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Original Research Article

Association of GSTM1, GSTT1 and CYP2E1 Gene Polymorphisms with Antituberculosis Drug Induced Hepatotoxicity in North Indian Population

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ABSTRACT

Background and Aims: Antituberculosis drug-induced hepatotoxicity (anti-TB-DIH) is a growing problem of serious concern in many countries around the world. Drug metabolizing enzymes have been proposed to play an influential role in the pathogenesis of anti-TB-DIH. Therefore, the main aim of this study was to investigate the possible association of *GSTM1*, *GSTT1* and *CYP2E1* gene polymorphisms with anti-TB-DIH.
Methods: A prospective case-control study was conducted in 244 North Indian TB patients receiving anti-TB treatment. These patients were prospectively followed up both clinically and biochemically, before and during the treatment period, for the diagnosis of anti-TB-DIH. The frequencies of *GSTM1*, *GSTT1* and *CYP2E1* gene polymorphisms were determined from genomic DNA, utilizing multiplex polymerase chain reaction and restriction fragment length polymorphism methods respectively. Logistic regression analysis was used to determine the association between gene polymorphisms and anti-TB-DIH.
Results: Anti-TB-DIH developed in 35 patients (cases). Out of 209 patients (without anti-TB-DIH), only 100 were taken as controls. As a result of genotype analysis, the frequencies of *GSTM1* and *GSTT1* null genotypes were found to be higher in cases as compared to controls. However, statistically significant difference was observed only for *GSTT1* (Adjusted-OR=2.39; 95%CI=1.06-5.39; *P*=0.04). Further, combined effects of these two gene polymorphisms on the risk of anti-TB-DIH were analysed. However, the effects were insignificant. Also, no significant difference was reported in the frequencies of three genotypes (C1/C1, C1/C2 and C2/C2) of *CYP2E1* between cases and controls.
Conclusions: *GSTT1* null polymorphism may be a potential risk factor for anti-TB-DIH in North Indian TB patients.

Keywords: Tuberculosis patients; Antituberculosis drugs; Hepatotoxicity; Genetic risk factors; *GSTM1*, *GSTT1* and *CYP2E1* polymorphisms.

INTRODUCTION

Tuberculosis (TB) is one of the foremost public health problems causing an enormous burden of suffering and deaths. [(1)] World health organization (WHO) reported that there were almost 9 million new TB

cases and 1.4 million TB deaths in 2011, of which more than 90% of global TB cases and deaths occurred in the developing world. [(2)] It is estimated that India has the highest burden of TB of any county in the world, accounting for nearly two million cases in 2010, one-fifth of the world's total. [(3)]

For effectual control of TB, a number of countries adopting DOTS (directly observed treatment short course) strategy, which is endorsed by the world health organization (WHO) and based on diagnosis and treatment of infectious cases, produces cure rates of up to 95% even in the poorest countries and prevents new infections by curing infectious patients. Isoniazid, rifampicin, pyrazinamide, ethambutal and streptomycin are the first line anti-TB drugs, traditionally used in this DOTS strategy. [(4)]

Due to wide spread application of DOTS strategy in TB treatment, it is imperative to evaluate their risk on human health. Anti-TB chemotherapy under DOTS produces many adverse effects such as hepatotoxicity, skin reactions, gastrointestinal and neurological disorders. Hepatotoxicity is one of the potential serious adverse effects related to anti-TB drugs. [(5)] Multidrug resistance, treatment failure, severe liver injury and even death, caused by hepatotoxicity during anti-TB therapy, is growing problem of serious concern in many countries around the world. [(6,7)]

