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

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

Dyah Aryani Perwitasari, Malinda Noverliyani, Endang Darmawan, Uly Adhi Mulyani, Jarir Atthobari, Bob Wilfert

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 play an 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 are 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 3813687, 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. In 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 (Tesfahunegno 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 (Balakp, 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 (Gaudie 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 resistance status of NAT2 gene could affect 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; Guo et al., 2014; An, et al., 2011). The study in Moroccos population revealed that the most prevalent phenotype of NAT2 and CYP2E1 was slow acetylators (72.3%) which had hepatotoxicity risk (Guo et al., 2014).
In Brazilian population, the hepatotoxicity experienced by 6.7% patents 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 Polymorphism) 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 915906 (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 1 is 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, Isomiazid, Pyrazinamide 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 NAT2 and CYP2E1 genes were designed using Sequenom 1Mplex SNP Genotyping and also according to the previous studies (Krishnakumar et al., 2010; Guoqta et al., 2014; Malina et al., 2013; Sheng et al., 2014; Xiang et al., 2014; Rama 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 2408238, rs 2515642, rs 3813867, rs 6413432, rs 7092584, rs 7435335, rs 8192772, rs 915906, rs 2031920, rs 2515641, rs 8192775, rs 2248694, rs 2248695, rs 2480259, rs 7435334 and rs 915907. SNPs of NAT2 were rs 6984020, rs 1208, rs 1799929, rs 1799931, rs 1799930, rs 1801279 and rs 1801280.

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 average is 48.96 (SD = 12.2). Most of the patients are male (63.16%). The average of AST and ALT measured at the end of the intensive treatment are 27.23 U/L (SD = 13.36 U/L) and 7.25 U/L (SD = 13.14). However, the average of ALT and AST increased at the end of the intensive treatment are 51.12 (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 regression linear 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-A13 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 to 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 CYP2E1 associated with DILI

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotype</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NAT2</strong></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><strong>CYP2E1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs 2031920</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 6413452</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 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, NAT*11</td>
<td>CC</td>
<td>C &gt; T</td>
<td></td>
</tr>
<tr>
<td>rs1799931, NAT*7</td>
<td>GG</td>
<td>G &gt; A</td>
<td></td>
</tr>
<tr>
<td>rs1801279, NAT*14</td>
<td>GG</td>
<td>G &gt; A</td>
<td></td>
</tr>
<tr>
<td>rs1801280, NAT*5</td>
<td>TT</td>
<td>T &gt; C</td>
<td></td>
</tr>
</tbody>
</table>

*(Guhoa et al., 2014)*

In the other hand, there were some 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-hepatotoxic group.

Of the 37 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 ethmic studies (Guhoa et al., 2014; Mehta et al., 2013; Sheng et al., 2014; Xiang et al., 2014; Rao et al., 2014; Gupta et al., 2013; Santos et al., 2013; Lv et al., 2013; 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, *11 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 (Guhoa et al., 2014). NAT2*5 and *7 were known as the slow acetylator in Asian Population (Xiaohen 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 (64%).
Table III. Genotype variants of CYP2E1 gene in 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, rs698420 and rs11996129, 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 rs8192775, 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 rs6413432 in Indonesia and India population has a role in DILI. According to the rs8192772, 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 rs207076, 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 24.3% 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 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 rs207076, rs1320149, rs3813867, rs6413432, rs8192772, rs2031920, rs2515641, rs8192775 of CYP2E1 gene are associated with DILI.

