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Genetic polymorphisms of *CYP*2E1 and *GSTM*1 loci and susceptibility to anti-tuberculosis drug-induced hepatotoxicity

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SUMMARY

BACKGROUND: Host genetic factors that influence predisposition to anti-tuberculosis drug-induced hepatotoxicity (DIH) are not clear in the Indian population.

OBJECTIVE: To investigate the possible association of DIH with polymorphism at the *Rsa*I site of the 5-prime untranslated region of *CYP*2E1 and *GSTM*1 'null' mutations.

METHODS: In this prospective study, 113 tuberculosis (TB) patients with DIH and 201 TB patients receiving anti-tuberculosis treatment without developing hepatotoxicity (non-DIH) constituted cases and controls, respectively. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to analyse genetic polymorphisms of *CYP450* 2E1 at the *Rsa*I site and 'null' *GSTM*1 mutations. PCR-RFLP results were compared between 185 non-DIH and 105 DIH patients.

RESULTS: A high frequency of c1c1 genotypes of *CYP2E1* was commonly encountered, and the difference between DIH and non-DIH patients was not significant (75.14% vs. 77.14%). The genotypic distribution of c2c2 was significantly higher in the DIH than in the non-DIH group (4.8% vs. 0.5%, OR 8.58, $P = 0.03$). However, adjustment for age, sex and serum albumin differences yielded an OR of 2.75, making it non-significant ($P = 0.26$). Homozygous 'null' mutation frequencies at the *GSTM*1 gene in DIH and non-DIH patients were observed that were not significantly different (40% and 37%, respectively, $P = 0.61$).

CONCLUSION: *Rsa*I variants of the *CYP*2E1 gene and *GSTM*1 'null' mutation were not associated with risk of DIH in a north Indian population.

KEY WORDS: DIH; *GSTM*1 'null'; *CYP*2E1; genetic susceptibility; PCR-RFLP

INDIA has the world's highest burden of tuberculosis (TB), accounting for one fifth of global incidence.[1] Among the various anti-tuberculosis drugs, isoniazid (INH) has been extensively studied to understand the mechanism of hepatotoxicity.[2] Metabolic intermediates of INH, rather than INH per se, are believed to be the cause of hepatotoxicity.[3] In the liver, INH is first metabolised into acetylisoniazid via *N*-acetyltransferase (NAT), followed by hydrolysis to acetylhydrazine, which is then oxidised into hepatotoxic intermediaries by cytochrome P450 2E1 (*CYP*2E1).[4]

Genetic polymorphisms are known to be involved in the modification of drug metabolism in the liver and thus may predispose individuals to drug-induced hepatotoxicity (DIH) or provide protection against DIH. A meta-analysis has suggested that *CYP*2E1 *Rsa*I/*Pst*I polymorphism may affect susceptibility to DIH, particularly among Chinese and Korean populations.[5] However, available information in the literature regarding the role of *CYP*2E1 polymorphism on the risk of DIH development during anti-tuberculosis treatment is conflicting. Three genotypes of *CYP*2E1 (c1/c1, c1/c2 and c2/c2) are known to exist in humans, as demonstrated by restriction fragment length polymorphism (RFLP) using *Rsa*I as the restriction enzyme.[6]

The *GSTM*1 gene is located on chromosome 1p13.3; 20–50% of individuals do not express the enzyme due to a homozygous gene deletion known as the *GSTM*1*0, or null, allele.[7] A recent meta-analysis has suggested that the *GSTM*1 null genotype may be associated with an increased risk of DIH, particularly among the Chinese population.[8] Conflicting reports have been published on the role of glutathione *S*-transferase loci (*GSTM*1) in the detoxification of toxic metabolites of INH in humans.

The issue of genetic susceptibility to DIH due to INH has long been addressed by phenotypic studies involving acetylator status only. The present study was designed to assess the role of the 'null' mutation

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of *GSTM*1 and *RsaI* polymorphism of *CYP*2E1 in conferring susceptibility to DIH during anti-tuberculosis treatment.

