



Pharmacogenetics

The roles of *GSTM1* and *GSTT1* null genotypes and other predictors in anti-tuberculosis drug-induced liver injury

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Received 2 March 2012, Accepted 19 June 2012

Keywords: adverse event, drug related, genetic polymorphisms, pharmacogenetics

SUMMARY

What is known and Objective: Anti-tuberculosis drugs (ATD), although highly effective, often cause liver injury. Glutathione S-transferases (GST) play a crucial protective role in the detoxifying mechanisms of drugs. Several studies have investigated the genetic null variants of *GSTM1* and *GSTT1* as possible risk factors for ATD-induced liver injury; however, those findings are inconsistent. We investigated *GSTM1* and *GSTT1* null genotypes in Brazilian patients with tuberculosis (TB), adjusting for other possible predictors of ATD-induced liver injury.

Methods: This was a prospective cohort study with patients who were treated for TB from 2006 to 2011. *GSTM1* and *GSTT1* gene deletions were analysed from genomic DNA by polymerase chain reaction (PCR). Demographic, clinical and laboratory data were extracted from medical records and possible predictors of liver injury were evaluated.

Results and Discussion: This study enrolled 177 patients. Anti-tuberculosis drugs-induced liver injury incidence was 33.3%. Hepatitis B infection (HBV) and increased alanine aminotransferase (ALT) baseline were significant predictors. Neither *GSTM1* nor *GSTT1* null genotypes were associated with ATD-induced liver injury; nevertheless, the comparison among four different liver toxicity grades showed that *GSTM1* non-null genotype was significant more frequent among the higher grades of liver toxicity.

What is new and Conclusion: *GSTM1* and *GSTT1* null genotypes do not seem to play important roles in ATD-induced liver injury in Brazilians. However, there was evidence that *GSTM1* polymorphisms were possibly related to the intensity of toxicity. Active HBV and initial high ALT could predict ATD-induced liver injury.

WHAT IS KNOWN AND OBJECTIVE

Standard tuberculosis (TB) treatment with rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E) is highly effective; however, the multi-drug regimen for TB often causes

adverse events. Hepatotoxicity is one of the most frequent and most serious adverse effects observed during TB treatment.^{1,2} As hepatotoxicity induced by anti-tuberculosis drugs (ATD) can lead to treatment interruption^{3,4} and result in therapeutic failure,² it is important to understand potential predictors of ATD-induced liver injury.

Drug metabolism pathways are classified as phase I and II reactions. Phase I reactions frequently produce toxic intermediates.² Glutathione S-transferases (GST) are multi-gene families of enzymes involved in phase II reactions that play a crucial protective role in the detoxifying mechanisms of drugs and xenobiotics.^{5–7} Mammalian soluble GST of mu (M) and theta (T) classes, *GSTM1* and *GSTT1*, respectively, are polymorphic in the human population. Carriers of *GSTT1* and *GSTM1* null genotypes lack any functional enzyme activity and fail to express the protein.^{5,8} The effects of these null genotypes on the risk of adverse events during TB treatment should be considered, as it has been suggested that GST enzymes may be important in R and H biotransformation processes.^{9,10}

Independent studies with different populations have examined the association of both gene deletions with drug-induced liver injury^{1,6,7,11–13}; however, their findings are conflicting. In Brazil, investigations focusing on probable implications of GST enzyme activity deficiencies on ATD-induced liver injury are scarce.

To study a probable genetically determined incidence of ATD-induced liver injury, we investigated *GSTM1* and *GSTT1* null genotypes in Brazilian TB patients adjusting for other factors that could affect drug-induced liver injury.

METHODS

Study design and Settings

This was a prospective cohort study of patients who were treated for active tuberculosis at Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil.

This investigation was approved by the institutional ethics committee and review board on 22 September 2003, registered at National System of Information about Ethics in Research: 0013-0-009-000-03/2003 and it was performed from 2006 to 2011.