Many factors are found to predispose patients towards hepatotoxicity of anti-TB drug such as advanced age, sex, poor nutritional status, liver disease, inappropriate use of drugs, infection with hepatitis B virus (HBV), hepatitis C virus and human immunodeficiency virus (HIV) and high alcohol intake.[(5)] However, many previous studies[(8,9)] have explored genetic risk factors for the development of anti-TB-DIH, such as polymorphisms in *N* acetyltransferase 2 (*NAT2*), cytochrome P450 2E1 (*CYP2E1*) and glutathione S-transferase (*GST*) genes. Among these, *CYP2E1* is one of the most important phase I drug metabolizing enzymes, which play a significant role in the production of hepatotoxic metabolites.[(9)] Therefore, higher activity of *CYP2E1* may increase higher production of hepatotoxic substances and thereby leads to the risk of anti-TB-DIH. Some previous studies have demonstrated the association between the *CYP2E1* genotype and anti-TB-DIH. [(10-12)] Similarly, glutathione S-transferase (*GST*), an important phase II drug metabolizing enzyme, play a significant role in the detoxification of hepatotoxic metabolites by conjugating the toxic products with glutathione which are water-soluble and can be excreted from the body. [(13,14)] Human cytosolic *GST* system consists of seven multigene classes, designated as alpha, mu, pi, theta, sigma, omega, and zeta. The *GSTM1* gene is classified into the mu class and the *GSTT1* gene belongs to the theta class.[(15)] The effects of GST polymorphisms on genetic susceptibility to anti-TB-DIH have been investigated particularly for *GSTM1* and *GSTT1* genes. Therefore, genetic variability of these drug metabolizing enzymes (*GST* and *CYP2E1*) might modulate the susceptibility to anti-TB-DIH.

In India the rate of anti-TB-DIH has been reported to be much higher (11.5%) compared to other developed countries (4.3%). [(16)] Therefore, anti-TB-DIH is very important issue for India and this present was designed to evaluate the role of genetic polymorphism of *GSTM1*, *GSTT1* and *CYP2E1*genes, in the pathogenesis of anti-TB-DIH, which may be further used for the early detection of anti-TB-DIH among individuals, who are subjected to anti-TB drugs. To the best of our knowledge, very limited investigation has been reported on North Indian population, to explore the

association of *GSTM1*, *GSTT1* and *CYP2E1* genotypes with the risk of anti-TB-DIH.

MATERIALS AND METHODS

Patients

A total of 244 TB patients taking anti-TB drugs under DOTS were registered in this prospective case-control study, which was conducted in the department of pulmonary medicine, King George Medical University, Lucknow, India. The diagnosis of TB was made on WHO criteria including chest X-ray, presence of acid-fast bacilli on sputum smear and positive sputum culture for *M. tuberculosis*. Inclusion criteria for the patients were as follows: (i) they were going to start anti-TB drugs (ii) not receiving any other hepatotoxic drugs parallel with anti-TB treatment, (iii) no history of chronic alcohol intake, (iv) normal findings of liver function parameters at the beginning of the treatment, and (v) negative for hepatitis B/C. Informed consent was taken from all the patients included in the study. The study was approved by the Ethical Committee. For all patients involved in this study a complete history were taken including demographic characteristics, full details of all anti-TB drugs with doses, concomitant use of other drugs, diseases characteristics and any clinical manifestations of anti-TB-DIH.

Follow up

Liver function parameters including alanine transaminase (ALT), aspartate transaminase (AST) and bilirubin were estimated in TB patients, before the initiation of treatment. Auto-analyzer was used for measuring liver function parameters from blood serum. Normal range in the laboratory was 9-43 U/L for ALT, 9-43 U/L for AST and 0.4-1.2 mg/dl for bilirubin.

After the initiation of drug therapy, these biochemical parameters along with clinical observation were performed every two weeks during the first two month and then monthly up to the end of the treatment. These biochemical parameters were repeated later, whenever symptoms indicative of hepatotoxicity like nausea, anorexia, vomiting occurred.

Criteria for the diagnosis of anti-TB-DIH

For the diagnosis of anti-TB-DIH, presence of at least one of the following criteria was used: [17]

i. A rise to more than 2 times the normal level of ALT and/or AST. ii. A rise in total serum bilirubin over 1.5 mg/dl. iii. Any increase in ALT and/or AST above pretreatment levels together with anorexia, nausea, vomiting, and jaundice.

