International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

N-Acetyl Transferase (NAT) 2 Polymorphisms and Susceptibility to Antituberculosis Drug-induced Hepatotoxicity in Indonesia

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Abstract

Tuberculosis (TB) treatment, based on the use of isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), shown to cause drug-induced hepatotoxicity (DIH). Recent studies have demonstrated that genetic variations may associate with the risk of DIH, such as INH acetylation status, related to N-acetyl transferase (NAT) 2 polymorphism, which slow acetylation are more prone to side effects from drugs. The proportion of rapid and slow acetylator vary remarkably in populations of different ethnic or geographic origin which has been described in the various study, but, there is still limited information on our population. The objective of this study is to investigate the contribution of NAT2 polymorphisms to the anti-TB DIH in our population. This case-control study conducted at the Cipto Mangunkusumo Hospital, Jakarta and Omni Hospital Alam Sutera, Tangerang, Indonesia from January 2015 - December 2016. We recruited 38 patients with DIH and 37 patients without DIH. A complete liver function profile, total serum bilirubin, indirect bilirubin and direct bilirubin were measured. Polymorphisms *NAT2**5, *6 and *7 were selected to perform genotyping with the representative SNP are respectively rs1799929, rs1799930 and rs1799931.

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Results of this study showed that 10.67% (8/75) of the subjects were *NAT2* mutant homozygotes. There is a statistical difference in acetylator status between slow and rapid acetylator with OR for *NAT2* slow acetylators compared with rapid acetylators was 4.183 (95% CI 1.416 – 12.354, $P = 0.007$). In a subanalysis, between slow acetylator polymorphism, *NAT2*6* have been shown to predominate the risk for DIH, as shown in other Asian populations. In conclusion, slow acetylator status of *NAT2* was a significant susceptibility risk factor for anti-TB DIH in Indonesia.

Keywords: TB; NAT2; Polymorphisms; slow acetylators; drug-induced hepatotoxicity.

1. Introduction

Drug-induced hepatotoxicity (DIH) is defined as a significant abnormality in liver function test (LFT), with an elevation of serum aminotransferases or bilirubin, along with clinical features such as anorexia, nausea, vomiting and jaundice during treatment [1]. Its spectrum ranges from hepatic adaptation to severe hepatotoxicity. Hepatic adaptation is a physiological phenomenon whereby the activation of various constitutional genes helps in averting drug- or toxin-related injury. It manifests as asymptomatic transient elevations of transaminases, and with persisting insult, may lead to hepatocellular injury [2]. The pathogenesis of DIH caused by these offending drugs is still enigmatic, and various mechanisms have been postulated.

Tuberculosis (TB) treatment, based on the use of isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), shown to cause adverse drug reaction (ADRs). One of the most serious ADRs is DIH, associated with high morbidity and mortality, as well as with increased treatment costs [3]. Other ADRs are gastrointestinal intolerance, kidney failure and cutaneous and hematological reaction which can lead to therapy discontinuation or more severe morbidity and mortality [4]. The interval between the start of anti-TB and the appearance of DIH varied from 1 week to 12 months, with the median being around eight weeks. Recovery usually occurs if drugs withdrawn before a severe liver injury happens [5].

The incidence of anti-TB DIH ranges from 1% to 36% [6]. A meta-analysis by Steele and his colleagues revealed a higher incidence of hepatotoxicity with concurrent administration of INH and RMP than with RMP or INH alone [7]. It has speculated that this is due to a drug to drug interaction in which induction of hepatic microsomal enzymes by RMP results in the production of increased concentration of a hepatotoxic metabolic product of INH. Thus, increased plasma RMP levels may occur due to the displacement of the drug from plasma protein binding sites by INH. This pharmacokinetic interaction between the two drugs may explain their added toxicity [7].

Advanced age, female gender, alcoholism, malnutrition, co-infection with Human Immunodeficiency Virus (HIV), pre-existent liver disease and drug interactions are common risk factors for DIH [8]. Recent studies have demonstrated that genetic variations may associate with the risk of DIH [5]. Among them, genetic polymorphism in the drug-metabolizing enzyme (DME) genes, such as INH acetylator status, is also an important factor that predisposes certain fraction of the population to DIH [9].