The eligibility criteria were (i) signed written consent; (ii) sputum smear with acid-fast bacilli or culture positive for *Mycobacterium tuberculosis*; (iii) ongoing TB treatment; (iv) Human

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Immunodeficiency Virus (HIV), Hepatitis B and C virus (HBV and HCV) serology results; and (v) laboratory liver function tests. The exclusion criteria were (i) no more than one visit registered, (ii) pregnancy and (iii) age <18 years.

Demographic, clinical and laboratory data were extracted from medical records. Possible predictors of ATD-induced liver injury analysed were age (years); gender; ethnicity; TB clinical form; multiple sexual partners; HIV infection; HAART use; HCV infection; HBV infection; *GSTM1* and *GSTT1* genotypes; tobacco use; alcoholism; baseline alanine aminotransferase (ALT) levels [IU/L; baseline CD4⁺ cell counts (cells/ μ L); and plasma HIV viral load levels (copies/mL).

Ethnicity was defined as White or non-White; TB clinical form defined as pulmonary, extra-pulmonary (TB affecting areas other than the lungs), pulmonary/extra-pulmonary or disseminated (simultaneous occurrence of TB in two or more non-contiguous sites); multiple sexual partners defined as 'yes', if two or more sexual partners in 1 year, or 'no', otherwise; HIV and HCV infection defined by positive or negative serology; HBV defined by serological detection of HBV surface antigen (HBsAg); tobacco use defined as current use reported by the patient; and alcoholism defined by a positive CAGE questionnaire.¹⁴

Following the Council of International Organizations of Medical Sciences (CIOMS), liver injury was defined as an increase in serum ALT levels beyond twice the normal upper limit (ALT \geq 42 international units [IU]/L), or at least a twofold increase in ALT initial levels for those patients with a baseline ALT of >84 IU/L, during the treatment period.

The severity of hepatotoxicity was classified according to the World Health Organization (WHO) Toxicity Classification Standards.²

The main outcome of interest was ATD-induced liver injury, taking into account the chronological relationship between start of drug intake and the development of liver injury for establishing causality.

GST genotyping

Genomic DNA was extracted from five millilitres whole blood using the QIAamp[®] DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions. *GSTM1* and *GSTT1* DNA fragments were amplified by polymerase chain reaction (PCR) assays.

GSTM1 genotyping was performed using primers *GSTM1*-I6F and *GSTM1*-I7R designed for Intron 6 and Intron 7 of *GSTM1*,

respectively, and 12HD8S and 12HD8R designed for Intron 8 of Vitamin D receptor gene as the internal amplification control (Table 1). The 25- μ L reaction mixture consisted of Template DNA (90 ng), PCR buffer (1 \times), MgCl₂ (1.5 mM), deoxynucleotide triphosphates dNTPs (0.2 mM), *GSTM1*-I6F (0.4 μ M), *GSTM1*-I7R (0.4 μ M), 12HD8S (0.4 μ M), 12HD8R (0.4 μ M) and Taq DNA Polymerase (Invitrogen by Life Technology[®], Carlsbad, CA, USA) (2U).

GSTT1 genotyping was performed using primers *GSTT1*F and *GSTT1*R designed for Exon 4 and Exon 5 of *GSTT1*, respectively, and AlbF and AlbR for Albumin gene¹⁵ as the internal amplification control (Table 1). The 25- μ L reaction mixture consisted of Template DNA (90 ng), PCR buffer (1 \times), MgCl₂ (1.5 mM), deoxynucleotide triphosphates dNTPs (0.2 mM), *GSTT1*F (0.8 μ M), *GSTT1*R (0.8 μ M), AlbF (0.4 μ M), AlbR (0.4 μ M), Taq DNA Polymerase (Invitrogen by Life Technology[®]) (2U).

PCR assays were carried out with the following conditions: Initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 50 s, annealing at 62 °C (*GSTM1*) or 64 °C (*GSTT1*) for 1 min, chain elongation at 72 °C for 30 s and final synthesis at 72 °C for 8 min.

Null genotypes were detected by the absence of a PCR product along with the presence of the internal control band on a 1.5% agarose gel containing 0.1 μ g/mL ethidium bromide, visualized by UV light and compared with the molecular weight marker. The DNA image was digitalized using a transilluminator with a system of image capture L-PIX-ST and L-PIX IMAGE 7.1 M Pixel. Images were captured with the software L-PIX IMAGE 1.0.1 (Loccus Biotecnologia, São Paulo, SP, Brazil).

