Acetylator Status on Tuberculosis Patients Receiving Isoniazid-Contained Antituberculosis Regimen

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Abstract: Hepatotoxic incidence after tuberculosis treatment might reach 48%. Isoniazid was the most important antituberculosis related to hepatotoxic effect. One of the important risk factor related to hepatotoxic is acetylator status that reflects the metabolism rate of isoniazid in the body. Slow acetylator was proven as significant risk factor of hepatotoxic effect after tuberculosis treatment. The acetylator status is related to genetic variation on NAT2 gene, whereas genetic variation of NAT2 gene is strongly related to ethnic or race. This study aimed to study the acetylator status on tuberculosis patients receiving isoniazid-contained antituberculosis regimen. Acetylator status was analyzed from the NAT2 genotypes (NAT2*5, NAT2*6 and NAT2*7). Detection of NAT2*5, *6, and *7 genotypes was performed using PCR/RFLP technique. From 35 DNA samples isolated from tuberculosis patients receiving antituberculosis, as many as 8 subjects (22.9%) and 27 subjects (77.1%) were categorized as slow acetylator and rapid acetylator, respectively. The dominant acetylator status in tuberculosis patients was rapid acetylator.

Keywords: acetylator, NAT2, isoniazid

1. Introductions

The incidence of hepatotoxic effect after tuberculosis treatment was relatively high. It might reach 48% in several studies. Isoniazid is known to be antituberculosis that most responsible for the incidence of hepatotoxic.1,2

One of the contributing factor assumed to be related to this is the activity of antituberculosis-metabolizing enzyme, including N-acetyl transferase 2 (NAT2). NAT2 enzyme is responsible for acetylation step of isoniazid metabolism.3,4,5,6,7,8,9

The activity of NAT2 (acetylator status) was strongly related to genetic variation on NAT2 gene. People with slow acetylator characteristic would have higher risk of hepatotoxic effect after tuberculosis treatment. Several studies had been studied about the relationship between acetylator status and risk of hepatotoxic; all of which showed a significant correlation.5,5,6,9,10,11,12

Otherwise, the proportions of slow acetylator were varies in several studies. Thus indicated the different risk for hepatotoxic effect in different population. As there wasn’t any study yet regarding the acetylator status in tuberculosis patients in Indonesia, it become very important to studied about the acetylator status on tuberculosis patients receiving isoniazid-contained antituberculosis regimen.

2. Methods

This study was a cross sectional study. The samples of this study were taken from the DNA that had been isolated from tuberculosis patients who attended pulmonology outpatient clinic of Sanglah Hospital between June to December 2014. This study was approved by Ethical Committee of Sanglah Hospital. Acetylator status was analyzed from the NAT2 genotypes (NAT2*5, NAT2*6 and NAT2*7). Detection of NAT2*5, *6, and *7 genotypes was performed using PCR/RFLP technique. The forward and reverse primers were 5'-GGA ACA AAT TGG ACT TGG-3' and 5'-TCT AGC ATG AAT CAC TCT GC-3', respectively. PCR was conducted on 35 cycles of reaction and the condition was set on:

| Step       | Temperature (°C) | Time (minutes) |
|------------|------------------|----------------|
| Initial denaturation | 94               | 5              |
| Denaturation        | 94               | 1              |
| Annealing           | 50               | 1              |
| Extention           | 72               | 1              |
| Final extention     | 72               | 10             |

PCR products were restricted using KpnI, TaqI and BamHI restriction enzymes. Incubation was performed at 37°C for 90 minutes. The restriction products were visualized on 2% agarose gel. Acetylator status was categorized into 2 groups: slow and rapid acetylator. It was considered as slow acetylator if the NAT2 genotypes contained two mutant alleles; whereas rapid acetylator if the NAT2 genotypes contained one or two wild type alleles (NAT2*4).

