Occurrence of CYP2B6 516G>T polymorphism in patients with ARV-associated hepatotoxicity

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Abstract

Background: Hepatic enzyme cytochrome P450 2B6 (CYP2B6) plays a role in the metabolism of efavirenz drugs. CYP2B6 516G>T variation showed an implication for HIV treatment.

Methods: CYP2B6 516G>T polymorphism was genotyped in a total 165 HIV patients that include 34 with and 131 without hepatotoxicity and 155 healthy individuals by the PCR-RFLP.

Results: In patients with hepatotoxicity, the prevalence of CYP2B6 516TT genotype was higher as compared to healthy individuals (35.3% vs. 30.5%, OR = 1.74). Patients with hepatotoxicity using tobacco had a higher prevalence of genotypes CYP2B6 516GT, 516TT, 516GT+TT as compared to healthy individuals (28.57% vs. 25.93%; 57.14% vs. 29.63%; 85.71% vs. 55.56%). Likewise, hepatotoxicity in patients consuming alcohol showed higher distributions of CYP2B6 516GT, 516TT, 516GT+TT genotypes (57% vs. 25.93%; 42.86% vs. 33.33%; 71.43% vs. 59.26%). Nevirapine users with hepatotoxicity overrepresented genotypes CYP2B6 TT and 516GT+TT as compared to efavirenz users (47.83% vs. 45.45%, OR = 6.88, 65.22% vs. 54.55%, OR = 1.56). Similarly, in nevirapine +alcohol users with hepatotoxicity, the frequency of CYP2B6 516GT, 516GT+TT genotypes was higher than with nevirapine +alcohol nonusers (40.0% vs. 11.11%, OR = 8.00, 80.0% vs. 27.78%, OR = 4.00). In HIV patients, nevirapine users had higher frequency of CYP2B6 516GT, 516GT+TT genotypes as compared to efavirenz users (42.02% vs. 25.00%, OR = 2.53; 72.27% vs. 58.33%, OR = 1.86). Likewise, in HIV patients, genotypes CYP2B6 516GT, 516GT+TT were predominant with nevirapine +alcohol users as compared to nevirapine +alcohol nonusers (57.89% vs. 34.57%, OR = 2.46; 78.95% vs. 69.14%, OR = 1.67). In multivariate logistic regression, taking nevirapine had a protection for severity of ARV-associated hepatotoxicity (OR = 0.23, p = 0.005).

Conclusions: No significant association was detected between CYP2B6 516G>T polymorphism and susceptibility to ARV-associated hepatotoxicity.

Keywords
ARV-associated hepatotoxicity, CYP2B6, genetic polymorphism, HIV patients, NNRTI drugs
1 | INTRODUCTION

Antiretroviral therapy (ART) has modified the natural history of HIV infection in both rich and limited-resource countries (LRCs) like India. Two lines of treatment are widely available to treat the HIV patients and almost no test, aside from the CD4 cell count, is performed for monitoring treatment outcome. Long-term efficacy and toxicity are major challenges when selecting an antiretroviral (ARV) regimen for treatment of AIDS. A common adverse drug reaction (ADR) is hepatotoxicity which leads to treatment interruptions in HIV patients (Dyke, Wang, & Williams, 2008). Individuals using nevirapine were associated with higher incidence of liver toxicity than efavirenz (van Leth et al., 2004). A 10.8% grade 3 or 4 hepatotoxicity was observed with efavirenz-treated group and 8.9% in the nevirapine-treated group (Reisler, Servoss, & Sherman, 2001). However, the incidence of hepatotoxicity due to nevirapine was 3.19% (Nagpal, Tayal, Kumar, & Gupta, 2010).