Selection of cases and controls

Using the criteria, for the diagnosis of anti-TB-DIH, we prospectively identified patients who developed anti-TB-DIH and they were considered as cases. For the purpose of comparison controls were selected, from the same cohort, who showed no evidence of anti-TB-DIH.

GSTM1 and GSTT1 genotyping analysis

Genomic DNA was extracted from the peripheral blood leucocytes, using salting out method with slight modification. [18] To determine the presence or absence of *GSTM1* and *GSTT1* genes, two separate multiplex polymerase chain reactions (PCR) were performed. The forward and reverse primers used for *GSTM1* were: 5' GAACTCCCTGAAAAGCTAAAGC 3' and 5' GTTGGGCTCAAATATACGGTGG 3'. For *GSTT1* the primers used were 5' TTC CTTACTGGTCCTCACATCTC 3' and 5' TCACCGGATCATGGCCAGCA 3'. *CD36* gene was used as an internal positive control for *GSTM1* and for this gene the primers were 5'- ACTCACCCTGAACCCCTTC-3'and 5' AGCCTCTGAGTAGTTGGGGCC 3'. Similarly, *CYP1A1* gene was used as an internal positive control for *GSTT1* and the primers were 5' ACTCACCCTGAACCCCTTC 3' and 5' AGCCTCTGAGTAGTTGGGGCC 3'. PCR was performed in a volume of 15 µl

containing DNA (100-150 ng), 5 pmol of each primer set, 7.5µl master mix (MBI-Fermentas, USA) and nuclease free water per tube. Amplification condition consisted of denaturation step at 94^0C for 5 min, followed by 35 cycles at 94^0C for 30 s, 64^0-66^0C for 30 s and 72^0C for 30 s. The final extension was carried out at 72^0C for 10 min. PCR products were checked on 2% agarose gel containing ethidium bromide, and then visualized by gel documentation system. Finally, *GSTM1* and *GSTT1* null genotypes were identified by the absence of 230bp band and 458bp band, respectively with the help of internal controls (*CD36*: 401bp band; *CYP1A1*: 196bp band).

CYP2E1 genotyping analysis

The genotypes of *CYP2E1* were grouped into three types C1/C1, C1/C2 and C2/C2. C1 was classified into wild type allele and C2 was classified into mutant allele. The three genotypes of *CYP2E1* gene were determined by PCR-RFLP (Restriction Fragment Length Polymorphism). Initially, PCR was performed in a volume of 15 µl containing DNA (100-150 ng), 10 pmol of each primer set, 7.0µl master mix (MBI-Fermentas, USA) and nuclease free water per tube. Amplification condition consisted of denaturation step at 95^0C for 5 min, followed by 35 cycles at 95^0C for 30 s, 56.5^0C for 30 s and 72^0C for 30 s. The final extension was carried out at 72^0C for 10 min. The forward and reverse primers used for CYP2E1 were 5'CCAGTCGAGTCTACATTGTCA3' and 5'TTCATTCTGTCTTCTAACTGG3' respectively. The PCR products were then digested with restriction enzyme *Rsa I* for 18 h at 37^0C. The digested fragments were checked by polyacrylamide gel electrophoresis (PAGE) with ethidium bromide staining and finally visualized by gel documentation system.

Statistical analysis

The data collected was entered in Microsoft Excel computer program and checked for any inconsistency. The descriptive statistics such as mean, standard deviation and percentages were calculated. The categorical variables were analyzed by using Chi-squire test and continuous variables were compared by using independent t-test. Odds ratio (OR) and its 95% confidence interval (CI) were calculated to find out the association of *GST and CYP2E1 gene* polymorphisms with anti-TB-DIH. The p value less than 0.05 was considered significant. All the analysis was carried out by using SPSS 15.0 version.

RESULTS

Baseline characteristics of cases and controls

During the treatment period, out of 244 patients, only 35 developed anti-TB-DIH (cases), detected by clinical examination and confirmed by abnormal pattern of liver function parameters. Remaining 209 patients showed no evidence of anti-TB-DIH. Out of 209, only 100 patients were randomly taken as controls for the comparison with cases. The baseline characteristics of cases and controls are illustrated in Table 1. A significance difference was observed between cases and controls only with respect to sex (P <0.0001), body mass index (P=0.001) and disease classification (P=0.02). However, cases and controls showed no significance difference in terms of age (P=0.09) and socio-economic status (P=0.40).