Statistical Analysis

The continuous variables age, baseline ALT levels, CD4⁺ and HIV viral load were described by median and interquartile range (IQR, 25th to 75th percentile).

Pearson's chi-square or Fisher's exact test was used to compare categorical variables, and rank sum test was performed to compare the continuous variables. Relative risk (RR) with 95% confidence interval (CI) was calculated to estimate the strength of association. The statistical tests were based on a two-tailed probability with a significance threshold of 0.05. A survival analysis with a Cox proportional hazard model was conducted. Different nested Cox models were tested by the deviance statistics, with a significance threshold of 0.05. All analyses were performed using the Statistical Package for the Social Sciences,

Table 1. PCR primers

Genotyping	Primers		Fragment size (bp)
<i>GSTM1</i>	<i>GSTM1</i> -I6F	5'GAA TGA GAT CTG TTT TGC TTC ACG3'	381
	<i>GSTM1</i> -I7R	5'GC GAG ATA ATT CTG TTA CCT TAC TGG3'	
Vitamin D receptor	12HD8S	5'CC AAG ACT ACA AGT ACC GCG TCA3'	795
	12HD8R	5'AGC GGA AGA GGT CAA GGG TCA3'	
<i>GSTT1</i>	<i>GSTT1</i> F	5'GTG AGC CAG TAT CTC CCC AGA CA 3'	517
	<i>GSTT1</i> R	5'CTG CTT TAT GGT GGG GTC TGC A3'	
Albumin ^a	AlbF	5'GCC CTC TGC TAA CAA GTC CTAC3'	350
	AlbR	5'GCC CTC TGC TAA CAA GTC CTAC3'	

^aAlbumin primers defined by Arand *et al.*¹⁵

SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) and the R-project software (Windows version 2.14, packages *epicalc* and *rms*, R Development Core Team, Vienna, Austria).¹⁶

RESULTS AND DISCUSSION

Patients' characteristics

A total of 234 patients were screened and 177 patients were enrolled into this study. The median (IQR) age was 37 (30–49.0) years. Table 2 shows patient's characteristics. Most of them were men (66.1%), non-White subjects (61.0%) with no more than one sexual partner in 1 year (71.2%).

Most patients presented pulmonary TB (50.9%), followed by extra-pulmonary (22.6%) and disseminated (17.5%) clinical forms.

Given that about 13% of TB cases occur among people living with HIV,¹⁷ TB and HIV co-infection is significant in this context. Almost half the population studied 79/177 (44.63%) was HIV-positive; as this investigation was conducted in a reference centre for infectious diseases treating a population with specific characteristics, the high prevalence of HIV infection is clarified.¹⁸

The majority of HIV-infected patients 63/79 (79.8%) were taking concurrent antiretroviral therapy (ART) and TB treatment. The most common HAART combination used was zidovudine + lamivudine + efavirenz (AZT + 3TC + EFZ); few patients 11/63 (17.5%) were treated with protease inhibitors (PI), such as ritonavir (RTV) and saquinavir (SQV).

HCV prevalence was 6.8%, whereas HBsAg was present in 2.3% of patients. Concerning tobacco use and alcoholism, the identified rates were 29.4% and 15.8%, respectively.

GST genotypes

GSTM1 null genotype was identified in 31.1% of patients and *GSTT1* null genotype was identified in 22.0%.

The comparison of the null genotypes according to ethnicity showed no significant differences between White and non-White individuals. Among the White subjects, the frequency of *GSTM1* null genotype was 22/69 (31.9%) whereas in the non-White ethnicity was 33/108 (30.6%). Additionally, 16/69 (23.2%) of White individuals were carriers of the *GSTT1* null genotype whereas 23/108 (21.3%) of non-White subjects were carriers of the same genotype.

