ARV-Associated Hepatotoxicity

Drug-induced adverse effects have been reported with the use of all antiretroviral (ARV) drugs [1]. Hepatotoxicity is a relatively common adverse drug reaction (ADR) leading to the treatment interruptions in human immunodeficiency virus (HIV-) infected patients on different drug regimens [2], with consequent immunologic compromise [3]. The severity of the hepatic injury and drug-induced liver injury (DILI) depends upon the toxic molecules (reactive oxygen species) and the counterbalance of cytokines (TNF-α, IL-1β, IL-10, and IL-1RA) [4-6]. 10.8% in the efavirenz-treated group while 8.9% in the nevirapine-treated group reported having grade 3 or 4 hepatotoxicity [7]. While in another report, nevirapine use was associated with a higher incidence of hepatotoxicity than efavirenz use [8]. Nagpal et al. [9] reported a prevalence of nevirapine-induced hepatotoxicity (3.19%) in HIV-infected individuals in India. The activity of drug metabolizing enzymes was found to be altered in some infectious and inflammatory states such as bacterial pneumonia, viral infections, surgery, and trauma [10]. The effect of infection on the drug metabolizing enzymes is carried by the few cytokines such as IL-1β, IL6, tumor necrosis factor alpha (TNF-α), and interferon (INF) α or γ [11, 12]. IL-1 receptor antagonist (IL-1RA) restricted the induction of HIV replication in vitro [13-15], and the treatment with ARV drugs resulted in an increased level of circulating IL-1RA [16].

Interleukins (ILs) are proinflammatory cytokines produced by leukocytes, macrophages, monocytes, and epithelial cells. The IL-1 family consists of eleven members including IL-1α, IL-1β, IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1RA) [17]. IL-1α and IL-1β are the most influencing proinflammatory cytokines, and IL-1RN is a
naturally occurring anti-inflammatory cytokine [18, 19]. The IL-1RA also regulates biological activity of IL-1α and IL-1β [19–21]. ILs promote the interaction of endothelial cells with circulating leukocytes and induce the proliferation of monocytes and macrophages.

IL-1 cytokine cluster spanning ~430 kb region which is located on chromosome 2q13-21, consists of IL-1α, IL-1β, and IL-1RN genes [22]. IL-1β plays a pivotal role in driving inflammation by binding to the IL-1 receptor to induce more proinflammatory cytokines. The proinflammatory cytokine IL-1β and its antagonist IL-1RA are strongly induced by infection and are encoded by polymorphic genes [23]. IL-1β production is tightly regulated by two steps: induction of pro-IL-1β gene expression and caspase-1-mediated pro-IL-1β cleavage [24]. Polymorphisms have been reported in all the three genes [25]. IL-1RN gene contains 86-bp variable number tandem repeat (VNTR) polymorphism in intron 2 region [26]. IL-1RN alleles 1 and 2 are the most common, while other alleles occur at a combined frequency of <5%. The role of IL-1RN (VNTR) has been studied in the development of inflammatory disorders [27–29]. Witkin et al. [30] suggested that the presence of IL-1RN 2/2 homozygous genotype reduced the viral load (P < 0.05) in the treatment-naive HIV-infected individuals. There was no relation between IL-1RN alleles and CD4 levels. Plasma IL-1RA concentrations were higher with borderline significance (P = 0.058) in treatment-naive HIV-infected adults [31].

In IL-1β, three biallelic polymorphisms are reported representing C-T base transitions, at positions −511, −31, and +3954 bp from the transcriptional site. IL-1β-511C/T polymorphism has been associated with various inflammatory diseases and in linkage disequilibrium with the IL-1RN polymorphism [32].

IL-1β promoter (−511C/T) and exon 5 (+3953C/T) polymorphisms may influence the gene expression and also found to be associated with various inflammatory diseases [33, 34]. Asensi et al. [35] suggested that IL-1β 3954T allele was less frequent in the patients with lipodystrophy syndrome compared with those without (17.8% versus 27.0%, P = 0.03). However, the IL-1β 3954T allele was associated in patients with lipodystrophy syndrome who are under highly active antiretroviral therapy (HAART) (P = 0.02). The carriage alleles of IL-1α-889, IL-1β-3953, and IL-1β-3’ UTR did not differ between HIV-associated dementia (HAD) patients and controls [36].

