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Hepatic Injury and Glutathione s-transferase Deletion Related to Antituberculosis Use: An Observational Study in Balinese Population, Indonesia

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Abstract

BACKGROUND: Glutathione S-transferase (GST), together with other drug-metabolizing enzymes (N-acetyltransferase and cytochrome P450), plays a crucial role in the metabolism of isoniazid (isonicotinic acid hydrazide). Among five isoforms of GST, GSTM1, and GSTT1 had been proved to involve in isoniazid metabolism.

AIM: We aimed to investigate association between GST deletion and hepatic injury in the Indonesian population.

METHODS: This is a cross-sectional study. The total participants' number was 70. Our whole blood samples were collected from adult pulmonary tuberculosis patients who received antituberculosis treatment category one in Bali. Detection for *GSTM1* and *GSTT1* deletion performed with the polymerase chain reaction technique using internal standard β-globin. Data analysis performed with the Chi-square test.

RESULTS: The proportion of *GSTM1* null was 71.4% whereas the *GSTT1* null was 34.3%. The proportion of combined *GSTM1* null and *GSTT1* null was 22.9%. There was no significant difference in liver damage incidence between *GSTM1* null and wild-type ($p > 0.005$). There was also no significant difference in liver damage incidence between *GSTT1* null and wild-type ($p > 0.005$).

CONCLUSIONS: Neither *GSTM1* nor *GSTT1* deletion proved to be associated with liver injury regarding antituberculosis use.

Introduction

Glutathione S-transferase (GST) is widely known as one of the principal enzymes involved in the metabolism of many drugs or substances in the human body [1]. This broad class of enzymes especially takes part in phase two metabolism of drugs through catalyzing conjugation reaction to alter drugs or substances into a more polar metabolite; thus, it will be easier to excrete [1]. GST, together with other drug-metabolizing enzymes, N-acetyltransferase (NAT), and cytochrome P450 (CYP), plays a crucial role in the metabolism of isoniazid (isonicotinic acid hydrazide) [2]. NAT enzyme takes part in isoniazid metabolism through catalyzing acetylation reaction, whereas CYP catalyzing oxidation reaction [2], [3].

GST enzyme is a soluble enzyme located in the cytosol, microsome, and mitochondria. GST enzyme in mammalian has some isoforms, including GSTA (alpha), GSTM (mu), GSTP (pi), and GSTT (theta) [4], [5]. Each isoform is encoded by a specific gene. Among the five isoforms of GST, GSTM1, and GSTT1 had been proved to involve in isoniazid metabolism [6]. GSTM1 enzyme is encoded by the *GSTM1* gene located on chromosome one, while the GSTT1 enzyme is encoded by the *GSTT1* gene located on chromosome 22 [6].

Some polymorphisms might potentially occur in such genes. The major type of polymorphism found in *GSTM1* and *GSTT1* gene is deletion [5]. Some evidence had shown that GST deletion contributed to hepatic injury induced by antituberculosis. However, there was a controversy regarding this finding in several populations [6]. Some researches represented a significant correlation between GST deletion and hepatic injury, whereas other evidence performed the opposite [5], [6]. Until now, there has been no study reporting the association between GST deletion and hepatic injury in the Indonesian population. Thus, it needs to be confirmed about this in our local population.

Methods

Samples selection and collection

This study was an analytical observational study with a cross-sectional design. Our samples were whole blood from adult pulmonary tuberculosis patients who received antituberculosis treatment category one in Bali (which had been already collected on our previous research and preserved at Integrated Biomedical Laboratory of Medical Faculty, Udayana University). Inclusion criteria, including pulmonary tuberculosis patients in Bali, age ≥18 years old, received antituberculosis treatment category one and had already signed the informed consent. Sampling method using a purposive sampling technique. The total participants' number was 70. Our study had been approved by the Ethics Committee of Medical Faculty Udayana University/Sanglah Hospital.

Detection of GSTM1 and GSTT1 deletion

To detect the *GSTM1* and *GSTT1* deletion, we performed the polymerase chain reaction (PCR) technique using internal standard β-globin. PCR was set on early denaturation temperature 94°C (for 5 min), followed by 35 cycles of denaturation (94°C for 45 s), annealing (55°C for 45 s), and elongation (72°C for 45 s). Finally, the PCR temperature was ended with the final elongation at 72°C for five min. In the end, we identified the PCR product by identifying the fragment length for each gene: 268 bp for the β-globin gene; 215 bp for the *GSTM1* gene; and 968 bp for *GSTT1* gene. The forward and reverse primer for each gene was represented in Table 1.