ACKNOWLEDGEMENT

The author thank to the Head and staffs of Public Health Centers and Lung Hospital of Yogyakarta and Lampung who assisted the researcher during the study procedures.
REFERENCES
https://extranetwho.int/src/Reports/op=Re
plet&name=%2FWHO_HQ_Reports% 2F2G%2FPROD%2FEXT%2FTRCoun
tryProfile%2fIS02=ID&LAN=EN&outty
pe=html, it was accessed on 10
September 2015
An HR, Wu XQ, Wang ZY, Zhang JX, Liang
Y. 2012. NAT2 and CYP2E1 polymorphisms
associated with anti-
tuberculosis drug-induced hepatotoxicity
Attobhair J, Mulyani UA, Perwitasari DA.
2013. Early Drug-Induced Liver Injury
After Intensive Phase of TB Treatment
in Indonesian Primary Care Centers and
Lung Hospital Study. Drug Safety. 36(9):693
Babahk A, Aria H, Bakker N, Alica S, Ong
K, Kizilg G, Cetinoglu G, Cilgur NC.
2012. Management of and risk factors
related to hepatotoxicity during
tuberculosis treatment. Tuber Bact.
Toxik. 60(2):136-44.
Chrimotro JG, Gastagnino JP, Musella RM,
Nogueira M, Amara FA, Frias A, Vieira M,
Aidar O, Peres S, de Laranjanga GP. 2013.
Sex, ethnicity, and slow acetylator profile are the major
causes of hepatotoxicity induced by
antituberculosis drugs. J. Gastroenterol
Hepatol. 28(2):323-8.
Costa CNO, Luz, AVM, Cathia VN et al.
2012. Genetic interaction between
NAT2, GSTM1, GSTT1, CYP2E1, and
environmental factor is associated with
disease reactions to anti-tuberculosis
drugs. Mol Diag Ther. 16(4):1-10
Drug-induced hepatitis and the risk
factors for liver injury in pulmonary
tuberculosis patients. J Family Med Prim
Guoana S, Ratbi I, Laarabi FZ, Elhaddani SC,
Distribution of allele and genotypic
frequencies of NAT2 and CYP2E1 variants in
Moroccan population. BMC Genet.
19:15:156.
Gupta VH, Singh M, Amaranpurkar DN, Sasi
P, Joshi JM, Banat R, H R PK,
Amarapurkar AD, Joshi K, Wangkar PP.
2013. Association of GSTM1
polymorphism with anti-tuberculous drug
induced hepatotoxicity in Western
Indian population. Ann Hepatol. 12(6):
959-65.
Huguchi N, Tahara N, Yamagishi K,
2007. NAT2 6A, a haplotype of the N-
acetyltransferase 2 gene, is an important
biomarker for risk of anti-tuberculosis
drug-induced hepatotoxicity in Japanese
patients with tuberculosis. World J.
Gastroenterol. 13(45):6003-6008
Jeong J, Park JH, Cho YJ, Yoon HH, Song J,
Lee CT, Lee JH. 2015. Drug-induced
hepatotoxicity of anti-tuberculosis drugs
Kishnakumar D, Umamaheswaran G,
Kayathi et al. 2010. Genetic
polymorphism of drug-metabolizing
enzymes CYP2E1, CYP2A6 and
CYP3A5 in south indian patients,
Fundamental & Clinical Pharmacology, 26:
295-306
Lv X, Tang S, Xia Y, Zhang Y, Wu S, Yang
Z, Li X, Tu D, Chen Y, Deng P, Ma
Y, Chen D, Chen R, Zhao S. 2012.
NAT2 genetic polymorphisms and anti-
tuberculosis drug-induced hepatotoxicity
in Chinese community population
Ann Hepatol. 11(3):700-710.
Mishra S, Daschakraborty S, Sisakia P,
Kapoor P, Agarwal R. 2013. N-
acetyltransferase and cytochrome P450
2E1 gene polymorphisms and
susceptibility to antituberculosis drug
hepatotoxicity in an Indian population.
Nehwan, Stella P, Jula CML. 2014, Kadar
serum glutamic oxaloacetate transaminase
dan serum glutamic pyruvic transaminase
pada pasien tuberculosis paru selama dua
bulan berjalannya pemberian obat anti-
tuberculosis komubasi dosis tetap, J.E-
Clinic, 20(3):3-6.
Pharmacogenetics of isoniazid-induced

Volume 27 Issue 1 (2016)
Cytchrome p450 2E1 gene polymorphism haplotypes and anti-


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

ORIGINALITY REPORT

9%

SIMILARITY INDEX

PRIMARY SOURCES

1. indonesianjpharm.farmasi.ugm.ac.id
   Internet
   29 words — 1%

   18 words — 1%

3. www.deepdyve.com
   Internet
   16 words — 1%

4. Omics for Personalized Medicine, 2013.
   Crossref
   14 words — 1%

   Crossref
   11 words — < 1%

6. www.vitals.com
   Internet
   11 words — < 1%

   Crossref
   10 words — < 1%


Terahara, K.. "Differences in integrin-dependent phagocytosis among three hemocyte subpopulations of the Pacific oyster "Crassostrea gigas"", Developmental and Comparative Immunology, 2006


So Yamada. "Genetic variations of NAT2 and CYP2E1 and isoniazid hepatotoxicity in a diverse population", Pharmacogenomics, 09/2009

Worldwide MDPI. (Internet)
Chen, Ru, Jing Wang, Yuan Zhang, Shaowen Tang, and Siyan Zhan. "Key factors of susceptibility to anti-tuberculosis drug-induced hepatotoxicity", Archives of Toxicology, 2015.