Till now, the association of IL-1RN (VNTR) and IL-1β (−511C/T) polymorphisms has not been described with the severe hepatotoxicity and its prevalence in HIV-infected individuals on NNRTI-containing ART and healthy controls. Hence, the present study is intended to evaluate the association of IL-1RN (VNTR) and IL-1β (−511C/T) polymorphism with severe hepatotoxicity and its prevalence in HIV-infected individuals and healthy controls. Its interaction with environmental factors was also analyzed.

2. Material and Methods

2.1. Participants. This is a case-control study, carried out from November 2012 to February 2015. A total of 162 HIV-infected individuals were recruited. Out of that, 34 HIV-infected individuals with hepatotoxicity (grade III/IV) under NNRTI-containing ART regimen, 128 without hepatotoxicity-confirmed liver function test (LFT), and age-matched 152 healthy individuals were consecutively recruited from outpatient clinics of National AIDS Research Institute, Pune. Nevirapine was given in the standard dosage of 200 mg twice a day. Nevirapine was given in the standard dosage of 200 mg twice a day. In HIV-infected individuals with hepatotoxicity cases, having hepatitis B, hepatitis C, tuberculosis, and concurrent untreated opportunistic infections, immune reconstitution syndrome and under any other known hepatotoxic drugs were excluded from the case group. In the group of HIV-infected individuals without hepatotoxicity, individuals having evidence of hepatotoxicity, hepatitis B, hepatitis C, tuberculosis, and receiving any other known hepatotoxic drugs were excluded. Similarly, 152 (those from the same family were excluded), free of Hepatitis B and C and tuberculosis, age-matched and serum negative individuals from HIV-ELISA test were recruited. Clinical data were obtained by questionnaire, personal interviews, and review of case records. LFT was done to evaluate the status of the liver enzyme. Total bilirubin > 3.22 mg/ml, SGOT > 93.8 U/ml, SGPT > 229.5 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 were considered as HIV-infected control. Estimation of the CD4 count was done by fluorescence-activated cell sorting (FACS). ELISA for hepatitis C and HBsAg testing was performed using Ortho HCV ELISA test system (Ortho Clinical Diagnostics, Buckinghamshire, UK) and Murex HBsAg Confirmatory Version 3 (DiaSorin, Dartford, UK) ELISA. The status of tobacco and alcohol usage was also recorded in the questionnaire. The study was approved by the institutional ethics committee board and informed written consent was taken from all eligible participants.

2.2. DNA Extraction. A 2 ml peripheral blood sample was collected and stored at ~70°C before DNA extraction. Extraction of genomic DNA was done using AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA) according to the protocol given by the manufacturer.

2.3. Genotyping

2.3.1. IL-1RN (86 bp VNTR) Polymorphism. IL-1RN (86 bp repeats) polymorphism was determined by polymerase chain reaction (PCR) analysis as described by Tarlow et al. [26]. The reaction mixture for PCR was prepared in a total volume of 25 μl with 10 pmol primers, 10x standard Taq buffer, genomic DNA (100–150 ng), 2.5 mM deoxynucleotide triphosphates (dNTPs), and one unit of Taq DNA polymerase (New England Biolabs, USA). The reaction conditions for IL-1RN (VNTR) were initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 30 sec, and a final
extension at 72°C for 10 min. The amplified PCR product of *IL-1RN* was genotyped on 3% agarose gel. The genotypes were determined as allele 1, 410 bp (four repeats); allele 2, 240 bp (two repeats); allele 3, 500 bp (five repeats); allele 4, 325 bp (three repeats); and allele 5, 595 bp (six repeats) [37].

2.3.2. *IL-1β* (-511C/T) Polymorphism. *IL-1β* (-511C/T) polymorphism was assessed by PCR followed by the restriction digestion (PCR-RFLP) method. PCR reaction was prepared in a total volume of 25 μl with primers (10 pmol) as described by Rad et al. [38], 10x standard Taq buffer, genomic DNA (100–150 ng), 2.5 mM deoxynucleotide triphosphates, and 1 unit of Taq DNA polymerase (New England Biolabs, USA). PCR conditions were as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 51°C for 30 sec, extension at 72°C for 45 sec, and a final extension at 72°C for 7 min. Further, the amplified product was digested using *Ava* restriction enzyme (MBI Fermentas Inc., Hanover, MD, USA). The genotype of *IL-1β* (-511C/T) was determined on 10% polyacrylamide gel after staining with ethidium bromide with reference to molecular weight markers. Genotypes were assessed as follows: 304 bp for TT, 190 and 114 bp for CC and 304, and 190 and 114 bp for CT. All PCR reactions were performed in a Veriti 96-well thermal cycler (Applied Biosystems, CA, USA). To avoid the data discrepancy in genotyping, 20% of samples from patients and controls were regenotyped by another laboratory personnel, and also 10% of samples were assessed by sequencing. No inconsistency was noticed with the previously recorded genotypes.