MATERIAL AND METHODS

In this prospective study, patients aged 16–65 years were recruited from the out-patient department or the in-patient facility of the All India Institute of Medical Sciences (AIIMS) Hospital, New Delhi, India. A total of 113 consecutive patients who developed clinical and/or laboratory evidence of DIH while on anti-tuberculosis treatment between March 2007 and February 2011 constituted cases (DIH), while 201 TB patients without DIH constituted controls (non-DIH).

Written informed consent was obtained from all patients. The AIIMS New Delhi Ethics Committee approved the study protocol. A total of 155 patients who developed clinical and/or laboratory features suggestive of DIH while on anti-tuberculosis drugs were screened. Two patients died, while 40 patients were excluded. Reasons for exclusion included chronic alcoholism ($n = 7$), concomitant intake of hepatotoxic drugs ($n = 3$), acute viral hepatitis ($n = 20$) and human immunodeficiency virus (HIV) enzyme-linked immunosorbent assay (ELISA) test positivity ($n = 10$).

The diagnosis of pulmonary TB was based on the presence of acid-fast bacilli on sputum smear or *Mycobacterium tuberculosis* on sputum culture. In patients with smear-positive TB, culture was not performed unless multidrug-resistant TB was strongly suspected. Sputum cultures were performed in all smear-negative pulmonary TB patients. In patients with negative smears and cultures, TB was diagnosed based on symptoms, chest radiographic infiltrates in the upper lobes, and clinical and radiographic response to anti-tuberculosis drugs. Disseminated TB was diagnosed on the basis of histopathological and/or microbiological evidence of TB from two non-contiguous sites. Patients with disseminated TB exhibiting classic miliary mottling on chest radiograph were included as miliary TB. The dose levels of each anti-tuberculosis drug administered to the TB patients are shown in Table 1.

The diagnosis of DIH was based on previously published criteria.[9,10] Briefly, 1) an increase of five times the upper limit of normal (50 international units [IU]/l) of serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) levels on one occasion or more than three times (>150 IU/l) on three consecutive occasions; 2) serum total bilirubin level >1.5 mg/dl; 3) any increase in serum AST and/or ALT above pre-treatment values, together with anorexia, nausea, vomiting and jaundice; 4) absence of serological evidence of infection with hepatitis viruses A, B, C or E; and 5) improvement in liver function (serum bilirubin <1 mg/dl, AST and ALT <100 IU/l) after the withdrawal of anti-tuberculosis drugs. DIH was diagnosed if any one of criteria 1), 2) or 3) were present along with criteria 4) and 5). Non-DIH subjects were observed during the same study period, with regular follow-up of liver function tests.

Laboratory investigations including haemogram and blood biochemistry with detailed liver function tests (serum bilirubin, AST, ALT, alkaline phosphatase [ALP], serum total protein and serum albumin) were performed in all patients using standard laboratory procedures. Markers of acute viral hepatitis immunoglobulin IgM anti-hepatitis A virus (Immuno LISA, Orgenics, Yavne, Israel), IgM anti-hepatitis B core antigen and/or hepatitis B surface antigen (Microelisa System, bioMerieux, Zaltbommel, The Netherlands), IgM anti-hepatitis C virus antibodies (Microelisa System, bioMerieux) and IgM anti-hepatitis E virus (Immuno LISA, Orgenics) were performed in all patients who developed features suggestive of DIH while on anti-tuberculosis treatment. Serological testing for evidence of HIV 1 or 2 infection using ELISA was also performed. Abdominal ultrasonography was performed in all patients to rule out fatty liver or chronic liver disease.

Those patients whose serum samples tested positive for markers of viral hepatitis and/or who were receiving other potentially hepatotoxic drugs or had ultrasonography evidence of chronic liver disease were excluded from the study. HIV-infected and chronic alcohol-dependent patients who had consumed >48 g of alcohol/day for at least 1 year were excluded. Patients receiving other potentially hepatotoxic drugs (e.g., methotrexate, phenytoin, valproate, fluconazole), pregnant women and subjects who did not provide written informed consent were also excluded from the study.