Mainly, drug metabolism and detoxification enzymes can be present in the liver and these are susceptible to injury. Single nucleotide polymorphism (SNP) in drug metabolizing enzyme genes may cause liver toxicity (Bissell, Gores, Laskin, & Hoofnagle, 2001). CYP2B6 (OMIM: 614,546) is an isoenzyme found on exon 4 of chromosome 19 and is characterized by wide interindividual and interethnic variation in expression and activity in the human liver in vitro (Saitoh et al., 2007). Drug metabolizing enzyme including CYP2B6 plays a major role in the hydroxylation of EFV and NVP (Erickson, Mather, Trager, Levy, & Keirns, 1999; Ward et al., 2003). A total of 29 alleles of CYP2B6 (*1A [wild-type] to *29) have been described (CYP2B6 allele nomenclature. www.cypalleles.ki.se/cyp2b6.htm 102), many of which are associated with increased, decreased, or abolished enzyme activity (Watanabe et al., 2010; Zanger et al., 2007; Zhang et al., 2011). CYP2B6*6 516G>T and 785A>G polymorphism were associated with “slow metabolism” of EFV (Zhang et al., 2011; Desta et al., 2007). The CYP2B6*6 516G>T gene polymorphism is accountable for aberrant splicing, resulting in a high-splice variant and low CYP2B6 expression phenotype (Hofmann et al., 2008). The CYP2B6 516 G>T gene polymorphism is associated with higher plasma EFV concentrations leading to increased drug-related side effects (Haas et al., 2005; Nyakutira et al., 2008; Ribaudo et al., 2006; Rodriguez-Novoa et al., 2005; Sadiq, Fredericks, Khoo, Rice, & Holt, 2005). A G>T change at position 516 of CYP2B6 gene results in a Gln–His (Glutamine–Histidine) amino acid change which is associated with a significant reduction in CYP2B6 catalytic activity (Penzak et al., 2007). The 516TT genotype of CYP2B6 is associated with reduced enzyme activity (Gatanaga et al., 2007).

Studies have reported an association between CYP2B6 genotypes and the pharmacokinetics of EFV and NVP in HIV-infected patients of European and African ethnicities (Cabrera et al., 2009; Haas et al., 2009; Mahungu et al., 2009; Motsinger et al., 2006; Ribaudo et al., 2007; Rotger et al., 2007; Schipani et al., 2011; Wyen et al., 2008). Also, similar studies have been published in other ethnically diverse patients (Carr, Porte, Pirmohamed, Owen, & Cortes, 2010; Chen et al., 2010; Lindfelt, O’Brien, Song, Patel, & Winslow, 2010; Puthanakit, Tanpaiboon, Aurrupil, Cressey, & Sirisanthana, 2009; Ramachandran et al., 2009; To et al., 2009). In India, it is believed that we have different gene pools in south, north, east, and west regions. The frequency of CYP2B6 516G>T polymorphism was reported only in South Indian population (Ramachandran et al., 2009). Hence, we evaluated the prevalence of CYP2B6 516G>T polymorphism in West Indian HIV patients.

2 | MATERIAL AND METHODS

2.1 | Subjects

This is a case–control study, undertaken between November 2012 and February 2015, at the outpatient clinics of National AIDS Research Institute, Pune. The study included 34 patients with hepatotoxicity (Grade III/IV) under NNRTI containing ART regimen, 131 HIV patients without hepatotoxicity confirmed by liver function test (LFT), and 155 age-matched healthy controls. Patients with hepatotoxicity having hepatitis B, hepatitis C, tuberculosis, and concurrent untreated opportunistic infections, immune reconstitution syndrome and under any other known hepatotoxic drugs were excluded from cases. HIV patients having evidence of hepatotoxicity, hepatitis B, hepatitis C, tuberculosis, and receiving any other known hepatotoxic drugs were excluded. One hundred and fifty-five individuals (those from the same family were excluded), HIV, Hepatitis B, C and Tuberculosis free, age-matched and serum negative from HIV-ELISA test were recruited. Clinical research proforma was filled to obtain clinical data by questionnaire, personal interviews, and review of case records. Liver function test was done to evaluate the status of liver enzyme. Total Bilirubin >3.22 mg/ml, SGOT >93.8 U/ml, SGPT >29.5 U/ml, and Alkaline phosphatase >550.8 U/ml for male hepatotoxicity cases and total Bilirubin >3.22 mg/ml, SGOT >163.2 U/ml, SGPT >173.4 U/ml, and Alkaline phosphatase >550.8 U/ml for female hepatotoxicity were considered as cases. Total Bilirubin <1.24 mg/ml, SGOT <32 U/ml, SGPT <34 U/ml, and Alkaline phosphatase <108 U/ml for male and female HIV-infected control were considered. Estimation of CD4 count was done by fluorescence-activated cell sorter (FACS). CD4 status was used to classify patients into different subgroups. CD4 ranges from <200 cells/mm³ were defined as an advanced stage, 201–350 cells/mm³ as an intermediate stage, and >350 cells/mm³ onward as an early stage. ELISA for
hepatitis C and HBsAg testing was performed using Ortho HCV ELISA test system and Murex HBsAg confirmatory (Diasorin) ELISA. Environmental exposures such as tobacco and alcohol usage were also recorded in the questionnaire. The study was approved by the local institutional ethics committee and written informed consent was taken from all eligible participants.