2.4. Data Analysis. The mean age variable was considered with an appropriate standard deviation. Deviations from Hardy-Weinberg equilibrium in control were assessed by chi-square (χ²) goodness-of-fit test. Chi-squared test was also used to compare genotype frequencies in HIV-infected individuals with hepatotoxicity, without hepatotoxicity, and healthy controls. SHEsisPlus online analysis tool was used to compare haplotype frequency between HIV-infected individuals with hepatotoxicity, without hepatotoxicity, and healthy controls [39–41]. Odds ratios (ORs) and 95% confidence interval (CI) were determined by multivariate unconditional binary logistic regression using SPSS software version 17.0 (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.) Data was analyzed for the two-sided statistical significance, and a *P* value less than 0.05 was considered for the statistically significant difference. Linkage disequilibrium (LD) was assessed between both the loci by calculating the relative LD value (D') as D = Dij/Dmax [42]. The Dij values were compared between HIV-infected individuals with hepatotoxicity versus without hepatotoxicity, HIV-infected individuals with hepatotoxicity versus healthy controls, and HIV-infected individuals without hepatotoxicity versus healthy controls by comparison of confidence intervals.

### 3. Results

Thirty-four HIV-infected individuals with hepatotoxicity, 128 without hepatotoxicity, and 152 healthy controls were included in the study. The mean age difference between all study groups were $35.14 \pm 8.96$, $39.29 \pm 1.34$, and $36.75 \pm 8.50$ years, respectively. Characteristics of HIV-infected individuals with, without hepatotoxicity, and healthy controls are shown in Table 1.

| Subjects | HIV-infected individuals with hepatotoxicity (grade III and IV) | HIV-infected individuals without hepatotoxicity | Healthy controls |
|----------|-------------------------------------------------------------|-------------------------------------------------|------------------|
| Number   | N = 34                                                       | N = 128                                        | N = 152          |
| Mean age (range) | 35.14 ± 8.96                                               | 39.29 ± 1.34                                  | 36.75 ± 8.50     |
| Females  | 16                                                          | 44                                             | 40               |
| Males    | 18                                                          | 84                                             | 112              |
| **Alcohol habit (N = 162)** |                                                    |                                                  |                  |
| User (N = 50) | 7 (14.0)                                                    | 43 (86.0)                                      | 0                |
| Nonuser (N = 112) | 27 (24.11)                                                 | 85 (75.89)                                     | 0                |
| **Tobacco habit (N = 162)** |                                                  |                                                  |                  |
| User (N = 49) | 7 (14.28)                                                   | 42 (85.71)                                     | 0                |
| Nonuser (N = 113) | 27 (23.89)                                                 | 86 (76.11)                                     | 0                |
| **CD4+ status (N = 162)** |                                                |                                                  |                  |
| <200 (N = 92) | 16 (17.39)                                                  | 76 (82.61)                                     | NA               |
| 201–350 (N = 50) | 17 (34.0)                                                   | 33 (66.0)                                      | NA               |
| >350 (N = 20)  | 1 (5.0)                                                     | 19 (95.00)                                     | NA               |

| NNRTI regimen | HIV-infected individuals with hepatotoxicity (grade III and IV) | HIV-infected individuals without hepatotoxicity | Healthy controls |
|---------------|-------------------------------------------------------------|-------------------------------------------------|------------------|
| Efavirenz (N = 23) | 11 (47.8)                                                   | 12 (52.2)                                      | NA               |
| Nevirapine (N = 139) | 23 (16.50)                                                  | 116 (83.50)                                    | NA               |

**Table 1: Characteristics of HIV-infected individuals with hepatotoxicity, without hepatotoxicity, and healthy controls.**
Table 2: Frequency distributions of IL-1RN (VNTR) and IL-1β (-511C/T) genotypes and alleles between HIV-infected individuals with versus without hepatotoxicity and hepatotoxicity versus healthy controls.

