of antioxidant enzymes (Perwitasari et al., 2014).

Our study objectives was to evaluate the polymorphisms profile of N-AT2 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 N-AT 2 and CYP2E1 genes were designed using Sequenom iPLEX SNP Genotyping and also according to the previous studies (Krishnakumar et al., 2010; Guoaua 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 rs2070676, rs1329149, rs1410897, rs1961456, rs2070675, rs2070677, rs2408258, rs2515642, rs3813867, rs6414342, rs7092584, rs743535, rs8192772, rs915906, rs2031920, rs2515641, rs8192775, rs2249694, rs2249695, rs2480259, rs743534 and rs915907. SNPs of N-AT2 were rs6984200, rs1208, rs1799929, rs1799931, rs1799930, 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 diagnosed TB patients with the age average is 38.11 (SD= 14.57) and body weight average 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.
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 \( \text{NAT2} \) and \( \text{CYP2E1} \) genes.

According to the previous studies, the SNPs of the \( \text{NAT2} \) and \( \text{CYP2E1} \) 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 \( \text{NAT2} \) 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 \( \text{NAT2}^{*}12A \), the subjects with A>G had risk to experience DILI. The pattern presents in \( \text{NAT2}^{*}11, *7, *14 \) and *5 in Moroccan population. In our study, we did not find variants of rs 1041983, \( \text{NAT2}^{*}13A \) which had higher risk of DILI in Moroccan population (Guaoua et al., 2014).

\( \text{NAT2}^{*}5 \) and *7 were known as the slow acetylator in Asian Population (Xiaozhen et al., 2012). However the \( \text{NAT2}^{*}11 \) and *12 A were assigned as rapid acetylator (Sharma et al., 2010). According to the phenotypes of \( \text{NAT2}^{*}12A, *11, *7, *5 \) and *14, most of our patients in this study are slow acetylator (54%).

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**Table I.** Most frequent genotype in each SNPs of \( \text{NAT2} \) and \( \text{CYP2E1} \) associated with DILI

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotype</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{NAT2} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1208</td>
<td>AA</td>
<td>70.0</td>
</tr>
<tr>
<td>rs1799929</td>
<td>CC</td>
<td>74.0</td>
</tr>
<tr>
<td>rs1799931</td>
<td>GG</td>
<td>54.0</td>
</tr>
<tr>
<td>rs1801279</td>
<td>GG</td>
<td>92.0</td>
</tr>
<tr>
<td>rs1801280</td>
<td>TT</td>
<td>66.0</td>
</tr>
<tr>
<td>rs 6984200</td>
<td>AT</td>
<td>34.0</td>
</tr>
<tr>
<td>rs 11996129</td>
<td>CT</td>
<td>38.0</td>
</tr>
<tr>
<td>( \text{CYP2E1} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs 203120</td>
<td>CC</td>
<td>72.0</td>
</tr>
<tr>
<td>rs 2515641</td>
<td>CC</td>
<td>44.0</td>
</tr>
<tr>
<td>rs 8192775</td>
<td>GG</td>
<td>60.0</td>
</tr>
<tr>
<td>rs 6413432</td>
<td>TT</td>
<td>52.0</td>
</tr>
<tr>
<td>rs 8192772</td>
<td>TT</td>
<td>46.0</td>
</tr>
<tr>
<td>rs 915908</td>
<td>AA</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**Table II.** Variants of SNPs in \( \text{NAT2} \) gene in Indonesia and Moroccan population associated with DILI

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Indonesian population</th>
<th>Moroccan population*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1208, NAT*12A</td>
<td>AA</td>
<td>A &gt; G</td>
<td></td>
</tr>
<tr>
<td>rs1799929, NAT2*11</td>
<td>CC</td>
<td>C &gt; T</td>
<td></td>
</tr>
<tr>
<td>rs1799931, NAT2*7</td>
<td>GG</td>
<td>G &gt; A</td>
<td></td>
</tr>
<tr>
<td>rs1801279 , NAT2*14</td>
<td>GG</td>
<td>G &gt; A</td>
<td></td>
</tr>
<tr>
<td>rs1801280 , NAT2*5</td>
<td>TT</td>
<td>T &gt; C</td>
<td></td>
</tr>
</tbody>
</table>

*(Guaoua et al., 2014)
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.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Indonesian population</th>
<th>Genotype</th>
<th>China population*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2031920</td>
<td>CC</td>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>rs2515641</td>
<td>CC</td>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>rs8192775</td>
<td>GG</td>
<td></td>
<td>AG</td>
</tr>
</tbody>
</table>

*(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. Even though 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 Morocco, 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 professionals 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 level 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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