In the liver, INH is first metabolized into acetyl-isoniazid via N-acetyltransferase 2 (NAT2), followed by

hydrolysis to acetyl hydrazine. Acetyl hydrazine further oxidized into hepatotoxic intermediates by cytochrome P450 2E1 (CYP2E1) [10]. Direct hydrolysis of INH also generates hydrazine, a potent hepatotoxin. Disposal of acetyl hydrazine also depends on further acetylation by NAT2 to form a non-toxic metabolite, diacetyl hydrazine. Glutathione S-transferase (GSTs) are detoxification enzymes that catalyze the conjugation of glutathione to several classes of molecules including toxic intermediates of the INH metabolism [11].

N-acetyltransferase 2 is located on chromosome 8p21.3 – 23.1 [3]. It refers to inter-individual differences in the acetylation capacity. Two main metabolic phenotypes have been described in human populations: the fast acetylator phenotype, association with a normal acetylators capacity, and the slow acetylator phenotype characterized by a decreased enzyme activity. Slow acetylators are more prone to side effects from drugs that are acetylated, due to the build-up of non-metabolized drugs [12, 13]. On the contrary, fast acetylators may exhibit therapeutic failure after standard doses [14].

The proportion of rapid and slow acetylators vary remarkably in populations of different ethnic or geographic origin. Therefore, routine screening of individuals for their acetylator status before initiation of therapy should permit to improve drug efficacy and reduce adverse events [14]. Genotyping is accepted as an accurate and efficient means to determine acetylator status since a high correlation between phenotype and genotype has been demonstrated in several studies [14]. Over 65 NAT2 variants possessing one or more SNPs in the 870-bp NAT2 coding region have been reported. The seven most frequent SNPs are rs1801279 (191G>A), rs1041983 (282C>T), rs1801280 (341T>A), rs1799929 (481C>T), rs1799930 (590G>A), rs1208 (803A>G) and rs1799931 (857G>A) [15]. The analysis of the seven most common SNPs in NAT2 as described above has been shown to be highly predictive of the acetylator phenotype with a prediction rate close to 100% [14]. Because of the high cost and several days are necessary to complete the analysis, the majority of studies investigate the NAT2 genotype assay for three SNPs: 481C>T, 590 G>A and 857 G>A [15]. Based on Sabbagh and Darlu on SNP selection at the NAT2 locus for an accurate prediction of the acetylation phenotype in Koreans with tree-based analysis, neural network analysis and multifactor dimensionality reduction analysis; combination of 341T>C, 590G>A and 857G>A had 100% accuracy for predicting acetylation phenotype compared with seven most common SNPs [14].

A relationship between gene polymorphisms and anti-TB DIH has been described in the various study, there is still limited information in our population, which has a heterogeneous genetic background. The objective of the present study is to investigate the contribution of NAT2 variants to the anti-TB DIH.

2. Materials and Method

2.1. Collection of Samples

The present study was carried out at the Cipto Mangunkusumo Hospital, Jakarta and Omni Hospital Alam Sutera, Tangerang, Indonesia from January 2015 to December 2016. All patients presenting to the clinic who fulfilled the inclusion/exclusion criteria were recruited. From this sample, we selected 38 patients with DIH and 37 patients without DIH. Study subjects were 15 – 70 years old, newly diagnosed with active TB, with lesions

of TB clearly visible on chest X-ray, or positive sputum smear. Patients who developed DIH were classified as cases, while the remainder were classified as controls. Drug-induced hepatotoxicity was defined according to the criteria established by the Council for International Organizations of Medical Sciences (CIOMS) as an increase in liver biochemical parameters more than two times the upper limit of normal occurring at any time during anti-TB treatment [6, 16]. The upper limit of generally used in the study was 31 IU/mL for serum alanine transferase (ALT), 32 IU/mL for serum aspartate transferase (AST) and 1.1 mg/dL for total bilirubin. Subjects excluded if fulfill the following criteria; Abnormal serum ALT, AST or bilirubin levels or symptoms related to abnormal liver function, such as jaundice, before anti-TB treatment. Alcohol- related liver disease or habitual alcohol drinking if daily consumption is more than 40 gr for at least five years [17]. Any other hepatic or systemic diseases that may cause liver dysfunction, such as carriers of the hepatic B or C virus, heart failure, respiratory failure, HIV infection, etc.. Concomitant use of hepatotoxic drugs. Pregnant women at the time of DIH. Each individual agreed to participate in the study by signing an informed consent and recruited as samples.

