Analysis of GSTM1 Deletion on Adult Pulmonary Tuberculosis Patients

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Abstract: It has been widely known that tuberculosis treatment might cause mild to severe side effects. One of which is hepatotoxic effect, that might potentially serious and might cause acute liver failure. Hepatotoxicity incidence in worldwide has reached 48% in many countries. One of the responsible factor contributing to this is genetical variation on drug metabolizing enzyme for antituberculosis, including GSTM1. Unfortunately, there’s no enough evidence regarding distribution of GSTM1 deletion in Indonesia. Meanwhile, the proportion of GSTM1 null in many countries were varies, reached 43-46%. This study aimed to identify the deletion of GSTM1 on tuberculosis patient receiving antituberculosis. GSTM1 deletion identification was conducted using PCR techniques. The proportion of GSTM1 null genotype in this study was 71.4%.

Keywords: GSTM1 null, deletion, antituberculosis liver injury

1. Introduction

Until now tuberculosis still become problem in many developing countries including Indonesia. It has been widely known that tuberculosis treatment might cause mild to severe side effects. One of which is hepatotoxic effect, that might potentially serious and might cause acute liver failure. Hepatotoxicity incidence regarding antituberculosis use in tuberculosis patient in many countries have shown varied number. The proportion in Brazil were 15.6% whereas the proportion in China and India were ranging between 14-48%,1,2,3,4,5,6 One of the contributing factor known to be responsible for hepatotoxicity incidence was genetical variation on antituberculosis-metabolizing enzyme, including GSTM1, NAT2 and CYP2E1.3,7,8,9,10 Genetic variation mostly found on GSTM1 was deletion. This polymorphism has already studied in many countries worldwide, but the result were varies. Afterall most studies reported that GSTM1 polymorphism were significantly related to hepatotoxicity incidence. Unfortunately in Indonesia there was no adequate studies regarding the distribution of GSTM1 polymorphism. That’s why it was very important to investigate the proportion of GSTM1 deletion on pulmonary tuberculosis patient.

2. Methods

This study was a cross sectional study. The subjects were selected from adult pulmonary tuberculosis patients that attended pulmonology outpatient clinic of Sanglah Hospital, using consecutive sampling technique. This study was approved by Ethical Committee of Sanglah Hospital. DNA sample of patient were isolated using guanidine isothiocyanatemethod from 5 mL of whole blood. Identification of GSTM1 deletion was performed using PCR technique via coamplification of GSTM1 and β-globin. Forward and reverse primer for GSTM1 were 5’-CTG CCC TAC TTG ATT GAT GGG-3’ and 5’-CTG GAT TGT AGC AGA TCA TGC TG-3’, respectively. PCR condition was set on initial denaturation temperature 94ºC for 5 minutes; followed by 35 cycles of: denaturation temperature on 94ºC for 45 seconds, annealing temperature on 55ºC for 45 seconds, elongation temperature on 72ºC for 45 seconds; ended with final elongation temperature on 72ºC for 5 minutes. Electrophoresis using 2% agarosegel. GSTM1 null and widtype genotype were identified when 1 (268 bp) and 2 bands (268 and 215 bp) performed on visualization, respectively.

3. Result and Discussion

As many as 35 samples were included in the study: 60% have positive initial BTA status; 40% have negative initial BTA status. As many as 20% were also received medications other than antituberculosis along the course of treatment.

One of the contributing factor known to be responsible for hepatotoxicity incidence was genetical variation on antituberculosis-metabolizing enzyme, including GSTM1, NAT2 and CYP2E1.3,7,8,9,10 Genetic variation mostly found on GSTM1 was deletion. This polymorphism has already studied in many countries worldwide, but the result were varies. Afterall most studies reported that GSTM1 polymorphism were significantly related to hepatotoxicity incidence. Unfortunately in Indonesia there was no adequate studies regarding the distribution of GSTM1 polymorphism. That’s why it was very important to investigate the proportion of GSTM1 deletion on pulmonary tuberculosis patient.

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Table 1: Subject characteristics in wild type and null GSTM1 genotype

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Wild type (n)</th>
<th>Null (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30 years old</td>
<td>3 (18.8)</td>
<td>13 (81.2)</td>
</tr>
<tr>
<td>≥ 30 years old</td>
<td>7 (36.8)</td>
<td>12 (63.2)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6 (30)</td>
<td>14 (70)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (26.7)</td>
<td>11 (73.3)</td>
</tr>
<tr>
<td>Initial BTA status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5 (23.8)</td>
<td>16 (76.2)</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (35.7)</td>
<td>9 (64.3)</td>
</tr>
<tr>
<td>Other medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (28.6)</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>No</td>
<td>8 (28.6)</td>
<td>20 (71.4)</td>
</tr>
</tbody>
</table>

GSTM1 genetic variation was assumed as one contributing factor responsible for hepatotoxic incidence due to GSTM1 role on isoniazid metabolism. As we know many factors related to hepatotoxicity regarding antituberculosis use including genetic, race, age, gender, acetylation status, type of treatment, dose of treatment, duration of treatment, alcohol consumption, comorbid disease (especially liver and renal disease) as well as interaction with other drugs. GSTM1 was responsible for phase II metabolism of isoniazid, specifically catalyzed the conjugation reaction. Isoniazid was a prodrug that require further metabolism into acetylsisoniazid (catalyzed by N-acetyltransferase) and hydrazine. Hydrazide acetylsisoniazid then will be converted into acetylhydrazine and further into diacetylhydrazine (catalyzed by N-acetyltransferase). Acetylhydrazine also metabolized by CYP2E1 into toxic metabolitethat will subsequently be excreted after detoxification by glutathione s-transferase (GST).13,14

Several studies have investigated the relationship between genetic variation on drug-metabolizing enzyme and hepatotoxicity incidence. Most have reported that GSTM1 deletion significantly related to hepatotoxicity incidence. This also have been studied in metaanalysis studies conducted by Sun et al., as well as by Cai et al. Study by Sun et al. has shown that GSTM1 null genotype, together with c1/c1 and slow acetylator NAT2 increased the incidence of hepatotoxicity significantly. Similar to Sun et al. result, study by Cai et al. reported thatslow acetylator NAT2, CYP2E1*1A allele andGSTM1 null related to the risk of mild hepatotoxicity.16

4. Conclusion

The proportion of GSTM1 null genotype in pulmonary tuberculosis patient was relatively high, 71.4%.

References