| IL-1RN (VNTR) Genotypes | HIV-infected individuals with hepatotoxicity \(N = 34\) (%) | HIV-infected individuals without hepatotoxicity \(N = 128\) (%) | \(P\) value | OR (95% CI) |
|-------------------------|-------------------------------------------------|-------------------------------------------------|-----------|-------------|
| 1/1                     | 19 (55.9%)                                      | 60 (46.9%)                                      | —         | 1 (reference) |
| 1/2                     | 10 (29.4%)                                      | 51 (39.9%)                                      | 0.36      | 0.62 (0.24–1.56) |
| 2/2                     | 3 (8.8%)                                        | 10 (7.8%)                                       | 0.78      | 0.95 (0.18–4.33) |
| 1/3                     | 2 (5.9%)                                        | 2 (1.6%)                                        | 0.56      | 3.16 (0.29–34.43) |

| IL-1RN (VNTR) Alleles | HIV-infected individuals with hepatotoxicity \(N = 68\) (%) | HIV-infected individuals without hepatotoxicity \(N = 256\) (%) | \(P\) value | OR (95% CI) |
|-----------------------|-------------------------------------------------|-------------------------------------------------|-----------|-------------|
| 1                     | 50 (73.52)                                      | 176 (68.75)                                     | —         | 1 (reference) |
| 2                     | 16 (23.52)                                      | 73 (28.51)                                      | 0.50      | 0.77 (0.39–1.50) |
| 3                     | 2 (2.94)                                        | 3 (1.17)                                        | 0.68      | 2.35 (0.27–17.89) |

| IL-1β (-511C/T) Genotypes | HIV-infected individuals with hepatotoxicity \(N = 34\) (%) | HIV-infected individuals without hepatotoxicity \(N = 128\) (%) | \(P\) value | OR (95% CI) |
|---------------------------|-------------------------------------------------|-------------------------------------------------|-----------|-------------|
| CC                       | 7 (20.6%)                                       | 21 (16.4%)                                      | —         | 1 (reference) |
| CT                       | 14 (41.2%)                                      | 61 (47.7%)                                      | 0.75      | 0.84 (0.29–2.43) |
| TT                       | 13 (38.2%)                                      | 46 (35.9%)                                      | 0.47      | 0.68 (0.24–1.93) |

| IL-1β (-511C/T) Alleles | HIV-infected individuals with hepatotoxicity \(N = 68\) (%) | HIV-infected individuals without hepatotoxicity \(N = 256\) (%) | \(P\) value | OR (95% CI) |
|-------------------------|-------------------------------------------------|-------------------------------------------------|-----------|-------------|
| C                       | 28 (41.17%)                                     | 103 (40.23%)                                    | —         | 1 (reference) |
| T                       | 40 (58.82%)                                     | 153 (59.76%)                                    | 0.88      | 1.04 (0.60–1.79) |

| IL-1RN (VNTR) Genotypes | Healthy control \(N = 152\) (%) | \(P\) value | OR (95% CI) |
|-------------------------|---------------------------------|-----------|-------------|
| 1/1                     | 19 (55.9%)                      | —         | 1 (reference) |
| 1/2                     | 10 (29.4%)                      | 0.30      | 0.58 (0.25–1.36) |
| 2/2                     | 3 (8.8%)                        | 0.78      | 0.67 (0.18–2.55) |
| 1/3                     | 2 (5.9%)                        | 0.69      | 2.39 (0.37–15.33) |

| IL-1RN (VNTR) Alleles | Healthy controls \(N = 304\) (%) | \(P\) value | OR (95% CI) |
|-----------------------|---------------------------------|-----------|-------------|
| 1                     | 50 (73.52%)                     | —         | 1 (reference) |
| 2                     | 16 (23.52%)                     | 0.28      | 0.68 (0.37–1.26) |
| 3                     | 2 (2.94%)                       | 0.93      | 1.62 (0.20–8.57) |

| IL-1β (-511C/T) Genotypes | Healthy controls \(N = 152\) (%) | \(P\) value | OR (95% CI) |
|---------------------------|---------------------------------|-----------|-------------|
| CC                       | 7 (20.6%)                       | —         | 1 (reference) |
| CT                       | 14 (41.2%)                      | 0.88      | 0.81 (0.29–2.23) |
| TT                       | 13 (38.2%)                      | 0.71      | 0.71 (0.26–2.00) |

| IL-1β (-511C/T) Alleles | Healthy controls \(N = 304\) (%) | \(P\) value | OR (95% CI) |
|-------------------------|---------------------------------|-----------|-------------|
| C                       | 28 (41.17%)                     | —         | 1 (reference) |
| T                       | 40 (58.82%)                     | 0.58      | 0.83 (0.49–1.42) |