2.2. Study design

In this case controlled study, the following data were recorded in the beginning of the study: demographics, nutritional status (body mass index [BMI]), duration of DIH occured and data on ALT, AST and bilirubin level. All patients enrolled in the study received a daily anti-TB regimen: INH 300 mg, RMP 600 mg (or 450 mg if body weight was < 50 kg), PZA 200 mg/kg body weight and EMT 800 mg daily for first 2 months. Patients blood sample were collected at the beginning of the study. A complete liver function profile including serum aminotransferases, serum total bilirubin, indirect bilirubin and direct bilirubin were measured using routine laboratory methods to defined DIH.

2.3. Polymorphism analysis

Genomic DNA was extracted from 6 mL EDTA-anticoagulant peripheral whole blood sample using the DNA isolation kit from *Taqman® Sample to SNP™ kit* (*Applied Biosystems®*). Following the manufacturer's instruction and was stored at -70^{o}C until used for genotyping, which was carried out by Prodia Laboratory, Jakarta. In this study, polymorphisms *NAT2**5, *6 and *7 were selected to perform genotyping. The representative SNP are respectively rs1799929, rs1799930 and rs1799931. All SNP genotyping was performed using the TaqMan® SNP genotyping assays (Applied Biosystems Inc, Foster City, CA, USA). Reactions were carried out according to the manufacturer's protocol (Taqman SNP Genotyping Assays, protocol, part number 4332856 Rev C). Probe fluorescence signal detection was performed using the CFX96 (Bio-Rad Laboratories Inc., Hercules, CA, USA) real-time polymerase chain reaction (rt-PCR) system.

Interpretation for NAT2 allele haplotype based on http://nat.mbg.duth.gr/Human%20 NAT2%20alleles_2013.htm (last updated on April 18, 2016). The *NAT2* alleles classified on the grounds of the current knowledge of the functional impact of the variant alleles. Consequently, the NAT2*4 alleles considered as functional alleles and the NAT2*5, *6 and *7 alleles as slow alleles. Individuals homozygous for rapid *NAT2* acetylator alleles or heterozygous (one rapid and one slow *NAT2* allele) were classified as rapid acetylators,

while those individuals homozygous for slow acetylator allele were considered slow acetylators [5, 14].

2.4. Statistical analysis

The Kolmogorov-Smirnov test was used to assess whether data were normally or non-normally distributed. Accordingly, the mean ± Standard Deviation (SD) or median and Inter Quartile Range (IQR) used in the descriptive statistics. Categorical variables were compared by chi-square or Fischer's exact test as necessary, and continuous variables were compared using Student's t-test or Mann-Whitney test. Odds ratio (OR) and 95% confidence intervals (95% CI) calculated. All statistical analyses performed with SPSS v 22 (Chicago, IL, USA). Statistical significance was considered at $p < 0.05$.

2.5. Ethical Clearance

Ethical approval for this study obtained from Research Ethics Committee, Mochtar Riady Institute for Nanotechnology Ethics Commitee (MRIN EC), Tangerang, West Java, Indonesia. No. : 005/MRIN-EC/0l/2015. Protocol no. : 04.1501023

3. Results

Characterisation of subjects

Seventy-five subjects diagnosed with TB. Among them, 38 diagnosed with DIH. There was no statistical difference in gender ($P = 0.131$) and nutrition status based on BMI ($P = 0.408$) between subjects with and those without anti-TB DIH.