Level of liver function parameters in cases and controls at before and during the treatment period

As shown in Table 1 pre treatment level of ALT, AST and bilirubin were almost similar in cases and controls (ALT=27.17±4.02 versus 26.13±4.02, P=0.19; AST=28.83±3.40 versus 29.03±2.88, P=0.74; bilirubin=0.58±0.09

versus 0.60±0.11, *P*=0.49). Whereas, during the treatment period, the level of ALT, AST and bilirubin were significantly higher in cases, when compared with controls (ALT=144.03±24.35 versus 32.79±3.22, *P*<0.0001; AST=141.63±21.02 versus 29.28±4.63, *P*<0.0001; bilirubin=1.27±0.36 versus 0.80±0.16, *P*<0.0001).

Table 1. Baseline characteristics and level of liver function parameters in cases and controls.

Characteristics	Cases (n=35)	Controls (n=100)	*P*-value
Age (mean±SD), years	31.29±13.18	35.54±12.54	0.09
Sex			
Male, no. (%)	10 (28.6)	63 (63)	<0.0001*
Female, no. (%)	25 (71.4)	37 (37)	
Socio-economic status			
Upper middle, no. (%)	1 (2.9)	7 (7)	0.40
Middle, no. (%)	4 (11.4)	18 (18)	
Lower, no. (%)	30 (85.7)	75 (75)	
Body mass index (mean±SD), Kg/m^2	19.50±2.08	21.15±2.51	0.001*
Disease classification			
Pulmonary, no. (%)	11 (31.4)	53 (53)	0.02*
Extra-pulmonary, no. (%)	24 (68.6)	47 (47)	
Before treatment			
ALT (U/L)	27.17±4.02	26.13±4.02	0.19
AST (U/L)	28.83±3.40	29.03±2.88	0.74
Bilirubin (mg/dl)	0.58±0.09	0.60±0.11	0.49
During treatment (Peak value)			
ALT (U/L)	144.03±24.35	32.79±3.22	<0.0001*
AST (U/L)	141.63±21.02	29.28±4.63	<0.0001*
Bilirubin (mg/dl)	1.27±0.36	0.80±0.16	<0.0001*

*Significant

Table 2. *GSTM1* and *GSTT1* genotype frequencies in patients with DIH (Cases) and without DIH (Controls)

	Cases (n=35)	Controls (n=100)	Unadjusted OR (95%CI), *P*-value	Adjusted OR[a] (95%CI), *P*-value
GSTM1				
Present	24 (68.6%)	71 (71%)	Reference	Reference
Null	11 (31.4%)	29 (29%)	1.12 (0.49-2.58), 0.79	1.19 (0.51-2.79), 0.69
GSTT1				
Present	19 (54.3%)	74 (74%)	Reference	Reference
Null	16 (45.7%)	26 (26%)	2.41 (1.08-5.34), 0.03*	2.39 (1.06-5.39), 0.04*
Combined genotypes				
Both GSTM1 and GSTT1 Present	15 (42.86%)	54 (54%)	Reference	Reference
GSTM1 present/ GSTT1 null	09 (25.71%)	17 (17%)	1.91 (0.71-5.13), 0.20	2.37 (0.80-7.00), 0.12
GSTM1 null/ GSTT1 present	04 (11.43%)	20 (20%)	0.72 (0.21-2.43), 0.60	0.86 (0.24-3.08), 0.82
GSTM1 null/ GSTT1 null	07 (20%)	09 (9%)	2.80 (0.89-8.77), 0.08	3.11 (0.96-10.07), 0.06

DIH: Drug induced hepatotoxicity; OR: Odds ratio; CI: Confidence interval; *: Significant

