Association of Genetic Polymorphism of GSTM1 and GSTT1 with the Susceptibility to Antituberculosis Drug-induced Hepatotoxicity in Chinese Population

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Abstract: Objective To investigate the relationship between the polymorphism of glutathione S transferase M1, T1(GSTM1, GSTT1) gene and the susceptibility to antituberculosis drug induced hepatotoxicity (ATDH) in patients with tuberculosis. Methods GSTM1 and GSTT1 gene polymorphisms in patients with or without liver toxicity after antituberculous treatment were analyzed using multiple PCR method. Results In ATDH group and control group, the proportion of GSTM1 gene deletion was 58.0% and 50.7% respectively, and the difference was not statistically significant (OR=1.322, 95%CI=0.921~1.878), the frequencies of GSTT1 deletion were 46.3% and 49.3%, respectively, and there was no significant difference between them. There was no significant difference in frequency of GSTM1 and GSTT1 variation between case group and control group (P> 0.05), and no synergistic effect of those two gene polymorphism were detected in the occurrence of antituberculosis drug-induced hepatotoxicity. Conclusion The polymorphisms of GSTM1 and GSTT1 genes may not be associated with the risk of ATDH.

Keywords: GSTM1; GSTT1; Tuberculosis; Hepatotoxicity; Genetic Polymorphism

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1. Introduction

Tuberculosis (TB) remains a global major infectious disease, especially in developing countries. World Health Organization (WHO) has declared TB as a public health emergency since 1993[1].

At present, Standard short-course chemotherapy based on isoniazid, rifampicin, pyrazinamide, and ethambutol has been accepted by many countries and is the basic scheme for the treatment of tuberculosis [2, 3]. A large number of clinical studies have shown that isoniazid, rifampicin and azininazinamid are to some extent susceptible to the occurrence of hepatotoxicity [3-8], especially when the above drugs were adminstored together, the incidence and severity of hepatotoxicity increased significantly. Antituberculosis drug-induced hepatotoxicity causes substantial morbidity and mortality with an incidence ranged from 2.5% to 34.9% [6,9]. The pathogenesis of antituberculosis drug-induced hepatotoxicity is unclear.

Currently, it is generally considered that the process of drug-induced hepatotoxicity is composed of multiple steps, including the entry of drugs into the liver tissue, the formation of enzyme catalyzed metabolic products, the metabolites as immunogens are then combined with endogenous proteins to induce immune damage or directly cause hepatocyte intoxication. In these steps, drug metabolism enzymes play a significant role in drug detoxification and activation, which exert important effect on drug efficacy and sensitivity to toxicity[10]. GSTM1 and GSTT1 are members of the GST family, and they both have detoxification functions for exogenous chemicals. Deletion homozygote of those two genes could cause the loss of enzyme activity [11]. There are reports that [12-18], GSTM1 and GSTT1 gene polymorphisms are related to the occurrence of anti tuberculosis drug hepatotoxicity, but the conclusions are not consistent. In this study, a case-control study was conducted to explore the association between GSTM1 and GSTT1 gene polymorphisms and susceptibility to antituberculosis drug-induced hepatotoxicity in Chinese population.

2. Study Objects

2.1 Study Object

Between May 2010 and March 2016, 600 patients with newly diagnosed tuberculosis who met the selection criteria were included. The case group consisted of 300 patients with hepatotoxicity after receiving first-line anti-tuberculosis treatment (2HRZE/4HR). Hepatic toxicity of anti-tu-

berculosis drugs is defined as asymptomatic or symptoms of hepatitis, such as loss of appetite, nausea, and vomiting after taking anti-TB drugs, together with at least one of the following conditions, (1) Serum AST and/or ALT is 3 times (or > 120 U/L) above the upper limit of normal, (2) In two consecutive blood samples, ALP test values is two times greater than the upper limit of normal , (3) Any increase in ALT, AST, ALP accompanied by progressively elevated bilirubin (>2.5 mg/dl). 300 patients with tuberculosis were selected as controls. These patients took the same anti-tuberculosis drugs but no hepatotoxicity appeared. All enrolled subjects must meet the following conditions: At the beginning of chemotherapy, liver function is normal and there are no other factors that may cause liver damage, such as malnutrition, HIV infection, alcohol abuse, viral hepatitis, liver disease, cardiac insufficiency, and the use other drugs, etc. In the course of treatment, changes in liver function were closely monitored in all subjects.

2.2 Methods

2.2.1 Human Peripheral Blood DNA Extraction

The whole blood sample of the tuberculosis patients mentioned above was collected, and a genome-wide extraction kit (Tiangen Biotech, Beijing, China) was used to extract DNA in blood according to the instructions, and the extract is immediately stored in a -20°C refrigerator for subsequent use.