Patients with DIH who satisfied the inclusion criteria were enrolled in the study. The hepatotoxic drugs INH, rifampicin (RMP) and pyrazinamide (PZA) were immediately stopped, and the patients were started on a regimen of non-hepatotoxic anti-tuberculosis drugs consisting of ethambutol, streptomycin and one of the fluoroquinolones. The patients were subsequently followed up on a weekly basis until clinical and biochemical parameters of acute liver injury stabilised, i.e., absence of vomiting and abdominal pain, both AST and ALT <100 IU/l and serum bilirubin <1.0 mg/dl. The time between stopping INH, RMP and PZA and achieving the above mentioned parameters was taken as the normalisation period.

GSTM*1 genotyping*

The *GSTM*1 homozygous 'null' genotype in DNA samples was detected by the absence of a polymerase chain reaction (PCR) product (230 base pair [bp]) on

Table 1 Dosages of anti-tuberculosis drugs administered to patients according to body weight

Anti-tuberculosis drug	Body weight kg	Dosage strength	Non-DIH dose ($n = 201$) mg/day mean ± SD	DIH ($n = 113$) mg/day mean ± SD	*P* value*
Rifampicin		mg/day			
	⩽35	300	537.31 ± 81.39	545.57 ± 82.79	0.39
	36–50	450			
	>50	600			
Isoniazid					
	⩽35	200	296.46 ± 18.56	297.51 ± 15.61	0.59
	>35	300			
Pyrazinamide		g/day			
	⩽50	1.0	1315.92 ± 241.75	1345.13 ± 232.22	0.29
	>50	1.5			
Ethambutol		mg/kg/day			
	—	15	845.77 ± 84.22	849.55 ± 86.73	0.70

* Based on Student's *t*-test.
DIH = drug-induced hepatotoxicity; SD = standard deviation.

2.0% agarose gel, together with the presence of an internal control band. The oligonucleotide primers (P1, P2, P3) used for PCR included 5′-CGC CAT CTT GTG CTA CAT TGC CCG-3′(P1), 5′-ATC TTC TCC TCT TCT GTC TC-3′ (P2) and 5′-TTC TGG ATT GTA GCA GAT CA-3′ (P3).[11] These three primers were used together in the reaction and formed two pairs, as follows: pair P1 and P2 (which anneal with homologous sequences of the *GSTM*1 and *GSTM*4 genes) yielded one 157 bp fragment used as an internal control for the reaction, and pair P1 and P3 (specific to the *GSTM*1 gene) produced a fragment of 230 bp. PCR was performed using Eppendorf Mastercycler ep gradient (Eppendorf North America Inc, Hauppauge, NY, USA); the PCR products were separated by electrophoresis in 2.0% agarose gel.

CYP2*E1 genotyping*

DNA was extracted from the patients' peripheral blood white blood cells using the Qiagen DNA Maxi manufacturer's Kit (Qiagen, Hilden, Germany). PCR with RFLP was used to genotype the *CYP*2E1 of patients and controls. Briefly, after initial amplification with the primer 5′-TTCATTCTGTCTTC-TAACTGG-3′ and 5′-CCAGTCGAGTCTACATTGT-CA-3′, the 410 bp PCR product was digested using restriction enzyme *Rsa*I (Fermentas, Burlington, ON, Canada).[6] The wild-type allele of *CYP*2E1 is c1, and the mutant is c2; individuals were accordingly grouped into one of the three *CYP*2E1 genotypes, c1/c1, c1/c2 and c2/c2.

Statistical analysis

Quantitative parameters such as anthropometric characteristics and liver function tests were compared between groups using Student's *t*-test or the Wilcoxon rank-sum test. The genotypic distribution was compared between cases and controls using Fisher's exact test. The odds ratio of DIH associated with c2c2 genotype was calculated using logistic regression. Multiple logistic regression was employed to adjust for backward differences between the DIH and non-DIH groups. Statistical analysis was carried out using Stata 11.2 (Stata Corp, College Station, TX, USA).