3 | DNA EXTRACTION

Two milliliters of peripheral blood sample was collected and stored at −70°C prior to DNA extraction. Genomic DNA extraction was done from peripheral blood leukocytes pellet using the AxyPrep Blood Genomic DNA Miniprep Kit according to the protocol given by the manufacturer.

3.1 | Genotyping

The CYP2B6 (516G>T) was genotyped using PCR-restriction fragment length polymorphism (PCR-RFLP) in subjects participants. The amplification of CYP2B6 (516G>T) primers was taken as reported (Ramachandran et al., 2009). PCR was performed in a total volume of 20 μl with 20 pmol of each primer, genomic DNA (100–150 ng), 10 mmol/L deoxynucleoside triphosphates, PCR buffer containing 100 mmol/L Tris-HCl, pH 8.6, 50 mmol/L KCl, 1.5 mmol/L MgCl2, and 1.5 units of Taq polymerase (Bangalore Genei, India). The reaction conditions for CYP2B6 516G>T were: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 7 min. Amplified product of CYP2B6 was digested using restriction enzyme BsrI (Fermentas Inc.). Genotyping of CYP2B6 was done in 15% polyacrylamide gel using molecular weight markers and visualized after staining with ethidium bromide. Twenty percent of samples from both patients and controls were re-genotyped by other laboratory personnel and no discrepancy in genotyping was noticed. Ten percent of samples were sequenced to assess the genotyping error.

3.2 | Data analysis

The age variable was expressed as mean ± SD. The deviation from Hardy–Weinberg equilibrium in controls was analyzed using χ² goodness-of-fit test. We compared the genotype frequency between HIV patients with hepatotoxicity versus without hepatotoxicity and HIV patients versus healthy controls using χ² statistic (Fisher's exact test for cell size <5). Tobacco, alcohol usages, and genotypes interaction were examined in all eligible HIV patients. Odds ratios (ORs) and 95% confidence interval (CI) were calculated by unconditional binary logistic regression. All statistical analysis was performed using spss software version 17.0 (SPSS) and tests of statistical significance were two-sided and taken as significant when p-value was less than 0.05.

4 | RESULTS

The study consisted of a total 165 HIV patients of which 34 patients had hepatotoxicity, 131 were without hepatotoxicity, and 155 were healthy controls. The mean age (years ±SD) of patients with hepatotoxicity, without hepatotoxicity, and healthy controls were 39.45 years ± 3.91, 38.39 years ± 4.23, and 36 years ±2.80, respectively. The demographic profile of HIV patients with, without hepatotoxicity, and healthy controls is shown in Table 1.

5 | GENOTYPE–PHENOTYPE CORRELATION

5.1 | CYP2B6 516G/T polymorphism and HIV patients with hepatotoxicity

The genotype and allele frequency of CYP2B6 516G>T polymorphism in total HIV patients and healthy controls and HIV patients with and without hepatotoxicity are shown in Table 2. The distribution of CYP2B6 516 G>T polymorphism was not significantly different between total HIV patients and healthy controls. Higher distribution of CYP2B6 516TT genotype was observed in HIV patients with hepatotoxicity as compared to without hepatotoxicity (35.3% vs. 30.5%, OR = 1.74, 95%CI: 0.66–4.60, p = 0.25). The CYP2B6 516GT genotype and 516T allele were not significantly different between both the groups (26.5% vs. 40.5% and 48.5%vs. 50.8%, respectively).