Association of GSTM1 and GSTT1 genotypes with anti-TB-DIH

The genotype frequencies of *GSTM1* and *GSTT1* are illustrated in Table 2. The frequencies of *GSTM1* and *GSTT1* null genotypes were found to be higher in cases as compared to controls (31.4% versus 29% and 45.7% versus 26%). However, the differences were statistically significant only for *GSTT1* (*P*=0.03, OR=2.41, 95% CI=1.08-5.34), not for *GSTM1* genotype (*P*=0.79, OR=1.12, 95% CI=0.49-2.58). A multivariable logistic regression analysis was then performed to adjust for the residual

effects of other variables such as age, sex, socio-economic status, body mass index and disease classification. After adjustment, having the *GSTT1* null genotype remained a significant (*P*=0.04, Adjusted OR=2.39, 95% CI=1.06-5.39) risk factor for anti-TB-DIH.

Further, combined effects of these two polymorphisms on the risk of anti-TB-DIH were also analysed in our study subjects. For this purpose, we distributed these genes into four groups (both *GSTM1*/*GSTT1* present, both *GSTM1*/*GSTT1* null, *GSTM1* present/*GSTT1* null, *GSTM1* null/*GSTT1* present). As shown in Table 2. the associated risk of anti-TB-DIH with combined *GSTM1* and *GSTT1* polymorphisms were not statistically significant.

Table 3. Characteristics of patients with DIH (Cases), stratified by *GSTM1* genotype

Characteristics	No. of patients (n=35)	GSTM1 genotype		
		Present (n=24)	Absent (n=11)	*P*-value
Age (mean±SD), years		32.21±14.14	29.27±11.13	0.54
Sex				
Male, no. (%)	10	7 (70.0)	3 (30.0)	0.90
Female, no. (%)	25	17 (68.0)	8 (32.0)	
Socio-economic status				
Upper middle, no. (%)	1	1 (100.0)	0(0.0)	0.26
Middle, no. (%)	4	4 (100.0)	0 (0.0)	
Lower, no. (%)	30	19 (63.3)	11 (36.7)	
Body mass index (mean±SD), Kg/m^2		19.00±1.77	20.58±2.39	0.03*
Disease classification				
Pulmonary, no. (%)	11	8 (72.7)	3 (27.3)	0.72
Extra-pulmonary, no. (%)	24	16 (66.7)	8 (33.3)	
Before treatment				
ALT level (U/L)		27.75±4.11	25.91±3.67	0.21
AST level (U/L)		29.00±3.20	28.45±3.93	0.66
Bilirubin level (mg/dl)		0.57±0.07	0.61±0.30	0.31
During treatment (Peak value)				
ALT (U/L)		142.33±24.76	147.73±24.14	0.55
AST (U/L)		142.29±21.88	140.18±19.96	0.78
Bilirubin (mg/dl)		1.29±0.39	1.23±0.30	0.60

DIH: Drug induced hepatotoxicity; *: Significant

Characteristics of patients with anti-TB-DIH (Cases), stratified by GSTM1 and GSTT1 genotypes

Baseline characteristics of hepatotoxic patients, including age, sex, body mass index, socio-economic status and disease classification were analysed, between *GSTM1* present and null genotypes (Table 3). Statistically significance difference was observed only with respect to body mass index (*P*=0.03). Pre and during treatment level of liver function parameters were also analysed between *GSTM1* present and null genotypes. We observed no significance difference in the pretreatment and during treatment level of liver function parameters with respect to *GSTM1* present and null genotypes.

Similarly, baseline line characteristics and level of liver function parameters of hepatotoxic patient were determined between *GSTT1* present and null genotypes (Table 4). No significant difference was observed, regarding baseline

characteristics and level of liver function parameters. However, during the treatment period, hepatotoxic patient with *GSTT1* null genotype showed higher level of ALT, AST and bilirubin as compared to patients with *GSTT1* present genotype.

