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# NAT2 Genotype Pattern among Tuberculosis Patients Receiving Fixed-Dose Combination of Antituberculosis

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**Abstract:** ***Drug-induced liver injury (DILI) has known to be very responsible for more than 50% of acute liver failure cases in United States. Nowadays DILI's treatment is only focused on supportive therapy as well as stopping the medication responsible for it. The incidence of antituberculosis liver injury (ATLI) in the world was ranging between 13-48%. Acetylator status and NAT2 genotype pattern related to it were two factors that have strong relationship to ATLI incidence. Slow acetylator was reported to significantly increase ATLI incidence in many studies. The proportion of slow acetylator were varies in many countries, ranging between 6-55% and these were strongly related to NAT2 genotype pattern. This study aimed to investigate NAT2 genotype pattern in tuberculosis patients receiving fixed-dose combination of antituberculosis. As many as 35 tuberculosis patients attending Outpatient Clinic of Sanglah Hospital were included in this cross sectional study. Identification of NAT2 genotype was performed with PCR-RFLP assay using KpnI and BamHI restriction enzymes. This study reveals the proportion of NAT2*4/*4; *4/*5 and *5/*5 genotype were 54.3%; 37.1%; dan 8.6%, respectively, whereas the proportion of NAT2*4/*4; *4/*7 and *7/*7 genotype were 11.4%; 71.4%; dan 17.2%, respectively.***

**Keywords:** antituberculosis, liver injury, N-acetyl transferase 2, acetylator, gene

## 1. Introductions

Drug-induced liver injury (DILI) has known to be very responsible for more than 50% of acute liver failure cases in United States. More than 75% DILI might result serious disease and required liver transplantation. Nowadays DILI's treatment is only focused on supportive therapy as well as stopping the medication responsible for it. DILI was the most frequent cause of drug withdrawal from the market. One of many drugs related to DILI was antituberculosis.[1,2,3]

The incidence of antituberculosis liver injury (ATLI) in the world was ranging between 13-48%. Acetylator status and NAT2 genotype pattern related to it were two factors that have strong relationship to ATLI incidence.[4,5,6,7,8,9]

Slow acetylator was reported to significantly increase ATLI incidence in many studies. The proportion of slow acetylator were varies in many countries, ranging between 6-55% and these were strongly related to NAT2 genotype pattern. Slow acetylator were dominant in Brazil and India.[4,10] Contrary to those result, the dominant acetylation status in China and Japan was fast acetylator.[5,6,8,11,12]

## 2. Methods

As many as 35 tuberculosis patients attended Pulmonary Outpatient Clinic of Sanglah Hospital between June to December 2014 were included in this cross sectional study. All subjects received fixed-dose combination of antituberculosis category 1. Subjects were selected using consequtive sampling technique. This study was approved by Ethical Committee of Sanglah Hospital.

DNA was isolated using guanidine isothiocyanate methods. The sequences for forward and reverse primer were 5'-GGA ACA AAT TGG ACT TGG-3' and 5'-TCT AGC ATG AAT CAC TCT GC-3', respectively. DNA chains were denatured at 94ºC for 5 minutes, followed by 35 cycle of reaction (94ºC denaturation for 1 minute, 50ºC annealing for 1 minute, 72ºC elongation for 1 minute). Finally ended by final extension at 72ºC for 10 minutes. PCR product was digested using KpnI and BamHI restriction enzyme. The mixture was incubated at 37ºC for 90 minutes. Electrophoresis of PCR-RFLP product using 2% agarose gel.

The wild type NAT2*4/*4, heterozygote *4/*5 and homozygote mutant *5/*5 genotype showed 2 bands (662 and 430 bp), 3 bands (1092, 662 and 430 bp), and 1 band (1092 bp), respectively. The wild type NAT2*4/*4, heterozygote *4/*7 and homozygote mutant *7/*7 genotype showed 2 bands (814 dan 278 bp), 3 bands (1092, 814 dan 278 bp), and 1 band (1092 bp), respectively.

## 3. Results and Discussions

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

**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) |

This study revealed the proportion of NAT2*4/*4; *4/*5 and *5/*5 genotype were 54.3%; 37.1%; and 8.6%, respectively (Table 2), whereas the proportion of NAT2*4/*4; *4/*7 and

*7/*7 genotype were 11.4%; 71.4%; and 17.2%, respectively (Table 3). These results indicated that most subjects were fast acetylator. It means in most subjects the metabolism were very fast and therefore had a lower risk of hepatotoxicity.[11]

**Table 2:** NAT2*5 genotype pattern in tuberculosis patients receiving fixed-dose combination of antituberculosis

| NAT2*5 genotype | Frequency (n) | Proportion (%) |
|---|---|---|
| Wild type | 19 | 54.3 |
| Mutant heterozygote | 13 | 37.1 |
| Mutant homozygote | 3 | 8.6 |

**Table 3:** NAT2*7 genotype pattern in tuberculosis patients receiving fixed-dose combination of antituberculosis

| NAT2*7 genotype | Frequency (n) | Proportion (%) |
|---|---|---|
| Wild type | 4 | 11.4 |
| Mutant heterozygote | 25 | 71.4 |
| Mutant homozygote | 6 | 17.2 |

On INH metabolism process, NAT2 enzyme together with CYP2E1, GSTM1 and GSTT1 catalyzed the metabolism of INH in liver. INH actually was a prodrug that required further biotransformation into its active form acetyl-INH (catalyzed by NAT2 enzyme) and hydrazine. Hydrazine and acetyl-INH would then be converted into acetylhydrazine and furthermore into diacetylhydrazine (by NAT2). Acetylhydrazine was also converted by CYP2E1 into toxic metabolite that required detoxification first (by GST enzyme) before excreted.[11,13,14]

The proportion of ATLI in Brazil was 15.6%[4], whereas in China, the proportion of ATLI in many studies revealed varies result ranging between 14-48%.[5,6,7,8,9]

Many factors responsible for ATLI incidence, one of which was genetic factor. Some genetic variations responsible for higher risk of ATLI, specifically on gene related to antituberculosis metabolism such as NAT2, CYP2E1, GSTM1 and GSTT1 gene. NAT2, CYP2E1, GSTM1 and GSTT1 were actually enzymes that needed for isoniazid (INH) metabolism.[1,3,6,15]

The relationship between NAT2 polymorphism and acetylator status in ATLI had also reported in many studies, but the results were inconsistent. Some of the studies revealed that there was no significant association between NAT2 polymorphism and ATLI.[5] In the other hand, many studies reported that slow acetylator of NAT2 was the most significant risk factor for ATLI incidence.[4,6,8,11,12,16] A metaanalyses conducted by Sun *et al*. (2008) showed that slow acetylator of NAT2 together with genotype c1/c1 CYP2E1 and GSTM1 *null* signifiantly increased ATLI incidence.[17]

The proportion of slow acetylator varied in many studies. Slow acetylator proportion in China ranging between 20-29%.[5,6,8] Different result was reported in Japan. The proportion of slow acetylator in Japan was only 6-9%.[11,12] Despite the different result, slow acetylator status in China and Japan were not the dominant acetylator status. Different result was showed in the study conducted in Brazil. Slow acetylator was the dominant acetylator status in Brazil with proportion 55%. Similar result was showed by study conducted in India, the proportion of slow acetylator was 55%.[4,10]

## 4. Conclusions

In this study the dominant genotype of NAT2 was *4/*4 (54.3%) and *4/*7 (71.4%).

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