RESULTS

According to the defined criteria, 113 DIH cases and 201 non-DIH controls were recruited into the study. In the DIH group, pulmonary TB (PTB) was present in 28 (24.7%) patients, extra-pulmonary TB in 59 (52.2%) and miliary/disseminated TB in 26 (23%). The non-DIH control group comprised sputum smear- and culture-confirmed PTB patients. The baseline demographic profile is shown in Table 2. Patients in the control group were generally younger. Table 3 compares pre- and post-treatment liver function in the two groups. To compare the repeat measure parameters (liver enzymes and bilirubin), the highest peak post-treatment values attained were analysed.

The PCR-RFLP results were compared in 185 non-DIH and 105 DIH patients, as all the isolated genomic DNA samples did not show observable amplification that can be attributed to the presence of possible PCR inhibitory elements in the unamplified template DNA or the minimal DNA concentration. The frequency of the variant c2c2 genotype of

Table 2 Baseline demographic characteristics of non-DIH and DIH patients

Characteristics	Non-DIH ($n = 201$) mean ± SD	DIH ($n = 113$) mean ± SD	*P* value*
Age, years	27.6 ± 10.0	35.2 ± 13.4	<0.001
Male/female sex ratio	145/56	63/50	0.003
BMI, kg/m^2	17.1 ± 2.4	17.1 ± 1.5	0.52
MAC, cm	22.0 ± 2.8	21.7 ± 3.7	0.28
TSFT, mm	6.5 ± 1.0	6.6 ± 0.9	0.55

* Based on Student's *t*-test.
DIH = drug-induced hepatotoxicity; BMI = body mass index; MAC = mid-arm circumference; TSFT = triceps skin-fold thickness.

Table 3 Pre- and post-treatment liver function test results in non-DIH and DIH patients

Variables	Pre-treatment			Post-treatment		
	Non-DIH ($n = 201$) mean ± SD	DIH ($n = 113$) mean ± SD	*P* value*	Non-DIH ($n = 201$) mean ± SD	DIH ($n = 113$) mean ± SD	*P* value†
Serum bilirubin, mg/dl	0.69 ± 0.13	0.64 ± 0.14	0.06	0.75 ± 0.13	2.1 ± 0.13	<0.001
AST, IU/l (normal <50)	34.49 ± 14.21	37.46 ± 12.91	0.06	39.06 ± 18.45	323.18 ± 279.32	<0.001
ALT, IU/l (normal <50)	34.00 ± 3.92	34.20 ±13.09	0.44	43.83 ± 12.36	339.36 ± 303.60	<0.001
SAP, IU/l (normal 80–280)	258.54 ± 108.18	196.00 ± 92.77	<0.001	225.58 ± 56.77	303.96 ± 161.42	<0.001
Serum protein, g/dl	8.10 ± 0.64	7.50 ± 0.75	<0.001	8.20 ± 0.54	8.07 ± 0.42	0.01
Serum albumin, g/dl	3.90 ± 0.64	3.40 ± 0.44	<0.001	3.8 ± 0.42	4.09 ± 0.56	<0.00
Serum globulin, g/dl	4.20 ± 0.87	4.10 ± 0.62	0.33	4.34 ± 0.52	4.11 ± 0.75	0.99

* Based on Student's *t*-test.
† Based on Wilcoxon rank-sum test.
DIH = drug-induced hepatotoxicity; SD = standard deviation; AST = aspartate aminotransferase; IU = international unit; ALT = alanine aminotransferase; SAP = serum alkaline phosphatase.

*CYP*2E1 in DIH group was significantly higher than in the non-DIH group ($P = 0.03$). However, adjustment for age, sex and baseline serum albumin differences yielded an OR of 2.75 ($P = 0.26$). The frequency of the homozygous 'null' mutation in the *GSTM*1 gene was not significantly different (Table 4).

DISCUSSION

The incidence of DIH due to anti-tuberculosis drugs ranges from 1% to 36%.[2,10,12] Various clinical risk factors for DIH include old age,[12] malnutrition,[13] alcohol[14] and hepatitis B.[15] Several studies have been conducted to interpret the role of host genetic factors with regard to *N*-acetyltransferase 2 (NAT2),[16] cytochrome P450 2E1 (*CYP*2E1) polymorphisms,[17] human leukocyte class II antigen-DQ[18] and glutathione *S*-transferase M1 'null' mutation[19] in DIH.