2.2.2 Determination of GSTM1 and GSTT1 Genes

According to the report [13], the GSTM1 and GSTT1 genes were simultaneously examined by multiplex PCR and β-globin was used as an internal control. The GSTM1 ((Upstream: 5'GAACTCCCTGAAAAGCTAAAGC3', Downstream: 5'CTTGGGCTCAAATATACGGTGG3', 219bp), GSTT1 (Downstream: 5' CTTGGGCTCAAATATACGGTGG 3', Downstream: 5' TCACCGGATCATGGCCAGCA 3', 459bp), and β-globin (Upstream: 5' CAACTTCATCCACGTTCACC 3', Downstream: 5' GAAGAGCCAAGGACAGGTAC 3', 68bp) gene amplification primers were constructed successively.

The PCR reaction is a 25 ul amplification system, the final concentrations of each substances were as follows: 0.2mmol/L of each of the 4 dNTPs, 0.3mmol/L primer, 10 to 100ng DNA template, and 1U Taq DNA polymerase. The conditions for PCR were pre-denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 40 seconds, and a final extension at 72°C for 10 minutes. The PCR products were separated by 2.0% agarose gel electrophoresis, and the results were observed on a gel imaging and analysis system.

2.3 Genotyping

β-globin was used as an internal control with an amplification product showed at 268 bp, no β-globin fragment means inefficient amplification. After amplification of the DNA sample by PCR, 219 bp fragments and 459 bp fragments were generated, which were defined as GSTM1(+) and GSTT1(+), respectively. No corresponding amplification products were considered to be homozygous deletions, namely GSTM1(-) and GSTT1(-).

2.4 Statistical Analysis

Spss 13.0 software, t-test, x2 test and other methods were used to analyze the basic parameters of the two groups, and x2 test, factorial analysis and other methods were used to analyze the electrophoresis results of PCR products. The test level is α=0.05.

3. Results

3.1 Analysis of Basic Parameters

There was no significant difference in gender and age between the patient group and the control group. The values of ALT, AST, DBIL, TBIL, GGT and ALP before treatment were within the normal range. The comparison of serum ALT, AST, DBIL, TBIL, GGT and ALP between the two groups before and after treatment are shown in Table 1.

Table 1. Comparison of Patients' Parameters in Case Group and Control Group

	case group	control group	P
Gender(M/F)	174/126	160/140	0.287
Age(years)	37.19±18.193	36.41±18.661	0.167
Before			
ALT(U/L)	18.64±10.887	14.21± 6.546	
AST(U/L)	23.16±9.017	19.43±6.326	
DBIL(μmol/L)	5.628±7.012	4.325±3.246	
TBIL(μmol/L)	12.756±7.687	10.294±7.426	
GGT(U/L)	24.25±5.147	26.12±4.923	
ALP(U/L)	64.52±20.156	58.43±18.915	
After			
ALT(U/L)	196.946±208.431	18.488±9.058	
AST(U/L)	180.047±268.417	20.475±6.721	
DBIL(μmol/L)	11.231±20.754	4.513±2.013	
TBIL(μmol/L)	23.742±30.457	11.126±5.621	
GGT(U/L)	46.41±12.156	27.13±9.867	
ALP(U/L)	100.45±38.426	64.87±20.124	

3.2 PCR Amplification

If GSTM1 and GSTT1 genes are both positive (labeled as GSTM1(+)/GSTT1(+)), the DNA samples will produce 219bp, 459bp and 268bp fragments after PCR amplification. Accordingly, GSTM1(+)/GSTT1(-) genotype showed two fragments of 219bp and 268bp, GSTM1(+)/GSTT1(-) genotype has two fragments of 459bp and 268bp, while GSTM1(-)/GSTT1(-) genotype only showed 268bp fragments, as showed in Figure 1.

3.3 Relationship between GSTM1 and GSTT1 Genotypes and Susceptibility to Antituberculosis Drug-induced Hepatotoxicity

In the case group and the control group, the GSTM1 deletion genotypes accounted for 58.0% and 50.7%, respectively, the difference was not statistically significant (P>0.05), but the OR value of drug-induced hepatic impairment in GSTM1 deletion genotypes subjects is 1.32 (95% CI: 0.921 to 1.878, P>0.05), and was higher than normal controls. The GSTT1 deletion genotype accounted for 46.3% and 49.3% in these two groups, respectively, and the OR value was similar between the two groups (P>0.05). The results are shown in Table 2.

3.4 Joint analysis of GSTM1 and GSTT1 genotypes

The phenotypes of GSTM1 and GSTT1 genes were combined to analyze their relationship with ADTH. GSTM1(+)/GSTT1(+) subjects were considered as reference group. The results showed that there was no significant difference in the frequency of GSTM1(-)/GSTT1(-) genotype between the case group and the control group (p>0.05). Compared with patients with a GSTM1-deleted genotype (OR: 1.212, 95% CI: 0.762 to 1.967, P>0.05) or GSTT1-deficient genotype(OR: 0.736, 95% CI: 0.462 to 1.243, P>0.05), there was no significant increase in the risk factor for anti-tuberculous drug-induced hepatic impairment in the GSTM1(-)/GSTT1(-) genotype (OR: 1.153, 95% CI: 0.722 to 1.872, P>0.05). The results are shown in Table 3.