Table 4. Characteristics of patients with DIH (Cases), stratified by *GSTT1* genotype

Characteristics	No. of patients (35)	GSTT1 genotype		
		Present (n=19)	Absent (n=16)	*P*-value
Age (mean±SD),years		27.68±8.64	35.56±16.38	0.07
Sex				
Male, no. (%)	10	5 (50.0)	5 (50.0)	0.74
Female, no. (%)	25	14 (56.0)	11 (44.0)	
Socio-economic status				
Upper middle, no. (%)	1	1 (100.0)	0 (0.0)	0.64
Middle, no. (%)	4	2 (50.0)	2 (50.0)	
Lower, no. (%)	30	16 (53.3)	14 (46.7)	
Body mass index (mean±SD), Kg/m^2		19.43±1.73	19.58±2.49	0.84
Disease classification				
Pulmonary, no. (%)	11	4 (36.4)	7 (63.6)	0.15
Extra-pulmonary, no. (%)	24	15 (62.5)	9 (37.5)	
Before treatment				
ALT (U/L)		27.00±4.65	27.38±3.24	0.78
AST (U/L)		28.79±3.84	28.88±2.92	0.94
Bilirubin (mg/dl)		0.54±0.08	0.59±0.10	0.91
During treatment (Peak value)				
ALT (U/L)		143.47±26.77	144.69±21.97	0.88
AST (U/L)		138.26±18.43	145.62±23.72	0.30
Bilirubin (mg/dl)		1.25±0.32	1.30±0.41	0.70

DIH: Drug induced hepatotoxicity

Table 5: Frequency of *CYP2E1* genotypes in patients with DIH (Cases) and without DIH (Controls).

	Cases (n=35)	Controls (n=100)	Unadjusted OR (95%CI)	*P*-value
CYP2E1				
C2/C2	2 (5.7%)	8 (8%)	Reference	
C1/C2	5 (14.3%)	22 (22%)	0.91 (0.15-5.66)	0.91
C1/C1	28 (80.0%)	70 (70%)	1.60 (0.32-8.01)	0.56
CYP2E1				
C1/C2 + C2/C2	7 (20%)	30 (30%)	Reference	
C1/C1	28 (80.0%)	70 (70%)	1.63 (0.64-4.16)	0.30

DIH: Drug induced hepatotoxicity; OR: Odds ratio; CI: Confidence interval

Association of three genotypes of CYP2E1 gene with anti-TB-DIH

80% of the individual in the case group and 70% of the individual in the control group exhibited wild type *CYP2E1* C1/C1 genotype. Whereas, the frequencies of the mutant *CYP2E1* genotypes C1/C2 and C2/C2 in the case group were 14.3% and 5.7%, respectively and in the control group 22% and 8%, respectively. As shown in Table 5. no significant difference was to be found in the frequencies of three genotypes of *CYP2E1*, between cases and controls. The frequency of wild-type genotype C1/C1 compared with the combined mutant genotypes C1/C2 + C2/C2 was found to be higher in cases as compared to controls. However, the difference was statistically insignificant (*P*=0.30, OR=1.63, CI=0.64-4.16).

Characteristics of patients with anti-TB-DIH (Cases), stratified by CYP2E1 genotype

Baseline characteristics of hepatotoxic patients including age, sex, body mass index, socio-economic status and disease classification were also determined between wild type *CYP2E1* (C1/C1) and mutant type (C1/C2+C2/C2) genotypes. No significant difference was observed, regarding baseline characteristics between wild and mutant type genotype frequencies. Similarly, when we determined pre and post treatment level of liver function parameters between hepatotoxic patients with wild type (C1/C1) and mutant genotypes (C1/C2+ C2/C2), insignificant difference was observed (Table 6).

Table 6. Characteristics of patients with DIH (Cases), stratified by *CYP2E1* genotypes.