*CYP*2E1 is immensely important in the metabolic activation of many drugs and carcinogens.[20] The *Rsa*I and *Pst*I restriction sites are in the transcription-regulation region of *CYP*2E1, which has been linked to gene expression.[21] *CYP*2E1 RFLP detected using *Dra*I or *Pst*I has been found to be closely associated with the RFLP of *CYP*2E1 by *Rsa*I digestion.[21] Only *Rsa*I RFLP was therefore performed in this study. *GSTM*1 is another candidate gene in which polymorphism has been shown to be related to drug-induced toxicities.[19] The potential role of *GSTM*1 'null' genotypes as genetic predictors of DIH is still unclear. Published reports have estimated that the genotype frequencies of *GSTM*1 'null' in the normal Indian population are in the range of 20–79%.[22,23] The present study was thus designed to investigate the role of *Rsa*I polymorphism in the 5′-untranslated region (UTR) of *CYP*2E1 and *GSTM*1 'null' mutation, keeping in mind that these polymorphisms could probably explain the high risk of DIH in north Indian TB patients receiving anti-tuberculosis treatment.

A significant association between the c2c2 genotype and development of DIH was observed in the present study; however, after adjusting for confounding factors age, sex and baseline serum albumin level differences, the association remains non-significant. This indicates that DIH may be due to these confounders rather than the c2c2 genotype. The adjusted OR is still of considerable magnitude (OR 2.75); a larger study with an adequate number of c2c2 genotypes could possibly confirm the association. No association was found between *GSTM*1 'null' mutations and DIH. It may therefore be assumed that the variant c2c2 genotype could increase the accumulation of intermediate anti-tuberculosis drug metabolites; however, it is not an independent risk factor for hepatotoxicity in the Indian population.

Table 4 Genotyping distribution of *CYP*2E1 (*Rsa*I) and *GSTM*1 'null' mutations in non-DIH and DIH patients

	Non-DIH ($n = 185$) *n* (%)	DIH ($n = 105$) *n* (%)	OR	*P* value
*Rsa*I (5′UTR-*CYP*2E1)				
c1c1	139 (75.14)	81 (77.14)	1.0	
c1c2	45 (24.32)	19 (18.10)	0.72	0.29
c2c2	1 (0.54)	5 (4.76)	8.58	0.03
*GSTM*1				
M1 null	68 (36.75)	42 (40)	1.14	0.61

*CYP*2E1 = cytochrome P450 2E1; *GSTM*1 = glutathione *S*-transferase mu1; DIH = drug-induced hepatotoxicity; OR = odds ratio; 5′UTR = 5-prime untranslated.

Table 5 Comparison of studies of *CYP2*E1-*Rsa*I polymorphism in DIH patients in different ethnic groups

Author, year, reference	Country	Cases vs. controls	Association
Huang, 2003[17]	Taiwan	DIH ($n = 49$) vs. non-DIH ($n = 318$)	c1c1, $P = 0.09$
Cho, 2007[25]	Korean	DIH ($n = 18$) vs. non-DIH ($n = 114$)	c1c1, $P = 1.00$
Lee, 2010[24]	Taiwan	DIH ($n = 45$) vs. non-DIH ($n = 95$)	c1c1, $P = 0.01$
Bose, 2011[26]*	India	DIH ($n = 41$) vs. non-DIH ($n = 177$)	*Dra*I C/D, $P = 0.01$
Present study	India	DIH ($n = 105$) vs. non-DIH ($n = 185$)	c2c2, $P = 0.03$