VNTR: variable number tandem repeat; \(N\): total number of subject participants; NS: not significant; OR: odds ratio; CI: confidence interval. Odds ratio (OR) and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele (1/1 genotype and 1 allele for IL-1RN) (CC genotype and C allele for IL-1β) with other genotypes/alleles. Zero percent genotype frequencies in either of variables are avoided in the analysis.
3.1. IL-1RN (VNTR) and IL-1β (-511C/T) Polymorphism and HIV-Infected Individuals with Hepatotoxicity. In single and both locus model, none of the genotypes/alleles of IL-1RN and IL-1β polymorphism was associated with severe hepatotoxicity. IL-1RN 1/3 genotype and allele 3 showed higher risk for severe hepatotoxicity (5.9% versus 1.6%, OR = 3.61, 95% CI: 1.33–9.00). The frequency of IL-1RN 1/2 genotype and allele 2 was lower in HIV-infected individuals with hepatotoxicity compared to without hepatotoxicity (29.4% versus 39.9%, OR = 0.62, 95% CI: 0.47–0.81, P = 0.001). The frequency of IL-1RN 2/2 genotype was observed to be almost similar in both the groups (8.8% versus 7.8%).

In the IL-1RN (-511C/T) polymorphism, the frequency of -511CT, -511TT genotypes, and -511T allele was found to be almost similar in both the groups (41.2% versus 47.7%, 38.2% versus 35.9%, and 58.82% versus 59.76%, respectively). The frequency of IL-1RN and IL-1β genotypes/alleles did not differ between HIV-infected individuals with hepatotoxicity and healthy controls. However, IL-1RN 1/3 genotype and allele 3 were overrepresented in HIV-infected individuals with hepatotoxicity compared with healthy controls (5.9% versus 2.0%, OR = 2.39, 95% CI: 1.37–4.19, P = 0.001) and 2.94% versus 1.64%, OR = 1.62, 95% CI: 1.20–2.19, P = 0.001) (Table 2).

3.2. IL-1RN (VNTR) and IL-1β (-511C/T) Polymorphism and HIV-Infected Individuals. The genotype/allele frequencies of IL-1RN (VNTR) and IL-1β (-511C/T) polymorphisms in HIV-infected individuals and healthy controls are shown in Table 3. The genotype distributions of IL-1RN (VNTR) and IL-1β (-511C/T) polymorphism followed Hardy-Weinberg equilibrium in the healthy control population (P = 0.67 and 0.91). In single and both locus model, the frequency of IL-1RN and IL-1β genotypes/alleles did not differ significantly between HIV-infected individuals and healthy controls. However, the prevalence of IL-1RN 1/4 genotype and allele 4 was observed to be higher in HIV-infected individuals compared with healthy controls (2.3% versus 0.7%, OR = 3.40, 95% CI: 1.30–8.72, and 0.32%, OR = 4.59, 95% CI: 1.43–16.81, respectively). The frequency of IL-1β-511CT genotype marginally differed in HIV-infected individuals compared with healthy controls (47.7% versus 40.8%).

3.3. IL-1RN (VNTR) and IL-1β (-511C/T) Polymorphisms and HIV Disease Stages. Genotype frequencies of IL-1RN and IL-1β polymorphisms in patients with different HIV disease stages and healthy controls are presented in Table 4.