Characteristics	No. of patients (n=35)	CYP2E1 genotypes		
		C1/C1(n=28)	C1/C2 +C2/C2 (n=7)	*P*-value
Age (mean±SD), years		31.64±13.17	29.86±14.56	0.75
Sex				
Male, no. (%)	10	7(70.0)	3 (30)	0.35
Female, no. (%)	25	21 (84)	4 (16)	
Socio-economic-status				
Upper middle, no. (%)	1	1 (100)	0 (0)	0.36
Middle, no. (%)	4	2 (50)	2 (50)	
Lower, no. (%)	30	25 (83.3)	5 (16.7)	
Body mass index (mean±SD), Kg/m^2		19.45±2.11	19.66±2.12	0.82
Disease classification				
Pulmonary, no. (%)	11	9 (81.8)	2 (18.2)	0.86
Extra-pulmonary, no. (%)	24	19 (79.2)	5 (20.8)	
Before treatment				
ALT (U/L)		27.50±3.69	25.86±5.24	0.34
AST (U/L)		28.64±3.53	29.57±2.94	0.52
Bilirubin (mg/dl)		0.58±0.09	0.61±0.11	0.36
During treatment (Peak value)				
ALT (U/L)		143.18±25.61	147.43±19.78	0.68
AST (U/L)		140.79±22.89	145.00±11.37	0.64
Bilirubin (mg/dl)		1.30±0.39	1.17±0.19	0.40

DIH: Drug induced hepatotoxicity

DISCUSSION

Hepatotoxicity is an adverse complication of anti-TB therapy, which is emerging as a significant threat to tuberculosis control, and if not recognized in time and managed properly, it can leads to permanent injury and death. A number of factors have been found, which may increase the risk of anti-TB-DIH. [19] Detection of risk factors for hepatotoxicity may play an important role in minimizing the incidence. Also, the identification of high-risk patients may be useful to allow early detection of anti-TB-DIH and reduce the morbidity and mortality of this condition.

Of the various anti-TB regimens, most cases of anti-TB-DIH have been attributed to the metabolism of isoniazid. [20] *CYP2E1* and *GST* are the two main enzymes, which contribute a key role in the metabolism of isoniazid. [21,22] Recent studies have demonstrated that genetic polymorphisms of *GST* and *CYP2E1*, which influence their activation capacity and the generation of metabolites in the liver, might predispose an individual to hepatic adverse reactions in terms of liver injury. [9,23] Therefore, in this present study, we examined the possible association of *GSTM1*, *GSTT1* and *CYP2E1* gene

polymorphisms with the risk of anti-TB-DIH, in North Indian population.

In our study, *GSTT1* genotype exhibited significantly higher frequency of gene deletion in cases as compared to controls. After adjustment for age, gender, body mass index and baseline values of liver function parameters, there was also a significant association between *GSTT1* null genotypes and anti-TB-DIH, which indicates that subjects with *GSTT1* null genotype had an increased risk of anti-TB-DIH. Whereas, we did not find any significant association between *GSTM1* null polymorphism and anti-TB-DIH.

The principal function of *GST* gene family is conjugations of hepatotoxic metabolites with reduced glutathione. The intracellular binding reaction with GSH is catalyzed by the *GST* and leads to stable GSH-toxic products conjugates being transported out of the cell and excreted via feces and urine and thus reduces the toxic effects. Individuals with the *GSTT*1 null genotype resulting in either decreased or altered enzyme activity. The change in catalytic activity may reduce in GSH-toxic products conjugates and excretion, which is further associated with enhanced rate of anti-TB-DIH. [(24)]

In agreement to our study, a recently published study [(25)] conducted on Caucasian subjects, with 35 cases and 60 controls, showed a significant association between the null *GSTT1* homozygosity and anti-TB-DIH, whereas no significant association was found between the homozygous *GSTM1* null polymorphism and anti-TB-DIH. In contrast to ours, Cai et al. (2012) suggested that subjects with *GSTM1* null genotype had an increased risk for anti-TB-DIH.[(26)] Similarly, other previous studies[(27,28)] also reported that homozygous null mutation at *GSTM1* loci was a significant risk factor for anti-TB-DIH.