Table 2. TB patients' characteristics and crude comparisons of categorical variables

Variable	<i>n</i>	[%]	ATD-induced liver injury (%)	RR	CI [95%]	<i>P</i> value
Gender						
Male	117	66.10	38.00	1.65	[0.99–2.75]	0.04
Female	60	33.90	23.00	1.00	–	–
Ethnicity						
White	69	38.98	41.00	1.41	[0.94–2.13]	0.11
Non-White	108	61.02	29.00	1.00	–	–
TB clinical form						
Disseminated	31	17.51	55.00	2.06	[1.29–3.29]	0.01
Extra-pulmonary	40	22.60	3.00	1.12	[0.63–2.02]	0.69
Extra-pulmonary/pulmonary	16	9.04	38.00	1.41	[0.68–2.89]	0.39
Pulmonary	90	50.85	27.00	1.00	–	–
Multiple sexual partners						
Yes	40	22.60	45.00	1.67	[1.07–2.61]	0.04
No	126	71.19	27.00	1.00	–	–
HIV						
Positive	79	44.63	46.00	1.94	[1.26–2.99]	<0.01
Negative	98	55.37	23.00	1.00	–	–
Anti-HCV						
Positive	12	6.78	25.00	0.74	[0.27–2.01]	0.56
Negative	165	93.22	34.00	1.00	–	–
HBsAg						
Positive	4	2.26	75.00	2.32	[1.26–4.24]	0.12
Negative	173	97.74	32.00	1.00	–	–
Tobacco use						
Yes	52	29.38	33.00	0.97	[0.61–1.54]	0.91
No	125	70.62	34.00	1.00	–	–
Alcohol						
Yes	28	15.82	39.00	1.22	[0.73–2.04]	0.47
No	149	84.18	32.00	1.00	–	–
<i>GSTT1</i>						
Null	39	22.03	28.00	0.81	[0.47–1.41]	0.45
Non-Null	138	77.97	35.00	1.00	–	–
<i>GSTM1</i>						
Null	55	31.07	38.00	1.23	[0.80–1.88]	0.36
Non-Null	122	68.93	31.00	1.00	–	–

RR, risk ratio; CI, confidence interval; *P* value, midp.exact; ATD, anti-tuberculosis drugs; TB, tuberculosis.

The *GSTM1* and *GSTT1* homozygous deletions genotypes in healthy individuals have been reported in different populations¹⁹ and these are rather variable among different ethnic groups. In Brazil, *GSTM1* and *GSTT1* null genotypes have been described in that order in Whites, 48% and 26%; Intermediates, 44% and 24%; and Blacks, 28% and 27%.²⁰

The *GSTM1* null frequency found in this study is lower than those described in other ethnic groups worldwide (35–100%),^{5,19} but it is consistent with the frequency described for African Americans (23–41%),¹⁹ and the frequency found among non-White subjects (30.6%), yet slightly higher, is similar to the described among Black Brazilian individuals (28%).²⁰

The frequency of *GSTT1* null genotype described here is in agreement with the variation described worldwide (10–62%),^{5,19} although it is somewhat lower than the frequency described for Black Brazilians (27%).²⁰ Furthermore, the frequency found among White and non-White subjects (23.2% and 21.3%) is lower, but similar to that described among Whites (26%) and Intermediate Brazilians (24%).²⁰

ATD-induced liver injury

Anti-tuberculosis drugs-induced liver injury incidence was 33.3% (59/177) and possible predictors are shown in Tables 2 and 3. The factors that showed a significant increase in risk of ATD-liver injury were male gender (RR 1.65) with a CI close to the decision threshold of 95%, disseminated TB (RR 2.06), multiple sexual partners (RR 1.67) and HIV infection (RR 1.94). Additionally, mean values of baseline ALT were significantly elevated in patients who developed ATD-induced liver injury ($P = 0.004$; Table 3).