| IL-1RN (VNTR) Genotypes | HIV-infected individuals N = 128 (%) | Healthy controls N = 152 (%) | P value | OR (95% CI) |
|--------------------------|--------------------------------------|-----------------------------|---------|-------------|
| 1/1                      | 60 (46.9%)                           | 68 (44.7%)                  | —       | 1 (reference) |
| 1/2                      | 51 (39.9%)                           | 61 (40.1%)                  | 0.93    | 0.95 (0.55–1.63) |
| 2/2                      | 10 (7.8%)                            | 16 (10.5%)                  | 0.56    | 0.71 (0.27–1.81) |
| 1/4                      | 3 (2.3%)                             | 1 (0.7%)                    | 0.54    | 3.40 (0.30–8.72) |
| 1/3                      | 2 (1.6%)                             | 3 (2.0%)                    | 0.87    | 0.76 (0.08–5.81) |
| 2/3                      | 1 (0.8%)                             | 2 (1.3%)                    | 0.90    | 0.57 (0.02–8.26) |

| IL-1RN (VNTR) Alleles | HIV-infected individuals N = 256 (%) | Healthy controls N = 304 (%) | P value | OR (95% CI) |
|-----------------------|--------------------------------------|-----------------------------|---------|-------------|
| 1                     | 176 (68.75)                          | 202 (66.44)                 | —       | 1 (reference) |
| 2                     | 73 (28.51)                           | 95 (31.25)                  | 0.56    | 0.88 (0.60–1.29) |
| 3                     | 3 (1.17)                             | 5 (1.64)                    | 0.88    | 0.69 (0.13–3.36) |
| 4                     | 4 (1.56)                             | 1 (0.32)                    | 0.29    | 4.59 (0.48–108.86) |

| IL-1β (-511C/T) Genotypes | HIV-infected individual N = 128 (%) | Healthy controls N = 152 (%) | P value | OR (95% CI) |
|---------------------------|-------------------------------------|-----------------------------|---------|-------------|
| CC                       | 21 (16.4%)                          | 25 (16.4%)                  | —       | 1 (reference) |
| CT                       | 61 (47.7%)                          | 62 (40.8%)                  | 0.62    | 1.18 (0.59–2.37) |
| TT                       | 46 (35.9%)                          | 65 (42.8%)                  | 0.64    | 0.85 (0.43–1.68) |

| IL-1β (-511C/T) Alleles | HIV-infected individuals N = 256 (%) | Healthy controls N = 304 (%) | P value | OR (95% CI) |
|-------------------------|--------------------------------------|-----------------------------|---------|-------------|
| C                       | 103 (40.23)                          | 112 (36.84)                 | —       | 1 (reference) |
| T                       | 153 (59.76)                          | 192 (63.15)                 | 0.46    | 0.87 (0.61–1.24) |

N: total number of subject participants; OR: odds ratio; CI: confidence interval. Odds ratios (OR) and 95% CIs derived by logistic regression models comparing the homozygous wild-type genotype/allele (1/1 genotype and 1 allele for IL-1RN) (CC genotype and C allele for IL-1β) with other genotypes/alleles.
**Table 4**: Frequency distribution of *IL-1RN* (VNTR) and *IL-1β* (-511C/T) genotypes in different HIV disease stages of HIV-infected individuals.

| Genotype *IL-1RN* (VNTR) | Healthy controls (N = 145∗ (%)) | Early HIV disease stage (N = 19 (%) OR (P)) | Intermediate HIV disease stage (N = 31 (%) OR (P)) | Advanced HIV disease stage (N = 71 (%) OR (P)) |
|--------------------------|----------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| 1/1                      | 68 (46.9)                        | 8 (42.1) Ref. (1.20 (0.74))                | 16 (51.6) (0.72 (0.46))                    | 36 (50.7) (0.42 (0.19)) |
| 1/2                      | 61 (42.1)                        | 9 (47.4) (0.97 (0.97))                    | 11 (35.5) (0.99 (0.99))                    | 31 (43.7) (0.85 (0.65)) |
| 2/2                      | 16 (11.0)                        | 2 (10.5) (0.97 (0.97))                    | 4 (12.9) (0.99 (0.99))                     | 4 (5.6) (0.42 (0.19)) |

**Table 5**: Frequency distribution of haplotype *IL-1RN* (VNTR) and *IL-1β* (-511C/T) in HIV-infected individuals with hepatotoxicity, without hepatotoxicity, and healthy controls.