5.2 | CYP2B6 516G/T polymorphism and HIV patients

The distribution of CYP2B6 516G>T polymorphism in HIV patients and healthy controls is presented in Table 3. CYP2B6 516G>T polymorphisms in healthy controls followed the Hardy–Weinberg equilibrium (p = 0.12). The CYP2B6 carriage 516TT genotype presented slightly higher in HIV patients as compared to healthy controls (30.5% vs. 27.1%, OR = 1.14, 95%CI: 0.63–2.06, p = 0.64). The difference in frequency of CYP2B6 516GT genotype and 516T allele was
### Table 1
Characteristics of HIV patients with hepatotoxicity, without hepatotoxicity, and healthy controls

| S. no. | HIV patients with hepatotoxicity (Grade III and IV) | HIV patients without hepatotoxicity | Healthy controls |
|--------|--------------------------------------------------|-----------------------------------|------------------|
| Number | $N = 34$                                         | $N = 131$                         | $N = 155$        |
| Mean age (range) | $40.70 ± 7.63$                                      | $39.41 ± 7.18$                     | $36.75 ± 8.50$   |
| Females | 15 (44.11%)                                       | 47 (35.87%)                        | 38 (25%)         |
| Males | 19 (55.88%)                                       | 84 (64.12%)                        | 117 (75.48%)     |
| NNRTI regimen |                                      |                                   |                 |
| Nevirapine $N = 142$ | 30 (88.23%)                                      | 112 (85.49%)                       | Not applicable (NA) |
| Efavirenz $N = 23$ | 4 (11.76%)                                        | 19 (14.50%)                        | NA               |
| Alcohol habit |                                         |                                   |                 |
| User $N = 51$ | 23 (67.64%)                                       | 28 (21.37%)                        | 13 (8.12%)       |
| Nonuser $N = 114$ | 11 (32.35%)                                      | 103 (78.62%)                       | 21 (13.12%)      |
| Tobacco habit |                                         |                                   |                 |
| User $N = 50$ | 23 (67.64%)                                       | 27 (20.61%)                        | 15 (9.37%)       |
| Nonuser $N = 115$ | 11 (32.35%)                                      | 104 (79.38%)                       | 27 (16.87%)      |
| CD4+ status |                                         |                                   |                 |
| <200 ($N = 95$) | 25 (73.52%)                                       | 61 (45.56%)                        | NA               |
| 201–350 ($N = 50$) | 9 (26.47%)                                        | 50 (38.16%)                        | NA               |
| >350 ($N = 20$) | 0 (0)                                             | 20 (15.26%)                        | NA               |

### Table 2
Frequency distribution of CYP2B6 516G/T genotypes in a total HIV patients and healthy controls and HIV patients with and without hepatotoxicity

| Genotypes CYP2B6 516 G/T | Total HIV patients $N = 165$ (%) | Healthy controls $N = 155$ (%) | $p$-value | OR (95%CI) |
|--------------------------|----------------------------------|--------------------------------|-----------|-------------|
| GG                       | 51 (30.90)                       | 45 (29.0)                       | 1         | Reference   |
| GT                       | 62 (37.57)                       | 68 (43.9)                       | 0.50      | 0.80 (0.46–1.41) |
| TT                       | 52 (31.51)                       | 42 (27.1)                       | 0.87      | 1.09 (0.59–2.01) |
| GT+TT                    | 114 (69.09)                      | 110 (71.0)                      | 0.89      | 1.00 (0.58–1.73) |

| Alleles | Total HIV patients $N = 330$ (%) | Healthy controls $N = 310$ (%) | $p$-value | OR (95%CI) |
|---------|----------------------------------|--------------------------------|-----------|-------------|
| G       | 164 (49.69)                      | 158 (51.0)                      | 1         | Reference   |
| T       | 166 (50.30)                      | 152 (49.0)                      | 0.80      | 1.05 (0.76–1.45) |

| Genotypes CYP2B6 516 G/T | HIV patients with hepatotoxicity $N = 34$ (%) | HIV patients without hepatotoxicity $N = 131$ (%) | $p$-value | OR (95%CI) |
|--------------------------|-----------------------------------------------|-----------------------------------------------|-----------|-------------|
| GG                       | 13 (38.2)                                     | 38 (29.0)                                     | 1         | Reference   |
| GT                       | 9 (26.5)                                      | 53 (40.5)                                     | 0.67      | 0.82 (0.32–2.07) |
| TT                       | 12 (35.3)                                     | 40 (30.5)                                     | 0.25      | 1.74 (0.66–4.60) |
| GT+TT                    | 21 (61.8)                                     | 93 (71)                                       | 0.40      | 0.66 (0.28–1.56) |

| Alleles | HIV patients with hepatotoxicity $N = 68$ (%) | HIV patients without hepatotoxicity $N = 262$ (%) | $p$-value | OR (95%CI) |
|---------|-----------------------------------------------|-----------------------------------------------|-----------|-------------|
| G       | 35 (51.5)                                     | 129 (49.2)                                   | 1         | Reference   |
| T       | 33 (48.5)                                     | 133 (50.8)                                   | 0.78      | 1.04 (0.80–1.35) |

Note. $N =$ number of subjects, (%) = frequency of genotypes/allele. Presence of GG for GT, TT, and GT+TT genotypes and G for T allele were taken as reference group for statistical analysis.