Almost half of the HIV patients, 46.0% (36/79), developed ATD-induced liver injury. Although statistically not significant, 69.4% (25/36) of them were receiving HAART ($P = 0.093$) and 16.0% (4/25) receiving PI ($P = 1.00$). HIV-infected TB patients have an increased risk of hepatotoxicity² and this adverse event is frequently observed in patients receiving both therapies,^{2,21,22} possibly owing to the combined TB/HIV treatment that is often complicated by overlapping toxicities and drug–drug interactions.² However, there is evidence that HIV infection itself, independently to ART use, can increase the risk of serious adverse events during anti-tuberculosis therapy.²³ Therefore, it could be

speculated that the ATD-induced liver injury observed here could be related to altered activities of oxidative pathways² induced during the course of HIV infection.²⁴

The incidence of ATD-induced liver injury was 1.7 times higher than 19.5% estimated from the 2003–2005 cohort,¹⁸ suggesting that this adverse event is possibly increasing over time in the TB population of our Institute. Even though the incidence rate of ATD-induced liver injury depends on the investigators' definition of hepatotoxicity, as well as the population studied, the 33.3% frequency found here is above the incidence range of variation that has been reported in several studies (2–28%),² possibly because of differences in drug regimens.

Cox Regression Analyses

After comparing several possible combinations of predictors, only initial ALT remained in the final model, which showed a HR of 1.15 (1.09–1.20). Thus, a model with five predictors previously defined with another set of patients was fitted (ethnicity; multiple sexual partners; TB clinical form; HBsAg; and baseline ALT).¹⁸ After adjustment, only two of the five predictors showed significance, HBsAg (positive HR 10.69) and baseline ALT (HR 1.60) (Table 4). There were small differences in four predictors and an elevated difference at HBsAg regression coefficient, which was not significant in the previous data. Also, including *GSTT1* and *GSTM1* as predictors in this model did not substantially change the remaining predictors HR (Table 5), neither substantially changed the crude RR of *GSTM1* and *GSTT1* estimates.

Our previous study identified White ethnicity, extra-pulmonary (or disseminated) TB, multiple sexual partners, presence of HBsAg and high initial ALT as predictors of liver toxicity.¹⁸ In contrast, in this investigation we did not find the same variables as predictors of ATD-liver injury, which could be explained by the small sample size along with the eleven missing values of 'multiple sexual partners'.

Relationship between GST genotypes and ATD-induced liver injury

The GST null genotypes were compared to liver toxicity grades² (Table 6). No association was found between *GSTT1* null

Table 3. Baseline characteristics and crude comparison of continuous variables

Variable	ATD- liver injury		Total	P value
	Present	Absent		
Age	37 (32.0–45.5)	38 (30.0–49.7)	37 (30.0–49.0)	0.673
Median (IQR)				
ALT	48 (35.5–66.5)	34 (28.0–41.5)	37 (29.0–53.0)	<0.001
Median (IQR)				
n1	59	118	177	–
CD4+	120 (52.5–299.0)	225 (102.0–358.0)	153 (61.0–359.2)	0.231
Median (IQR)				
HIV viral load	68 227 (242.0–240 000.0)	44 082 (10892.5–149 581.2)	52 000 (3989.0–204 234.0)	0.908
Median (IQR)				
n2	36	43	79	–

ALT, alanine aminotransferase; ATD, anti-tuberculosis drugs; TB, tuberculosis; Test statistics, rank sum; n1, all patients studied; ALT, baseline levels before TB treatment; n2, only HIV patients.

Table 4. Cox regression model for ATD-induced liver injury with five variables

Variable	HR	CI 95%
Ethnicity		
White	1.20	[0.61–2.36]
Non-White	1	–
Multiple sexual partners		
Yes	1.84	[0.94–3.61]
No	1	–
TB clinical form		
Disseminated	2.22	[0.99–4.97]
Extra-pulmonary	0.85	[0.37–1.93]
Extra-pulmonary/pulmonary	1.22	[0.45–3.29]
Pulmonary	1	–
HBsAg		
Positive	10.69	[2.10–54.25]
Negative	1	–
ALT		
[IU]/L	1.60	[1.23–2.07]

ALT, alanine aminotransferase; ATD, anti-tuberculosis drugs; TB, tuberculosis; HBsAg, hepatitis B surface antigen; HR, hazard ratio; CI, confidence interval; ALT, baseline levels before TB treatment.
 $R^2 = 0.243$.