Table 1: Forward and reverse primer for each gene

Gene	Forward primer	Reverse primer
β-globin	5'-GAA GAG CCA AGG ACA GGT AC-3'	5'-CAA CTT CAT CCA CGT TCA CC-3'
GSTM1	5'-CTG CCC TAC TTG ATT GAT GGG-3'	5'-CTG GAT TGT AGC AGA TCA TGC-3'
GSTT1	5'-CTC CCT ACT CCA GTA ACT CCC GAC T-3'	5'-CTG GTC ATG GTC TCT ATG CAA AAG A-3'

Assessment of liver injury

Hepatic injury was identified from the concentration of transaminase enzymes (including aspartate aminotransferase and alanine aminotransferase [ALT]) that had been assessed on our previous research. The transaminase level was measured with spectrophotometry technique. Hepatic injury incidence was categorized into two groups: With hepatic injury (at least one transaminase enzyme showed abnormal concentration) and without hepatic injury. The normal ranges of ALT and AST in the Indonesian population are <31 U/L and <45 U/L, respectively.

Data analysis

Our data were analyzed with statistical software. The comparison of hepatic injury between *GSTM1* or *GSTT1* null and wild-type was analyzed using the Chi-square test. $p < 0.05$ was considered as statistically significant.

Results

Subject characteristics

Of the total samples, most subjects were male and age above 30 years old (Table 2). Only 5.7% of subjects were alcoholics. There was no comorbid disease (including liver and kidney disease). Mostly no other medication taken by subjects.

Table 2: Subject characteristics

Characteristics	n (%)
Sex	
Male	40 (57.1)
Female	30 (42.9)
Age	
<30 years old	32 (45.7)
≥30 years old	38 (54.3)
Initial AFB status	
Positive	42 (60.0)
Negative	28 (40.0)
Alcohol consumption	
Yes	4 (5.7)
No	66 (94.3)
Comorbid disease	
Yes	0 (0.0)
No	70 (100.0)
Other medication	
Yes	14 (20.0)
No	56 (80.0)

AFB: Acid fast bacil.

The Pattern of GSTM1 and GSTT1 Genotype

The dominant genotype pattern for *GSTM1* was null-type, whereas *GSTT1* was wild-type (Table 3). The proportion of *GSTM1* null was 71.4%, whereas the *GSTT1* null was 34.3%. The majority of our samples had a combination of *GSTM1* null and *GSTT1* wild-type. The proportion of combined *GSTM1* null and *GSTT1* null was 22.9%.

Table 3. The pattern of *GSTM1* and *GSTT1* genotype

Genotype	n (%)
Genotype *GSTM1*	
Null (−)	50 (71.4)
Wild type (+)	20 (28.6)
Genotype *GSTT1*	
Null (−)	24 (34.3)
Wild type (+)	46 (65.7)
GSTM1 and *GSTT1* combination	
GSTM1 (−) *GSTT1* (−)	16 (22.9)
GSTM1 (−) *GSTT1* (+)	34 (48.6)
GSTM1 (+) *GSTT1* (−)	8 (11.4)
GSTM1 (+) *GSTT1* (+)	12 (17.1)

GSTM1: Glutathione s-transferase M1; GSTT1: Glutathione s-transferase T1.

Hepatic injury incidence in GSTM1 and GSTT1 deletion

As many as five subjects represented enhanced transaminase levels. There was no significant difference in liver damage incidence between *GSTM1* null and wild-type ($p > 0.05$). There was also no significant difference in liver damage incidence between *GSTT1* null and wild type ($p > 0.05$). There was no significant difference in liver damage incidence between combined *GSTM1* and/or *GSTT1* null and *GSTM1* wild-type/ *GSTT1* wild-type ($p > 0.05$) (Table 4).

Table 4: Hepatic injury incidence according to *GSTM1* and *GSTT1* deletion

Genotype	Hepatotoxic		p
	Yes n (%)	No n (%)	
GSTM1			
Null	6 (12.0)	44 (88.0)	0.610
Wild type	4 (20.0)	16 (80.0)	
GSTT1			
Null	4 (16.7)	20 (83.3)	1.000
Wild type	6 (13.0)	40 (87.0)	
GSTM1, GSTT1			
GSTM1 null and/*GSTT1* null	6 (10.3)	52 (89.7)	0.195
GSTM1 WT and *GSTT1* WT	4 (33.3)	8 (66.7)	

GSTM1: Glutathione s-transferase M1, GSTT1: Glutathione s-transferase T1, WT: Wild-type.

Discussions

For years, isoniazid has proved to be the primary antituberculosis drug that contributes to hepatic injury in tuberculosis patients. Approximately 10–20% of tuberculosis patients who received isoniazid will lead to an increase of transaminase enzyme, especially ALT, as many as three times the normal upper limit [2], [3].

One of the possible factor influences hepatic injury induced by antituberculosis is genetic variation. Specifically for isoniazid, genetic variations that mostly related to liver damage are genetic variations on the drug-metabolizing enzymes, including NAT2, CYP, GSTM1, and GSTT1 [4], [6].

GST, as the primary enzyme, plays a role in phase two metabolism of isoniazid, might demonstrate deletion, thus might result in enhanced risk of liver damage in tuberculosis patients who are taking antituberculosis. On isoniazid metabolism, GST enzymes detoxify the toxic metabolites of isoniazid into the non-toxic and excretable form [7], [8].