| Haplotype | HIV-infected individuals with hepatotoxicity (N = 66∗) (% P value OR (95% CI)) | HIV-infected individuals without hepatotoxicity (N = 244∗) (% P value OR (95% CI)) | P value OR (95% CI) |
|-----------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------|
| 1C        | 19 (0.28)                                                                        | 81 (0.34)                                                                        | 0.56 1 (reference)  |
| 1T        | 29 (0.4)                                                                         | 90 (0.35)                                                                        | 0.25 1.41 (0.79–2.36) |
| 2C        | 6 (0.09)                                                                         | 14 (0.05)                                                                        | 0.31 1.67 (0.61–4.53) |
| 2T        | 10 (0.15)                                                                        | 57 (0.22)                                                                        | 0.17 0.60 (0.29–1.25) |
| 3C        | 2 (0.03)                                                                          | 2 (0.06)                                                                         | 0.15 3.85 (0.53–27.83) |

| Haplotype | Healthy controls (N = 292∗) (% P value OR (95% CI)) | P value OR (95% CI) |
|-----------|------------------------------------------------------|--------------------|
| 1C        | 19 (0.28)                                                                 | 0.95 1 (reference)  |
| 1T        | 29 (0.43)                                                                 | 0.34 1.29 (0.75–2.20) |
| 2C        | 6 (0.09)                                                                 | 0.32 1.63 (0.61–4.31) |
| 2T        | 10 (0.15)                                                                 | 0.07 0.51 (0.25–1.02) |
| 3C        | 2 (0.03)                                                                 | 0.48 1.82 (0.34–9.5) |

**Table 4**: Frequency distribution of *IL-1RN* (VNTR) and *IL-1β* (-511C/T) genotypes in different HIV disease stages of HIV-infected individuals.

**Table 5**: Frequency distribution of haplotype *IL-1RN* (VNTR) and *IL-1β* (-511C/T) in HIV-infected individuals with hepatotoxicity, without hepatotoxicity, and healthy controls.

**IL-1RN** 2/2 genotype was less prevalent in patients with advanced HIV disease stage compared to healthy controls (5.6% versus 11.0%, OR = 0.42, 95% CI: 0.11–1.52, P = 0.19), while the frequency of *IL-1β* -511CT genotype was observed higher in patients with early HIV disease stage compared to healthy controls (57.9% versus 40.8%, OR = 2.66, 95% CI: 0.50–14.21, P = 0.25; 47.4% versus 40.8%, OR = 1.46, 95% CI: 0.56–3.81, P = 0.44).

**3.4. Gene-Gene Interaction.** In the gene-gene interaction analysis, the haplotype frequencies of *IL-1RN* (VNTR) and *IL-1β* (-511C/T) polymorphisms in HIV-infected individuals with hepatotoxicity, without hepatotoxicity, and healthy controls are shown in Table 5. The linkage disequilibrium (LD) values and comparison of LD values (Dij) between HIV-infected individuals with hepatotoxicity versus without hepatotoxicity, HIV-infected individuals with hepatotoxicity versus healthy controls, and HIV-infected individuals without hepatotoxicity versus healthy controls are provided.

*N* = number of subject participants; * Individuals with rare genotypes (<5%) were excluded in the analysis; OR: odds ratio; CI: confidence interval. Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (1/1 genotype for *IL-1RN*) (CC genotype for *IL-1β*) with other genotypes.
Table 6: Frequency distribution of IL-1RN (VNTR) and IL-1β (-511C/T) genotypes in tobacco and alcohol using HIV-infected individuals with and without hepatotoxicity.

### HIV-infected individuals with hepatotoxicity

| Genotypes     | Tobacco users | Tobacco nonusers | P value | OR (95% CI) |
|---------------|---------------|------------------|---------|-------------|
| **IL-1RN (VNTR)** |
| 1/1           | 6 (85.7%)     | 13 (48.1%)       | —       | 1 (reference) |
| 1/2           | 1 (14.3%)     | 9 (33.3%)        | 0.40    | 0.24 (0.01–2.79) |
| **IL-1β (-511C/T)** |
| CC            | 2 (28.6%)     | 5 (18.5%)        | —       | 1 (reference) |
| CT            | 2 (28.6%)     | 12 (44.4%)       | 0.84    | 0.42 (0.03–5.93) |
| TT            | 3 (42.9%)     | 10 (37.0%)       | 0.78    | 0.75 (0.06–9.47) |