*aChromosome 19 - NC_000019.10.*
not statically significant between HIV patients and healthy controls (40.5% vs. 43.9% and 50.8% vs. 49.0%, respectively).

5.3 | Genotype-HIV disease stage correlation

CYP2B6 516G>T polymorphism was not significantly distributed between HIV disease stages and healthy controls. However, the presence of CYP2B6516TT genotype was greater in advanced HIV disease stages compared with healthy controls (30.38% vs. 27.1%, OR = 1.29, 95%CI: 0.58–2.84, p = 0.62) (Table 4).

5.4 | Gene–environment interaction

In patients with hepatotoxicity along with tobacco usage genotypes CYP2B6516GT, 516TT and 516GT+TT genotypes were higher as compared to nonusers (28.57% vs. 25.93%, OR = 1.57, 95%CI: 0.12–21.71, p = 0.68; 42.86% vs. 33.33%, OR = 1.83, 95%CI: 0.18–20.78, p = 0.92; 71.43% vs. 59.26%, OR = 1.72, 95%CI: 0.22–15.77, p = 0.87, respectively). Likewise, the occurrence of CYP2B6516GT and 516GT+TT genotypes was higher in HIV patients with alcohol users as compared to nonusers (54.54% vs. 33.33%, OR = 2.03, 95%CI: 0.77–5.43, p = 0.17; 74.99% vs. 68.97%, OR = 1.35, 95%CI: 0.55–3.33, p = 0.60, respectively) (Table 5).

Table 6 shows the distribution of CYP2B6 516G>T polymorphism in patients with hepatotoxicity, without hepatotoxicity using nevirapine and efavirenz. In patients with hepatotoxicity, the distribution of CYP2B6 516TT, 516GT+TT genotypes appeared to be higher in nevirapine users as compared to efavirenz users (47.83% vs. 9.09%, OR = 6.88, 95%CI: 0.55–189.71, p = 0.19; 65.22% vs. 54.55%, OR = 1.56, 95%CI: 0.29–8.64, p = 0.82, respectively). Similarly, CYP2B6516GT, 516TT, 516GT+TT genotypes were observed predominantly at a higher level in HIV patients with nevirapine use as compared to efavirenz use (42.02% vs. 25.00%, OR = 2.53, 95%CI: 0.48–14.49, p = 0.38; 30.25% vs. 33.33%, OR = 1.36, 95%CI: 0.28–6.74, p = 0.93; 72.27% vs. 58.33%, OR = 1.86, 95%CI: 0.47–7.17, p = 0.49, respectively).

In patients with hepatotoxicity CYP2B6516GT, 516TT, 516GT+TT genotypes were found to be much prevalent in nevirapine +alcohol users as compared to nevirapine +alcohol

### Table 3

| Genotypes CYP2B6 516 G/T | HIV patients | Healthy controls | p-value | OR (95%CI) |
|-------------------------|--------------|-----------------|---------|------------|
| GG                      | 38 (29.0%)   | 45 (29.0%)      | 1       | Reference  |
| GT                      | 53 (40.5%)   | 68 (43.9%)      | 0.98    | 0.99 (0.52–1.87) |
| TT                      | 40 (30.5%)   | 42 (27.1%)      | 0.64    | 1.14 (0.63–2.06) |
| GT+TT                   | 93 (71.0%)   | 110 (71.0%)     | 0.89    | 1.00 (0.58–1.73) |

**Note.** N = number of subjects, (%) = frequency of genotypes/allele. Presence of GG for GT, TT genotypes and G for T allele were taken as reference group for statistical analysis.