Earlier reports have suggested that a rare mutant allele of *CYP2*E1 (c2 allele) that lacks the *Rsa*I restriction site is associated with higher transcriptional activity, protein levels and enzyme activity than the more common wild-type allele (c1 allele).[21] We were careful to recruit patients with DIH after excluding patients who were concomitantly using potential hepatotoxic agents or those suffering from viral hepatitis. However, recent studies among different Asian ethnic groups did not reveal any relationship between *Rsa*I polymorphism and basal *CYP2*E1 activity. In their study, Huang et al. showed that patients with the *CYP2*E1 c1/c1 genotype had higher *CYP2*E1 activity than those with the *CYP2*E1 c1/c2 or c2/c2 genotype under the inhibitory effect of INH.[17] However, the authors of this study did not clearly mention whether 1) patients concomitantly using other potential hepatotoxic drugs had been excluded, or 2) adjustments had been made for baseline confounders. Likewise, another published report by Lee et al. found an association of the c1c1 genotype with DIH in a Taiwanese population.[24] In this study, authors did not state whether patients concomitantly using other hepatotoxic drugs or those suffering from viral hepatitis (hepatitis A and E) had been excluded; furthermore, the authors did not specify whether the results were adjusted for baseline confounders. In a Korean study, Cho et al. did not find a significant association of the c1c1 genotype with DIH.[25] A study from India did not find an association of *CYP2*E-*Rsa*I polymorphism with DIH (Table 5).[26] A re-examination of these earlier reports, adjusting for confounders, might confirm our observation of the lack of any association between c2c2 and DIH.

Reports on the association of a *GSTM*1 'null' mutation with DIH are conflicting. In a study in an Indian population, homozygous 'null' mutation at *GSTM*1 loci was implicated in DIH;[19] however, this study was conducted among a small number of patients. A study from Taiwan subsequently reported an increased risk of DIH with the *GSTM*1 'null' genotype (OR 2.23, $P = 0.03$);[27] however, this study was also limited by the fact that less stringent criteria were used in case selection (no information was provided regarding the exclusion of patients who were concomitantly using hepatotoxic drugs). In a recent study among Caucasian subjects with 35 cases and 60 controls, no significant association was found between 'null' *GSTM*1 homozygosity and DIH (OR 0.73, $P = 0.48$),[28] similar to the findings of the present study. Another study among Indian subjects, involving 51 DIH cases and 100 non-DIH controls, showed no risk of DIH (OR 1.0, $P = 1.0$)[29] with the GSTM1 'null' genotype; these observations are also similar to the findings of the present study (Table 6).

The discordance between the findings of the present study and those of other studies conducted among Asian populations could be attributed to several factors. First, there were differences in study design. Second, different selection criteria for cases and controls were used: we followed stringent criteria for the enrolment of study participants, whereas other investigators likely included patients with acute viral hepatitis and possibly those concomitantly using other hepatotoxic drugs. Third, the Asian population is reported to demonstrate high ethnic diversity.[7] Furthermore, the Indian population is known to have a high degree of ethnic diversity, especially subjects from the northern part of the country, who differ significantly from those of the eastern and southern regions.[24,25] Finally, the sample size in most studies was small.

Based on the results of this study, we conclude that

Table 6 Comparison of studies among different ethnicities for *GSTM*1 'null' mutation in DIH patients

Author, year, reference	Country	Cases vs. controls	Association
Roy, 2001[19]	India	DIH ($n = 33$) vs. non-DIH ($n = 33$)	M1 'null', $P < 0.05$
Huang, 2007[27]	Taiwan	DIH ($n = 63$) vs. non-DIH (n=115)	M1 'null', $P = 0.01$
Lerio, 2008[28]	Caucasian	DIH (n =35) vs. non-DIH (n=60)	M1 'null', $P = 0.48$
Chatterjee, 2010[29]	India	DIH ($n = 51$) vs. non-DIH ($n = 100$)	M1 'null', $P = 1.00$
Present study	India	DIH ($n = 105$) vs. non-DIH ($n = 185$)	M1 'null', $P = 0.40$

* The authors examined *Rsa*I polymorphism and reported no significant association with the development of DIH; however, the statistical data were not shown. The authors also studied *Dra*I in addition to *Rsa*I.

*CYP2*E1 = cytochrome P450 2E1; DIH = drug-induced hepatotoxicity; *GSTM*1 = glutathione *S*-transferase mu1.

*CYP*2E1 *Rsa*I polymorphism is not an independent risk factor for DIH and that the *GSTM*1 'null' mutation does not confer increased susceptibility to DIH when on anti-tuberculosis treatment in the north Indian population. Further studies with larger sample sizes and adjustment for confounders are required to evaluate these risk factors in other populations.