Table 1: Characteristics of subjects with or without antituberculosis drug-induced hepatotoxicity

Parameter	**Case (n=38)**	**Control (n=37)**	***p*- value**
Gender, n(%)			
Male	12 (31.6)	18 (48.6)	
Female	26 (68.4)	19 (51.4)	0.131[a]
Age, n(%)			
< 45 years old	18 (47.4)	28 (75.7)	
≥ 45 years old	20 (52.6)	9 (24.3)	0.012[a]
Body Mass Index			
< 18.5 kg/m^2	10 (26.3)	13 (35.1)	
≥ 18.5 kg/m^2	28 (73.7)	24 (64.9)	0.408[a]
AST (U/mL)	251.5 (11-898)	19 (9-79)	< 0.001[b]
ALP (U/ml)	205.5 (14-686)	14 (4-59)	< 0.001[b]

[a] Chi Square test; [b] Mann Whitney AST: aspartate transaminase; ALP: alanine transaminase

A significant difference found between age group (p = 0.012) and transaminases level (p < 0.001) between subjects with and without DIH.

Table 2: N-acetyl transferase two genotype frequencies in subjects with and without antituberculosis drug-induced hepatotoxicity

Polymorphism	SNP id	Allele	Case (n)	Control (n)	*p* value
		1/2*	11/12/22	11/12/22	
NAT2*4			4	7	
NAT2*5	Rs1799929	C>T	30/7/1	29/8/0	0.586
NAT2*6	Rs1799930	G>A	16/16/6	22/15/0	0.031*
NAT2*7	Rs1799931	G>A	24/13/1	25/12/0	0.592

1- allele 1, 2 – allele 2; 11 – homozygous for allele 1; 12 – heterozygous; 22 – homozygous for allele 2

NAT2: N-acetyltransferase 2; SNP: single nucleotide polymorphism

In this study, Table 2 showed that 10,67% (8/75) of the subjects were NAT2 mutant homozygotes. The allele frequency of NAT2*4 in the whole subjects was 14,67% (11/75); it was 10.53% (4/38) in the case group and 18.91% (7/37) in the control group.

Table 3: N-acetyl transferase two rapid acetylator and slow acetylator genotypes and susceptibility to anti-tuberculosis drug–induced hepatotoxicity

	Case (n = 38) n (%)	Control (n=37) n (%)	OR (95% CI)
Rapid acetylators	21 (37.5)	35 (62.5)	1 (reference)
NAT2*4/*4	4	11	
NAT2*4/*5	2	5	
NAT2*4/*6	9	10	
NAT2*4/*7	6	9	
Slow acetylators	17 (73.9)	6 (26)	4.183
NAT2*5/*5	1	0	(1.416 – 12.354)*
NAT2*5/*6	2	2	
NAT2*5/*7	3	1	
NAT2*6/*6	6	0	
NAT2*6/*7	4	3	
NAT2*7/*7	1	0	

* *p-value* = 0.007

Our results showed that there is statistical difference in acetylator status between slow and rapid acetyltor with OR for *NAT2* slow acetylators compared with rapid acetylators was 4.183 (95% CI 1.416 – 12.354, $p = 0.007$).