Table 5. Final Cox regression model for ATD-induced liver injury with GST genotypes

Variable	HR	CI 95%
Ethnicity		
White	1.20	[0.61–2.36]
Non-White	1	–
Multiple sexual partners		
Yes	1.85	[0.94–3.64]
No	1	–
TB clinical form		
Disseminated	2.26	[1.00–5.10]
Extra-pulmonary	0.97	[0.41–2.27]
Extra-pulmonary/pulmonary	1.18	[0.43–3.25]
Pulmonary	1	–
HBsAg		
Positive	9.57	[1.81–50.62]
Negative	1	–
GSTT1		
Null	0.76	[0.34–1.68]
Non-Null	1	–
GSTM1		
Null	1.64	[0.83–3.25]
Non-Null	1	–
ALT		
[IU]/L	1.66	[1.27–2.16]

ALT, alanine aminotransferase; ATD, anti-tuberculosis drugs; GST, glutathione S-transferases; TB, tuberculosis; HR, hazard ratio; CI, confidence interval; ALT, baseline levels before TB treatment.
 $R^2 = 0.258$.

genotype and the four grades; on the other hand, the *GSTM1* null genotype was more frequent among individuals who presented grade 1 mild toxicity and the rate of the non-null

genotype increased with increasing severity of grades, so that the presence of at least one functional allele of *GSTM1* was significantly more frequent among the groups with higher grades of liver toxicity ($P = 0.007$).

It could be hypothesized that the presence of the enzyme would result in overactivity of *GSTM1* and therefore in glutathione (GSH) depletion, the substrate of GST.^{25,26} Because GSH depletion of up to 30% of the total GSH level can impair the conjugation defence against toxic actions, the overactivity of *GSTM1* conjugation may have become counterproductive.²⁶ Moreover, as the GSTs are multi-gene families of enzymes, the combined conjugation activities of all the GSTs would increase the GSH depletion and instead of protecting, the GSTs collectively may have exposed the cell to injurious effects, such as oxidative DNA damage and associated mutagenic lesions.²⁵ Hence, the non-null genotype would be a bad prognostic marker of ATD-induced liver injury.

Although *GSTM1* polymorphisms were positively associated with liver toxicity grades and the *GSTM1* null genotype showed some increase in risk of ATD-induced liver injury (RR = 1.23, HR = 1.64), yet not statistically significant, neither *GSTM1* nor *GSTT1* null genotypes seemed to play important roles in ATD-induced liver injury. These results are in discrepancy with the studies that found a positive association.^{1,6,7,12}

A limitation of this study is that other explanatory variables that could be correlated with one or more of the other potential predictors were not evaluated. Concomitant use of other drugs, diet and the genetic polymorphisms of other biotransformation enzymes, such as phase I enzymes (e.g. cytochrome P450 enzymes – CYP450), could be predicting ATD-induced liver injury.

Several studies have examined the association of both gene deletions with drug-induced liver injury; however, their findings are inconsistent. In Asia, the *GSTM1* null genotype has been associated with individual ATD-induced hepatotoxicity.^{7,12} More recently, in India¹¹ and Korea,¹³ neither *GSTM1* nor *GSTT1* null genotypes were associated with anti-TB drug-induced hepatotoxicity, showing results in disagreement with those of previous studies on Asian populations. On the other hand, the *GSTT1* homozygous null genotype has been associated with ATD-induced liver injury in Caucasian patients.¹ Alternatively, the double-null *GSTM1* and *GSTT1* genotype may have been important to susceptibility to hepatotoxicity related to a wide variety of drugs, in Spanish patients.⁶ Differently, the latest study conducted in Brazil did not find an association between *GSTM1* and *GSTT1* genotypes with ATD-induced hepatitis.²⁷

Nevertheless, those studies were conducted with different populations, study designs, genotyping methods, methods of defining liver injury and of choosing cases and controls. Therefore, it could be hypothesized that they are not comparable; additionally, most of those investigations excluded other variables that could have been confounders for ATD-induced liver injury. Thus, this study addressed hepatotoxicity in TB patients in a different way; we tested potential predictors such as HIV, HBV, HCV, alcoholism and tobacco use, which usually represent exclusion criteria, to verify the combined prediction ability of these characteristics along with the genetic ones (*GSTM1* and *GSTT1*).