Also, in this present study we investigated the combined effect of *GSTM1* and *GSTT1* polymorphisms on the risk of anti-TB-DIH. We found that the distribution of combined genotypes were not statistically significant between cases and controls. After adjustment potential confounders, there were also no significant association between the combinations of *GSTM1* and *GSTT1* polymorphisms and the risk of anti-TB-DIH. Chatterjee et al. (2010) reported that the combined effect of *GSTM1* and *GSTT1* null polymorphisms were not significantly associated with anti-TB-DIH. [(29)] Similarly, Leiro et al. (2008) also observed no relation between combined *GSTM1* and *GSTT1* null mutations and the risk of anti-TB-DIH. [(25)] The findings confirm that patients without *GSTT1* activity are more vulnerable to anti-TB-DIH, compared with those who possess *GSTT1* activity.

Drug metabolizing enzyme-*CYP2E1,* plays an imperative role in the progression of anti-TB-DIH. Because of genetic polymorphisms, *CYP2E1* metabolic enzyme differs greatly between individuals. The three genotypes of *CYP2E1* are classified as C1/C1, C1/C2 and C2/C2 by restriction fragment length polymorphism using *RsaI* restriction enzyme. Of the three genotypes of *CYP2E1*, particularly C1/C1 genotype is associated with higher activity of *CYP2E1*, which is further directly responsible to develop anti-TB-DIH.[(26)] In the present study, we observed that the frequency of wild type genotype *CYP2E1* C1/C1 compared with the mutant genotypes (C1/C2 and C2/C2), was found to be higher percentage in cases group as compared to controls. According to Huang et al. (2003), individual with wild type C1/C1 genotype exhibit higher activity of *CYP2E1* than those with mutant *CYP2E1* C1/C2 or C2/C2 genotypes and therefore, may produce more hepatotoxins and raises the possibility for the development of anti-TB-DIH. [(12)]

However, in our study, no significant association was to be observed between *CYP2E1* C1/C1 genotype and the susceptibility to anti-TB-DIH. Similar to the finding of our study, Cho et al. (2007) found no relationship between *CYP2E1* C1/C1 genotype and anti-TB-DIH in their Korean population. [(30)] These results are inconsistent with those of Cai et al. (2012), who showed positive correlation between *CYP2E1* C1/C1 genotype and anti-TB-DIH. [(26)] Similarly, other previous studies [(31,32)] reported the association of *CYP2E1* C1/C1 with the severity of anti-TB-DIH.

In our study, among the three genotypes of *CYP2E1*, the mutant type *CYP2E1* C2/C2 was found to be in minimum percentage in both groups. Similarly, one previous study [(9)] that was conducted on the Brazilian population showed rare percentage of mutant *CYP2E1* C2/C2 genotype and this genotype was not to be associated with the susceptibility of anti-TB-DIH. Rare cases of mutant *CYP2E1* C2/C2 was also observed in Taiwanese population, which was studied by Huang et al. (2003). [(12)]

Also, no significances difference was to observed in this present study, when hepatotoxic patient's age, sex, body mass index, socioeconomic status, disease classification and pre and post treatment level of liver function parameters were analysed between wild type *CYP2E1* (C1/C1) and mutant type *CYP2E1* (C1/C2 +C2/C2) genotypes. Similarly, Huang et al. (2003) observed no significance difference in patient's baseline characteristics and pretreatment level of liver biochemical parameters between these genotypes. Further, their study also revealed that patients with *CYP2E1* C1/C1genotype had higher posttreatment serum ALT levels than those with *CYP2E1* C1/C2 or C2/C2 genotypes. [(12)] Lee et al. (2010) also determined the characteristics of hepatotoxic patients with respect to wild and mutant type genotypes and observed that the post treatment level of AST was significantly higher in patients with *CYP2E1* C1/C1 genotype than patients with C1/C2 and C2/C2 genotypes. [(31)]

CONCLUSION

In conclusion, *GSTT1* null polymorphism may be a potential risk factor for anti-TB-DIH in North Indian population. The finding of this present work may be crucial for screening among individuals at high risk for anti-TB-DIH, which can be ultimately imperative to support TB control programs. Further, we observed that genetic polymorphism of *CYP2E1* and *GSTT1* were not significantly associated with the risk of anti-TB-DIH. Therefore, further studies with more samples are needed to substantiate the role of drug metabolizing enzymes on the risk of anti-TB-DIH.

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