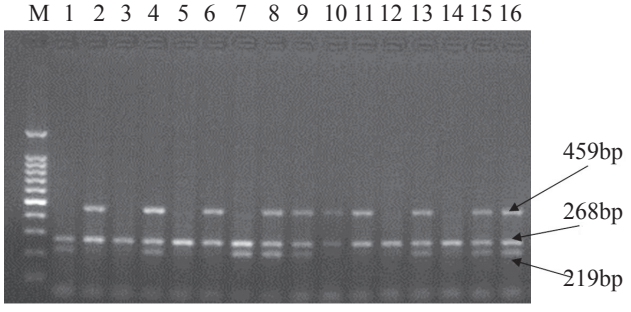

Figure 1. Agarose Gel Electrophoresis after PCR Amplification of GSTM1 and GSTT1

Table 2. Relationship between GSTM1 and GSTT1 Gene and ADTH in 600 Tuberculosis Patients

	GSTM1		GSTM1	
	+	-	+	-
case group	126	174	161	139
control group	148	152	152	148
OR(95%CI)	0.724 (0.508~1.028)	1.322 (0.921~1.878)	1.157 (0.799~1.560)	0.866 (0.618~1.217)
p	0.071		0.462	
$\chi 2$	3.251		0.541	

Table 3. Joint Analysis of GSTM1 and GSTT1 Genotypes with ATDH in 600 Tuberculosis Patients

genotype		case group	control group	OR(95%CI)	p	$\chi 2$
GSTM1	GSTT1					
+	+	72	74	1		
+	-	53	74	0.736(0.462~1.243)	0.210	1.573
-	+	92	78	1.212(0.762~1.967)	0.394	0.726
-	-	83	74	1.153(0.722~1.872)	0.537	0.382

4. Discussion

Glutathione S-transferase (GST) is an important phase II detoxification enzyme, it catalyzes the binding of the intermediate metabolites of the foreign compounds with reduced glutathione, and the resulting reduced glutathione conjugate is less toxic and easily excreted. Therefore, they have an important role in protecting cells from chemical attacks [19]. GSTM1 and GSTT1 are members of the GST family, both of which have detoxifying functions for exogenous chemicals, and its deletion homozygote could cause the loss of their corresponding enzyme activity [11].

A number of studies have been conducted to explore the relationship between GSTM1 and GSTT1 gene polymorphisms and anti-tuberculous drug-induced hepatotoxicity. In an Indian study of genetic polymorphisms of GSTM1 and GSTT1 in 33 patients with liver toxicity associated with antituberculosis drug and 33 treated TB patients without hepatotoxicity, Bidyut Roy et al[12] found that the risk for anti-tuberculosis drug-induced hepatic impairment in the GSTM1 deletion genotype was 2.13, so it might predicted the occurrence of hepatotoxicity against TB drugs. However, the difference in GSTT1 gene mutation and hepatotoxicity was not statistically significant between cases and controls. The above conclusion was supported by other researches, such as Yi-Shin Huang et al[13], but other studies have different conclusions. Virginia Leiro et al[14] conducted a retrospective study of 1,200 patients with active pulmonary tuberculosis in Spain from 1998 to 2006, they found that the GSTT1 homozygote deletion genotype was a risk factor for the development of hepatotoxicity of antituberculosis drugs (odds ratio 2.60), whereas GSTM1 was not significantly associated with hepatotoxicity. Furthermore, patients with both GSTM1 and GSTT1 deletion also had a lower risk factor for anti-tuberculosis drug-induced hepatic impairment than those with a single GSTT1 deletion. However, Studies by Sang-Heon Kim et al[15] in a Korean population showed that the GSTT1 and GSTM1 deletion variant genotypes were not associated with

the occurrence of anti-tuberculosis drug-induced hepatotoxicity, this conclusion were supported by Chatterjee S et al [20].

The results of this study show that the GSTM1 and GSTT1 gene polymorphisms are not related to the susceptibility of anti-TB drugs associated liver toxicity. The reason for the inconsistency between the findings of this study and various reports may be that there are large differences in genes from different regions and ethnicities, coupled with the small sample size of predecessors and differences in diagnostic criteria for hepatotoxicity. Moreover, anti-tuberculous drug hepatotoxicity is a multi-factor, multi-gene-related disease. When selecting cases and controls, the influence of various factors can easily be offset by each other, resulting in false negatives. Therefore, prospective randomized trials with large sample size and multivariate analysis are still needed to confirm the relationship between GSTM1 and GSTT1 and hepatotoxicity.

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