WHAT IS NEW AND CONCLUSION

The present investigation corresponds to the first effort made to study *GSTM1* and *GSTT1* null genotypes in Brazilians controlling

Table 6. GST null genotypes and liver toxicity grades

	Grade 1 (mild) <2.5 X ULN (ALT 84–105 IU/L) n (%)	Grade 2 (moderate) 2.5–5 X ULN (ALT 106–210 IU/L) n (%)	Grade 3 (severe) 5–10 X ULN (ALT 211–420 IU/L) n (%)	Grade 4 (serious) >10 XULN (ALT >420 IU/L) n (%)	P value
<i>GSTT1</i>					0.748
Non-Null	10 (90.91)	24 (75.00)	10 (90.91)	4 (80.00)	
Null	1 (9.09)	8 (25.00)	1 (9.09)	1 (20.00)	
<i>GSTM1</i>					0.007
Non-Null	2 (18.18)	23 (71.88)	9 (81.82)	4 (80.00)	
Null	9 (81.82)	9 (28.12)	2 (18.18)	1 (20.00)	
n	11	32	11	5	

ALT, alanine aminotransferase; Test statistics, Fisher's exact test; ULN, upper limit of normal; GST, glutathione S-transferases. Toxicity grades defined according to WHO Toxicity Classification Standards.²

for other factors that could simultaneously affect ATD-induced liver injury.

GSTM1 and *GSTT1* null genotypes were not associated with ATD-induced liver injury, suggesting that these genetic polymorphisms may not be important to this adverse event in TB patients. Nevertheless, ATD-induced liver injury is multi-factorial in origin, and other predictors of liver injury could be involved.

There was evidence that the *GSTM1* polymorphisms were possibly related to the intensity of toxicity. Moreover, active HBV and increased ALT baseline could predict ATD-induced liver injury.

REFERENCES

- Leiro V, Fernández-Villar A, Valverde D, Constenla L, Vázquez R, Piñeiro L, González-Quintela A. Influence of glutathione S-transferase M1 and T1 homozygous null mutations on the risk of antituberculosis drug-induced hepatotoxicity in a Caucasian population. *Liver Int*, 2008;**28**:835–839.
- Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *J Gastroenterol Hepatol*, 2008;**23**:192–202.
- Leung CC, Chan CK, Tam CM. HIV-related TB and adverse drug events. *Thorax*, 2007;**62**:923–924.
- Sharma SK, Singla R, Sarda P *et al.* Safety of 3 different reintroduction regimens of antituberculosis drugs after development of antituberculosis treatment-induced hepatotoxicity. *Clin Infect Dis*, 2010;**50**:833–839.
- Eaton DL, Bammler TK. Concise review of the glutathione S-transferases and their significance to toxicology. *Toxicol Sci*, 1999;**49**:156–164.
- Lucena MI, Andrade RJ, Martínez C *et al.* Glutathione S-transferase m1 and t1 null genotypes increase susceptibility to idiosyncratic drug-induced liver injury. *Hepatology*, 2008;**48**:588–596.
- Roy B, Chowdhury A, Kundu S, Santra A, Dey B, Chakraborty M, Majumder PP. Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. *J Gastroenterol Hepatol*, 2001;**16**:1033–1037.
- Xu S, Wang Y, Roe B, Pearson WR. Characterization of the human class Mu glutathione S-transferase gene cluster and the *GSTM1* deletion. *J Biol Chem*, 1998;**273**:3517–3527.
- Sodhi CP, Rana S, Mehta S, Vaiphei K, Goel RC, Mehta SK. Study of oxidative-stress in rifampicin-induced hepatic injury in growing rats with and without protein-energy malnutrition. *Hum Exp Toxicol*, 1997;**16**:315–321.
- Sodhi CP, Rana SV, Mehta SK, Vaiphei K, Attri S, Thakur S, Mehta S. Study of oxidative stress in isoniazid-induced hepatic injury in young rats with and without protein-energy malnutrition. *J Biochem Toxicol*, 1996;**11**:139–146.
- Chatterjee S, Lyle N, Mandal A, Kundu S. *GSTT1* and *GSTM1* gene deletions are not associated with hepatotoxicity caused by antitubercular drugs. *J Clin Pharm Ther*, 2010;**35**:465–470.
- Huang YS, Su WJ, Huang YH, Chen CY, Chang FY, Lin HC, Lee SD. Genetic polymorphisms of manganese superoxide dismutase, NAD(P)H:quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. *J Hepatol*, 2007;**47**:128–134.
- Kim SH, Kim SH, Yoon HJ *et al.* *GSTT1* and *GSTM1* null mutations and adverse reactions induced by antituberculosis drugs in Koreans. *Tuberculosis (Edinb)*, 2010;**90**:39–43.
- Mayfield D, McLeod G, Hall P. The CAGE questionnaire: validation of a new alcoholism screening instrument. *Am J Psychiatry*, 1974;**131**:1121–1123.
- Arand M, Mühlbauer R, Hengstler J, Jäger E, Fuchs J, Winkler L, Oesch F. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase *GSTM1* and *GSTT1* polymorphisms. *Anal Biochem*, 1996;**236**:184–186.