3. Results and Discussions

Human NAT2 gene is located on chromosome 8 (8p22).10 The NAT2*5, *6 and *7 genotype patterns were shown on Table 1.

| Genotype pattern | NAT2*5 | NAT2*6 | NAT2*7 |
|------------------|--------|--------|--------|
| Wild type        | 19     | 54.3   | 100    | 4      | 11.4   |
| Mutant heterozygote | 13    | 37.1   | 0      | 25     | 71.4   |
| Mutant homozygote | 3     | 8.6    | 0      | 6      | 17.2   |

Table 1: NAT2 genotype pattern in tuberculosis patients

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Based on the NAT2 genotype patterns, as many as 8 subjects (22.9%) were categorized as slow acetylator whereas 27 subjects (77.1%) were categorized as rapid acetylator. Slow acetylator was considered to be a significant risk factor related to hepatotoxic effect of antituberculosis. 3\textsuperscript{,}5\textsuperscript{,}6\textsuperscript{,}9\textsuperscript{,}10\textsuperscript{,}11\textsuperscript{,}12 The acetylator status of tuberculosis patients based on subject characteristics were shown on Table 2.

Table 2: Acetylator status of tuberculosis patients based on subject characteristics

| Subject characteristics | Rapid acetylator n (%) | Slow acetylator n (%) |
|-------------------------|------------------------|-----------------------|
| Age                     |                        |                       |
| - < 30 y.o.             | 13 (81.3)              | 3 (18.8)              |
| - ≥ 30 y.o.             | 14 (14.7)              | 5 (4.3)               |
| Gender                  |                        |                       |
| - Male                  | 15 (15.4)              | 5 (4.6)               |
| - Female                | 12 (11.6)              | 3 (3.4)               |
| Initial BTA status      |                        |                       |
| - Positive              | 18 (16.2)              | 3 (4.8)               |
| - Negative              | 9 (10.8)               | 5 (3.2)               |
| Other medication        |                        |                       |
| - Yes                   | 7 (5.4)                | 0 (1.6)               |
| - No                    | 20 (21.6)              | 8 (6.4)               |

Isoniazid was considered to be antituberculosis that most responsible for the incidence of hepatotoxic effect after tuberculosis treatment. 5\textsuperscript{,}6 The first phase metabolism of isoniazid (acetylation process) is catalyzed by NAT2. The genetic variation on NAT2 gene might subsequently caused changes on NAT2 enzyme activity especially on isoniazid metabolism. Thus, it might affect the risk of hepatotoxic effect after tuberculosis treatment. 10\textsuperscript{,}13\textsuperscript{,}14\textsuperscript{,}15 The role of NAT2 in isoniazid metabolism can be seen on Figure 1.

Slow acetylator had been proven in many studies as a significant risk factor related to hepatotoxic effect of antituberculosis. This included several studies conducted in China, Japan, India, and Brazil. 3\textsuperscript{,}5\textsuperscript{,}6\textsuperscript{,}9\textsuperscript{,}10\textsuperscript{,}11\textsuperscript{,}12 This study showed the major acetylator status in tuberculosis patients were rapid acetylator. This indicated that the risk of hepatotoxic effect after tuberculosis treatment in most of our subjects was relatively not high.

![Figure 1: The role of NAT2 in isoniazid metabolism](image)

Compared to studies conducted in other countries, our result was similar to those in China and Japan. The proportion of rapid acetylator was also dominant in China (71-80%) and Japan (91-94%). 3\textsuperscript{,}5\textsuperscript{,}9\textsuperscript{,}10\textsuperscript{,}11 Contrary to our result, the slow acetylator was dominant in several populations. The proportion of slow acetylator was slightly higher than rapid acetylator on studied conducted in India (55%) and Brazil (55%). 3\textsuperscript{,}16 This indicated the higher risk for hepatotoxic effect after tuberculosis treatment. This also proved that genetic variation was strongly related to the ethnicity or race.

4. Conclusion

The dominant acetylator status in tuberculosis patients was rapid acetylator.

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