Our results demonstrated that the proportion of null-type *GSTM1* was higher than wild-type *GSTM1 (*71.4% vs. 28.6%). This was similar to evidence showed on a study in China, Japan, Thailand, and Turkey. The proportion of *GSTM1* null in China, Japan, Thailand, and Turkey was 57.9%, 53.5%, 60%, and 72% [9]. However, some studies revealed different results. The proportion of null-type *GSTM1* on research done by Teixeira *et al*. [10] and Santos *et al.* [11] in Brazil, and also by Gupta *et al*. [12] in India, was lower than wild-type *GSTM1*. The difference is highly related to the race variation among populations.

However, our finding revealed that there was no significant difference in hepatic damage incidence related to antituberculosis between wild-type and null-type of *GSTM1*. Our result was similar to several studies in China, India, Brazil, and Korea [12], [13], [14], [15]. Some studies demonstrated contrary findings. Some studies stated that *GSTM1* null-type increased the risk of hepatic injury related to antituberculosis use [16], [17]. Meta-analysis reports supported a significant correlation between *GSTM1* deletion and hepatic injury in tuberculosis who were taking antituberculosis [8], [18], [19], [20]. A variation on the association of *GST* deletion with hepatic injury might particularly be influenced by a variation on race and ethnicity. Our finding was similar to other studies conducted in Asian countries, including India, China, and Korea. Moreover, there are also some other important enzymes responsible for isoniazid metabolism, namely, CYP and NAT2. Genetic variation on these genes might largely contribute to hepatic injury related to antituberculosis use.

Regarding our finding for the *GSTT1* gene, the wild-type *GSTT1* was the dominant genotype in our samples. The proportion of *GSTT1* null-type in our research was 34.3%. This was consistent with the proportion of *GSTT1* null-type in India (11.8%) and Brazil (22%) [11], [12].

There was no significant difference in liver damage proportion between wild-type and null-type of *GSTT1* represented in our study. Several studies reported similar findings. Deletion on *GSTT1* reported no significant association with liver damage induced by antituberculosis in other Asian populations, namely, Chinese and Indian [10], [13], [16], [21], [22], [23]. This supported the concept that genetic variation mostly influenced by race or ethnicity.

Some meta-analysis also revealed that there was no significant difference in hepatic injury proportion between *GSTT1* null and wild-type in tuberculosis patients who were taking antituberculosis [8], [18], [19], [20]. In East Asian, *GSTT1* was also not considered as a risk factor for hepatic injury related to antituberculosis administration [8], [18], [19], [20].

Our finding was different from evidence in Caucasian, India and Brazil. In the Caucasian population, *GSTT1* deletion enhanced the risk of hepatic damage related to antituberculosis about 2.6 times, whereas in Indian, about 2.92 times [12]. Research conducted by Forestiero *et al.* [17] in Brazil reported a similar finding. It was revealed that *GSTT1* null was significantly related to a mild increase of transaminase enzyme on tuberculosis patients who received antituberculosis. A report by Santos *et al.* in Brazil also supported this finding [18].

Regarding combined deletion on *GSTM1* and *GSTT1*, our study found that there was no significant

difference in hepatic damage incidence between combined *GSTM1* null/*GSTT1* null and wild-type. Our result supported evidence performed by Chaterjee *et al.* [14] and Yang *et al*. [20]

Opposite results were stated by Gupta *et al.* [12] and Singla *et al*. [24]. Combined deletion on *GSTM1* and *GSTT1* gene was proved to be represented a significantly higher risk of hepatic damage induced by antituberculosis in the Indian population. The opposite result possibly related to the different proportion of *GSTM1* deletion between our study and study conducted by Gupta *et al*. In our study, *GSTM1* null was the dominant genotype for GSTM1, whereas in a study conducted by Gupta *et al*., *GSTM1* wild-type was the dominant genotype for *GSTM1* in the study population [12].

Isoniazid is a prodrug that should be converted first into acetyl isoniazid (catalyzed by NAT enzyme) and hydrazine. Hydrazine and acetyl isoniazid subsequently will be changed into acetyl hydrazine and finally forms diacetylhydrazine. Acetyl hydrazine is also metabolized by the CYP2E1 enzyme, forming toxic metabolite which then will be detoxified by GST before excreted from our body. Deletion on *GSTM1* and *GSTT1* might result in a decrease or loss of GST enzyme activity. This might lead to liver damage following the increase of isoniazid toxic metabolites. A variation on the association of *GST* deletion with hepatic injury might particularly be influenced by a variation on race and ethnicity [3], [4], [5], [6].

Conclusions

The majority of our subjects showed null-type *GSTM1* and wild-type *GSTT1*. Neither *GSTM1* nor *GSTT1* deletion proved to be associated with liver injury induced by antituberculosis. Combined *GSTM1* null/*GSTT1* null also revealed no significant correlation with hepatic damage related to antituberculosis use.

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