ACKNOWLEDGEMENTS

The authors thank the medical, technical and nursing staff of the Evandro Chagas Clinical Research Institute (IPEC) for their support, and Leonardo Rosadas and Patricia Silva for technical assistance. This work is supported by grants from the Foundation for Research Support of Rio de Janeiro State (FAPERJ).

CONFLICTS OF INTEREST

The authors declare there are no conflicts of interest.

16. R Development Core Team. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2011.
17. World Health Organization. *Global tuberculosis control: WHO report 2011*. WHO/HTM/TB/2011.16. Geneva, Switzerland: WHO, 2011.
18. de Castro L, do Brasil PE, Monteiro TP, Rolla VC. Can hepatitis B virus infection predict tuberculosis treatment liver toxicity? Development of a preliminary prediction rule *Int J Tuberc Lung Dis*, 2010;**14**:332–340.
19. Roy PD, Majumder M, Roy B. Pharmacogenomics of anti-TB drugs-related hepatotoxicity. *Pharmacogenomics*, 2008;**9**:311–321.
20. Suarez-Kurtz G, Vargens DD, Struchiner CJ, Bastos-Rodrigues L, Pena SD. Self-reported skin color, genomic ancestry and the distribution of GST polymorphisms. *Pharmacogenet Genomics*, 2007;**17**:765–771.
21. Martinez E, Blanco JL, Arnaiz JA *et al*. Hepatotoxicity in HIV-1-infected patients receiving nevirapine-containing antiretroviral therapy. *Aids*, 2001;**15**:1261–1268.
22. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. *J Am Med Assoc*, 2000;**283**:74–80.
23. Marks DJ, Dheda K, Dawson R, Ainslie G, Miller RF. Adverse events to antituberculosis therapy: influence of HIV and antiretroviral drugs. *Int J STD AIDS*, 2009;**20**:339–345.
24. Wanchu A, Rana SV, Pallikkuth S, Sachdeva RK. Short communication: oxidative stress in HIV-infected individuals: a cross-sectional study. *AIDS Res Hum Retroviruses*, 2009;**25**:1307–1311.
25. Roodi N, Dupont WD, Moore JH, Parl FF. Association of homozygous wild-type glutathione S-transferase M1 genotype with increased breast cancer risk. *Cancer Res*, 2004;**64**:1233–1236.
26. Yu KD, Di GH, Fan L *et al*. A functional polymorphism in the promoter region of GSTM1 implies a complex role for GSTM1 in breast cancer. *FASEB J*, 2009;**23**:2274–2287.
27. Teixeira RL, Morato RG, Cabello PH *et al*. Genetic polymorphisms of NAT2, CYP2E1 and GST enzymes and the occurrence of antituberculosis drug-induced hepatitis in Brazilian TB patients. *Mem Inst Oswaldo Cruz*, 2011;**106**:716–724.