### Table 4

| Genotypes CYP2B6 516 G/T | Healthy controls | Early HIV disease stage | Intermediate HIV disease stage | Advanced HIV disease stage |
|-------------------------|-----------------|-------------------------|-------------------------------|---------------------------|
| GG                      | 45 (29.0%)      | 7 (36.84%)              | 11 (33.34%)                   | 20 (25.32%)               |
| GT                      | 68 (43.9%)      | 4 (21.05%)              | 14 (42.42%)                   | 35 (44.30%)               |
| TT                      | 42 (27.1%)      | 8 (42.11%)              | 8 (24.24%)                    | 24 (30.38%)               |

**Note.** N = number of subjects, (%) = frequency of genotypes. Presence of GG for GT, TT genotypes were taken as reference group for statistical analysis.
nonusers (40.0% vs. 11.11%, OR = 8.00, 95% CI: 0.28–449.98, 

\[ p = 0.41; 40.0\% \text{ vs. } 44.44\%, \text{ OR } = 2.00, 95\% \text{ CI: } 0.10–69.78, 

\[ p = 0.92; 80.0\% \text{ vs. } 27.78\%, \text{ OR } = 4.00, 95\% \text{ CI: } 0.31–110.70, 

\[ p = 0.46, \text{ respectively}. \]

In HIV patients, CYP2B6 516GT, 516GT+TT genotypes were distributed at a higher level with nevirapine + alcohol users in comparison to nevirapine + alcohol nonusers (57.89% vs. 34.57%, OR = 2.46, 95% CI: 0.84–7.31, \[ p = 0.10; 78.95\% \text{ vs. } 69.14\%, \text{ OR } = 1.67, 95\% \text{ CI: } 0.62–4.62, \text{ respectively} (Table 7).

### 5.5 | Risk factors of ARV‐associated hepatotoxicity: Multivariate logistic regression analysis

Correlation of age, sex, tobacco, alcohol, baseline CD4 counts, and CYP2B6 516G/T polymorphism with ARV-associated hepatotoxicity were done by multivariate logistic regression analysis. CYP2B6 516G/T polymorphism, age, sex, tobacco, alcohol usage, and baseline CD4 counts were not independent risk factors for ARV-associated hepatotoxicity. While comparing between HIV patients with and without hepatotoxicity, CYP2B6 516TT genotype, taking alcohol and male HIV patients with hepatotoxicity showed a risk for ARV-associated hepatotoxicity (OR = 1.79, \[ p = 0.27; \text{ OR } = 1.91, \text{ OR } = 1.90, \text{ p } = 0.16, \text{ respectively}) (Table 8).

### 6 | DISCUSSION

The differences in the activity of drug metabolizing enzyme contribute to interpatient variability in response to drugs. The SNPs of CYP450 enzymes are known to have a role in influencing the efficacy of therapy (Servoss et al., 2006). The

| Genotypes CYP2B6 516 G/T | Tobacco user \( N = 7 \) (%) | Tobacco nonuser \( N = 27 \) (%) | \( p \)-value | OR 95% CI |
|--------------------------|-----------------------------|-----------------------------|--------------|-------------|
| HIV patients with hepatotoxicity | | | | |
| GG | 1 (14.29%) | 12 (44.44%) | 1 | Reference |
| GT | 2 (28.57%) | 7 (25.93%) | 0.73 | 3.43 (0.18–117.72) |
| TT | 4 (57.14%) | 8 (29.63%) | 0.27 | 6.00 (0.45–170.81) |
| GT+TT | 6 (85.71%) | 15 (55.56%) | 0.30 | 4.80 (0.44–121.06) |
| HIV patients without hepatotoxicity | | | | |
| GG | 12 (27.91%) | 26 (29.55%) | 1 | Reference |
| GT | 20 (46.51%) | 33 (37.50%) | 0.70 | 1.31 (0.50–3.48) |
| TT | 11 (25.58%) | 29 (32.95%) | 0.88 | 0.82 (0.28–2.43) |
| GT+TT | 31 (72.09%) | 62 (70.45%) | 0.99 | 1.08 (0.45–2.63) |

| Genotypes CYP2B6 516 G/T | Alcohol user \( N = 7 \) (%) | Alcohol nonuser \( N = 27 \) (%) | \( p \)-value | OR 95% CI |
|--------------------------|-----------------------------|-----------------------------|--------------|-------------|
| HIV patients with hepatotoxicity | | | | |
| GG | 2 (28.57%) | 11 (40.74%) | 1 | Reference |
| GT | 2 (28.57%) | 7 (25.93%) | 0.68 | 1.57 (0.12–21.71) |
| TT | 3 (42.86%) | 9 (33.33%) | 0.92 | 1.83 (0.18–20.78) |
| GT+TT | 5 (71.43%) | 16 (59.26%) | 0.87 | 1.72 (0.22–15.77) |
| HIV patients without hepatotoxicity | | | | |
| GG | 11 (25.00%) | 27 (31.03%) | 1 | Reference |
| GT | 24 (54.54%) | 29 (33.33%) | 0.17 | 2.03 (0.77–5.43) |
| TT | 9 (20.45%) | 31 (35.63%) | 0.69 | 0.71 (0.23–2.22) |
| GT+TT | 33 (74.99%) | 60 (68.97%) | 0.60 | 1.35 (0.55–3.33) |