4. Discussion

Our results showed that there were significantly different between *NAT2* slow and rapid acetylators which the slow acetylator groups were found more prone to the anti-TB DIH. This result also performed on meta-analysis about *NAT2* polymorphisms and susceptibility to anti-TB DIH conducted by Wang PY and his colleagues with OR 4.967 (95% CI 3.291 – 6.705, $P < 0.001$) and Lee SW and his colleagues with OR 3.28 [95% CI 1.53-7.06) [3, 18]. Consistently, slow acetylators have associated with an increased risk of hepatotoxicity during INH treatment with ORs ranging between 2.6 – 4 [19, 20]. As a matter of fact, in slow acetylators the overall amount of the INH moiety that is available for direct hydrolysis to hydrazine is larger than that available in rapid acetylators [21]. Kinzing-Schippers MD, and his colleagues found that INH plasma concentration is higher (up to six-fold at the short interval) in those individuals with low activity alleles of *NAT2* [22]. Among the slow acetylator polymorphisms in our study, *NAT2*6* have been shown to predominate the risk for DIH, as shown in other Asian populations. Since the Chinese, Japanese, Korean and Thai populations show comparable NAT2 allele frequencies. This finding is also likely to hold in other populations along the Pacific Asian littoral. But, the most informative subset of SNPs for the prediction of acetylation phenotype in one population may not necessarily perform well in another if the population is sufficiently differentiated [14]. Some of the other environmental parameters which also been assessed in our study had various results. Gender was reported not affecting the incidence of DIH as shown in our study. But Xiang and his colleagues (2014) on Uyghur China population found that incidence of DIH was higher among men than women [23]. Some other studies have shown that women are at an increased risk of developing DIH during treatment [24], but most studies did not find an association with gender [18, 25]. Low nutritional status is considered to be one of the factors contributing to the relatively high incidence of DIH in studies from the developing countries [17]. But in our study, we found no correlation between BMI < 18.5 kg/m^2 and ≥ 18.5 kg/m^2. In our study, the following baseline clinical factors were significantly associated with anti-TB DIH: advanced age group, AST, and ALT. The same results were also shown by Mushiroda and his colleagues (2016) also demonstrated that in the logistic regression analysis of all factors significantly associated with anti-TB DIH, age was identified as independent predictors of the risk of anti-TB DIH [26]. Genotyping of *NAT2* was proven to be helpful in predicting hepatotoxicity risk in clinical practice, and modification of INH dosage based on *NAT2* genotyping was effective in reducing the incidence of hepatotoxicity [27]. Azuma and his colleagues also shown a great potential of the *NAT2* genotype-guided dosing stratification of INH in a randomized controlled trial for pharmacogenetic-based therapy [28]. Monte Carla simulation analysis by Coutts and his colleagues suggested that the INH-related hepatotoxicity risk might minimize by adopting different dosing strategies according to acetylator status. Interestingly, with the current standard dosage of 5 mg /kg/day, the estimated risk is very low among rapid acetylators, but rises to 50% among slow acetylators [21]. Although INH-induced hepatotoxicity was at first considered idiosyncratic, subsequent studies documented to dose-dependent effect due to the accumulation of toxic metabolites [29]. Cojutti and his colleagues seem to support this relationship as significantly higher INH exposure observed among patients who experienced hepatotoxicity than among those who did not [21]. Hepatotoxicity occurs at a greater frequency when INH and RMP are co-administered than

when either drug is given alone [7, 30]. Rifampicin induces the metabolism of INH to form hepatotoxic hydrazine metabolites and on the other, increased plasma RMP levels may occur due to the displacement of the drug from plasma protein binding sites by INH. This pharmacokinetic interaction between the two drugs may explain their added toxicity [31]. As described above, we are aware that this study has some limitations. Firstly, the potential role that RMP and PZA might have had in contributing to hepatotoxicity development were not assessed. Rifampicin may induce hepatocellular type liver damage as INH. Pyrazinamide is known as a dose-dependent hepatotoxin and causes hepatocellular injury like INH. However, little is known about the risk factors and genetic predisposition of PZA or RMP-induced hepatotoxicity, because most studies were undertaken with the combination therapy of anti-TB agents as also shown in this study [5]. Measuring the plasma INH, PZA, and total RMP levels, being a better predictor for the subsequent development of DIH later during treatment. Secondly, if raw data on all subjects had been available and adjustment by other covariants, including environmental factors and other gene polymorphisms took into account, the results of this study would have been more accurate.

5. Conclusion

In conclusion, this study showed that slow acetylator status of *NAT2* was a significant susceptibility risk factor for anti-TB DIH in Indonesia. The even further research was needed, we proposed here that *NAT2* genotyping which lead to individually designed doses of INH based on *NAT2* genetic information would be possible and may be a useful tool in order to to achieve the greatest success outcome for each patient in future.

Acknowledgements

We give our gratitude to all RSCM and OMNI Hospital staffs that have supported this study. Our appreciation also for all TB patients that have participated in this study.

6. Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare

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