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RESUME

CONTEXTE : Les facteurs génétiques liés à l'hôte qui influencent la prédisposition à l'hépatotoxicité induite par les médicaments anti-tuberculeux (DIH) ne sont pas clairs au sein de la population indienne.

OBJECTIF : Etudier l'association éventuelle entre la DIH et le polymorphisme sur le site *Rsa*I de mutations nulles 5-prime non traduites des régions *CYP*2E1 et GSTM1.

MÉTHODE : Dans cette étude prospective, 113 patients tuberculeux avec une DIH et 201 patients traités sans DIH ont constitué respectivement les cas et des témoins. Une réaction polymérase en chaîne du polymorphisme de la taille des fragments de restriction (PCR-RFLP) a été réalisée pour analyser le polymorphisme génétique de *CYP450* 2E1 sur le site *Rsa*I et les mutations nulles de *GSTM*1. On a comparé les résultats de la PCR-RFLP entre 185 patients non-DIH et 105 patients DIH.

RÉSULTATS : La fréquence du génotype c1c1 de *CYP*2E1 était souvent rencontrée et ne différait pas significativement entre DIH (75,14%) et non DIH (77,14%). La distribution génotypique de c2c2 était significativement plus élevée chez les DIH que chez les non DIH (4,8% vs. 0,5% ; OR 8,58 ; $P = 0,03$). Cependant, l'ajustement sur l'âge, le sexe et le sérum albumine aboutissaient à un OR de 2,75, c'est-à-dire non significatif ($P = 0,26$). Les fréquences des mutations homozygotes nulles sur le gène *GSTM*1 chez les patients DIH et non-DIH ne montraient pas de différence significative (40% et 37%, respectivement ; $P = 0,61$).

CONCLUSION : Le variant *Rsa*I du gène *CYP*2E1 et les mutations nulles du *GSTM*1 n'étaient pas associées à un risque de DIH dans la population de l'Inde du Nord.

RESUMEN

MARCO DE REFERENCIA: No se conocen bien los factores genéticos del huésped que influyen en la susceptibilidad a la hepatotoxicidad provocada por los medicamentos antituberculosos (DIH) en la población de la India.

OBJETIVO: Investigar la posible asociación entre esta reacción y el polimorfismo en la secuencia reconocida por la enzima *Rsa*I en la región no traducida del gen *CYP*2E1 y las mutaciones 'anuladoras' del gen *GSTM*1.

MÉTODOS: En el presente estudio prospectivo, se constituyó el grupo experimental con 113 pacientes con diagnóstico de tuberculosis (TB) que presentaron DIH y el grupo testigo con 201 pacientes que recibieron tratamiento antituberculoso y no presentaron DIH. Se analizaron los polimorfismos génicos de *CYP450* 2E1 en el punto *Rsa*I y las mutaciones 'anuladoras' de *GSTM*1 mediante una reacción en cadena de la polimerasa y análisis del polimorfismo en los fragmentos de restricción (PCR-RFLP). Los resultados del PCR-RFLP se compararon entre 105 pacientes del grupo experimental y 185 del grupo testigo.

RESULTADOS: Fue corriente observar el genotipo c1c1 de *CYP*2E1 y su frecuencia no presentó una diferencia significativa entre el grupo con DIH (75,14%) y el grupo testigo (77,14%). La distribución del genotipo c2c2 fue significativamente más alta en los pacientes con DIH que en el grupo testigo (4,8% contra 0,5%; OR 8,58; $P = 0,03$). Sin embargo, al corregir con respecto a la edad, el sexo y las diferencias en la albúmina sérica, el OR fue 2,75, con lo cual se pierde la significación estadística ($P = 0,26$). Las frecuencias de las mutaciones homocigóticas 'anuladoras' del gen *GSTM*1 no presentaron diferencia significativa entre los grupos (40% en pacientes con DIH y 37% en los testigos; $P = 0,61$).

CONCLUSIÓN: La variante *Rsa*I del gen *CYP*2E1 y la mutación 'anuladora' del gen *GSTM*1 no se asociaron con el riesgo de sufrir DIH en la población del norte de la India.