Note. \( N \) = number of subjects, (%) = frequency of genotypes. Presence of GG for GT, TT genotypes were taken as reference group for statistical analysis.
SNPs within the CYP2B6 gene are associated with altered hepatic CYP2B6 expression and activity (Tong et al., 2006). The interindividual differences in hepatic CYP2B6 expression and enzymatic activity may result in variable systemic exposure and therapeutic response to the drugs metabolized by CYP2B6.

The CYP2B6 gene is highly polymorphic (Lang et al., 2001) and encodes the major enzymes for hepatic elimination of efavirenz and nevirapine. The prevalence of T allele varies within the populations (Tong et al., 2006; Xu et al., 2001) and encodes the major enzymes for hepatic elimination and enzymatic activity may result in variable systemic exposure and therapeutic response to the drugs metabolized by CYP2B6.

The interindividual differences in hepatic CYP2B6 expression and activity (Tong et al., 2006). The gene–environment interactions determine the pathophysiology of the disease (Deng, Newman, Dunne, Silburn, & Mellick, 2004). However, for case–control association studies for environmental influences, cases must have matched controls (Greenland, 1980). It is assumed that a case study is always better to look at for the effect of gene–environment. Here, we have chosen the case-only analysis. Heavy alcohol consumption had a negative impact on the CD4 cell counts of HIV patients naïve to ART (Samet et al., 2007). In our study, CYP2B6 516TT genotype modulated the severity risk in patients with hepatotoxicity using tobacco (OR = 6.00, \( p = 0.27\)). Hepatotoxicity risk was revealed among alcohol users, with CYP2B6 516TT genotype (OR = 1.83, \( p = 0.92\)).

Similarly, presence of CYP2B6 516GT genotype among alcohol users resulted in the modulation of risk for acquisition of ARV-associated hepatotoxicity (OR = 2.03, \( p = 0.17\)).

CYP2B6 516TT genotype also indicated a risk for hepatotoxicity severity in nevirapine users (OR = 6.88, \( p = 0.19\)). Likewise, CYP2B6 516TT genotype among combined nevirapine + alcohol users has shown a risk for hepatotoxicity severity (OR = 2.00, \( p = 0.92\)). CYP2B6 516GT+TT combined genotype revealed a risk for acquisition of ARV-associated hepatotoxicity and its severity in combined alcohol and nevirapine users (OR = 1.67, \( p = 0.37\); OR = 4.00, \( p = 0.46\), respectively). In multivariate logistic regression, while comparing between HIV patients with and without hepatotoxicity, CYP2B6 516TT genotype showed a risk for ARV-associated hepatotoxicity (OR = 1.79, \( p = 0.27\)).

### Table 6: Frequency distribution of CYP2B6 516 G/T genotypes in NNRTI regimens using HIV patients with and without hepatotoxicity

| Genotypes CYP2B6 516 G/T | Nevirapine user \( N = 23 \) (%) | Efavirenz user \( N = 11 \) (%) | \( p\)-value | OR 95% CI |
|--------------------------|-------------------------------|-----------------------------|-------------|-----------|
| HIV patients with hepatotoxicity | | | | |
| GG | 8 (34.78%) | 5 (45.45%) | 1 | Reference |
| GT | 4 (17.39%) | 5 (45.45%) | 0.72 | 0.50 (0.06–3.82) |
| TT | 11 (47.83%) | 1 (9.09%) | 0.19 | 6.88 (0.55–189.71) |
| GT+TT | 15 (65.22%) | 6 (54.55%) | 0.82 | 1.56 (0.29–8.64) |
| HIV patients without hepatotoxicity | | | | |
| GG | 33 (27.73%) | 5 (41.67%) | 1 | Reference |
| GT | 50 (42.02%) | 3 (25.00%) | 0.38 | 2.53 (0.48–14.49) |
| TT | 36 (30.25%) | 4 (33.33%) | 0.93 | 1.36 (0.28–6.74) |
| GT+TT | 86 (72.27%) | 7 (58.33%) | 0.49 | 1.86 (0.47–7.17) |

Note. \( N \) = number of subjects, (%) = frequency of genotypes. Presence of GG for GT, TT and GT+TT genotypes were taken as reference group for statistical analysis.

In this study, the current CD4 count was considered as a substitute of current HIV disease stage. As the time points for HIV infection were not known, the results may be confounded by the duration of HIV infection. In our study, the distribution of CYP2B6 516G/T polymorphism was not significantly different between HIV disease stages and healthy controls.

TABLE 6 Frequency distribution of CYP2B6 516 G/T genotypes in NNRTI regimens using HIV patients with and without hepatotoxicity
individuals with CYP2B6 516TT genotype and alcohol and nevirapine usage are more prone to alcohol and drug-related hepatotoxicity.

Limitations of this study are as follows: (a) it could only define the association; (b) could not determine the causality; (c) could not estimate the plasma efavirenz and nevirapine level.

### TABLE 7

| Genotypes CYP2B6 516 G/T | Nevirapine + alcohol user N = 5 (%) | Nevirapine + alcohol nonuser N = 18 (%) | p-value | OR 95% CI |
|--------------------------|-------------------------------------|----------------------------------------|---------|-----------|
| HIV patients with hepatotoxicity |
| GG                       | 1 (20.0%)                           | 8 (44.44%)                             | 1       | Reference |
| GT                       | 2 (40.0%)                           | 2 (11.11%)                             | 0.41    | 8.00(0.28–449.98) |
| TT                       | 2 (40.0%)                           | 8 (44.44%)                             | 0.92    | 2.00(0.10–69.78) |
| GT+TT                    | 5 (80.0%)                           | 10 (27.78%)                            | 0.46    | 4.00(0.31–110.70) |

### TABLE 8

| Genotypes CYP2B6 516 G/T | Efavirenz + alcohol user N = 2 (%) | Efavirenz + alcohol nonuser N = 9 (%) | p-value | OR 95% CI |
|--------------------------|-------------------------------------|--------------------------------------|---------|-----------|

| Variables | B | S.E. | df | p-value | OR (95%CI) |
|-----------|---|------|----|---------|------------|
| 516GG     |   | 2    | 0.45 |  1.004 (1.002–1.007) |
| 516GT     | 0.001 | 0.516 | 1 | 0.99 | 0.99 (0.36–2.74) |
| 516 TT    | 0.586 | 0.540 | 0.27 | 0.07 | 1.79 (0.62–5.17) |
| Age       | 0.026 | 0.032 | 0.41 | 0.97 | 0.97 (0.91–1.03) |
| Sex       | 0.644 | 0.465 | 0.16 | 1.90 | 1.90 (0.76–4.73) |
| Tobacco user | 0.196 | 0.608 | 0.747 | 1.217 | (0.370–4.003) |
| Alcohol user | 0.647 | 0.637 | 0.637 | 1.91 | (0.766–4.739) |
| NNRTI drug user | 1.455 | 0.517 | 0.005 | 0.233 | (0.085–0.643) |
| Baseline CD4 count | 0.004 | 0.001 | 0.001 | 1.004 (1.002–1.007) |

Note. CYP2B6 516G/T polymorphism, Age 18–50 year, sex, tobacco user, alcohol user, NNRTI drug user, Baseline CD4. Significant values (<0.05) represented in bold.
in our subjects; (d) We had distributed a ratio of 1:4 for case controls. However, we could not complete matched enrollment in controls although our case–control ratio is around 1:3.

### 7 | CONCLUSION

The distribution of CYP2B6 516G>T polymorphism was not significantly different among HIV patients and healthy controls though, the prevalence of CYP2B6 516TT genotype was higher in patients with hepatotoxicity. CYP2B6 genotype along with nevirapine and alcohol usage may influence the acquisition of ARV-associated hepatotoxicity and hepatotoxicity severity. The prevalence of CYP2B6 516TT genotype varied among different ethnic groups. Hence, further study of correlation of gene polymorphism with drug levels should be done in HIV patients on efavirenz 400 mg in a larger sample size. Finding of study may help to predict the acquisition of hepatotoxicity and also may influence the choice of drug to maintain the doses and its responses.

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### CONFLICT OF INTEREST

None declared.

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