### HIV-infected individuals without hepatotoxicity

| Genotypes     | Tobacco users | Tobacco nonusers | P value | OR (95% CI) |
|---------------|---------------|------------------|---------|-------------|
| **IL-1RN (VNTR)** |
| 1/1           | 18 (42.9%)    | 42 (48.8%)       | —       | 1 (reference) |
| 1/2           | 17 (40.5%)    | 34 (39.5%)       | 0.86    | 1.17 (0.48–2.81) |
| 2/2           | 3 (7.1%)      | 7 (8.1%)         | 0.70    | 1.00 (0.18–5.06) |
| 1/4           | 2 (4.8%)      | 1 (1.2%)         | 0.48    | 4.67 (0.30–139.60) |
| **IL-1β (-511C/T)** |
| CC            | 4 (9.5%)      | 17 (19.8%)       | —       | 1 (reference) |
| CT            | 16 (38.1%)    | 44 (51.2%)       | 0.68    | 1.55 (0.40–6.40) |
| TT            | 22 (52.4%)    | 25 (29.1%)       | **0.05**| **3.74 (0.97–15.57)** |

### HIV-infected individuals with hepatotoxicity

| Genotypes     | Alcohol users | Alcohol nonusers | P value | OR (95% CI) |
|---------------|---------------|------------------|---------|-------------|
| **IL-1RN (VNTR)** |
| 1/1           | 6 (85.7%)     | 13 (48.1%)       | —       | 1 (reference) |
| 1/2           | 1 (14.3%)     | 9 (33.3%)        | 0.40    | 0.24 (0.01–2.79) |
| **IL-1β (-511C/T)** |
| CC            | 1 (14.3%)     | 6 (22.2%)        | —       | 1 (reference) |
| CT            | 3 (42.9%)     | 11 (40.7%)       | 0.84    | 1.64 (0.10–51.13) |
| TT            | 3 (42.9%)     | 10 (37.0%)       | 0.90    | 1.80 (0.11–56.75) |

### HIV-infected individuals without hepatotoxicity

| Genotypes     | Alcohol users | Alcohol nonusers | P value | OR (95% CI) |
|---------------|---------------|------------------|---------|-------------|
| **IL-1RN (VNTR)** |
| 1/1           | 21 (48.8%)    | 39 (45.9%)       | —       | 1 (reference) |
| 1/2           | 18 (41.9%)    | 33 (38.8%)       | 0.86    | 1.01 (0.43–2.38) |
| 2/2           | 1 (2.3%)      | 9 (10.6%)        | 0.22    | 0.21 (0.01–1.81) |
| 1/3           | 1 (2.3%)      | 1 (1.2%)         | 0.75    | 1.86 (0.0–72.41) |
| 1/4           | 1 (2.3%)      | 1 (1.2%)         | 0.75    | 1.86 (0.0–72.41) |
| 2/3           | 1 (2.3%)      | 2 (2.4%)         | 0.57    | 0.93 (0.0–14.35) |
| **IL-1β (-511C/T)** |
| CC            | 4 (9.3%)      | 17 (20.0%)       | —       | 1 (reference) |
| CT            | 21 (48.8%)    | 39 (45.9%)       | 0.27    | 2.29 (0.61–9.28) |
| TT            | 18 (41.9%)    | 29 (34.1%)       | 0.19    | 2.64 (0.68–11.06) |

N: number of subject participants; OR: odds ratio; CI: confidence interval. Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (1/1 genotype for IL-1RN) (CC genotype for IL-1β) with other genotypes. Significant P values (<0.05) and related OR and 95% CI are shown in bold.
versus healthy controls, and HIV-infected individuals versus healthy controls have no significant difference in both the genes (0.32, 0.32, and 0.39). It was expected that there might be the additive or synergistic effect of these variations with the development of ARV-associated hepatotoxicity and its severity.

Haplotype 1C (IL-1RN *1/IL-1β *C) was taken as reference. Haplotype 2T (IL-1RN *2/IL-1β *T) was underrepresented in cases of severe hepatotoxicity compared with HIV-infected individuals (15% versus 22%, OR = 0.60, 95% CI: 0.29–1.25, P = 0.17). The haplotypes 1T and 2C (IL-1RN *1/IL-1β *T and IL-1RN *2/IL-1β *C) showed risk for the cases of severe hepatotoxicity (43% versus 35%, OR = 1.41, 95% CI: 0.79–2.36, P = 0.25 and 9% versus 5%, OR = 1.67, 95% CI: 0.61–4.53, P = 0.31, resp.).

The frequency of haplotypes 2T (IL-1RN *2/IL-1β *T) differed between HIV-infected individuals with hepatotoxicity and healthy controls with borderline significance, and the risk was reduced for the cases of severe hepatotoxicity (15% versus 25%, OR = 0.51, 95% CI: 0.25–1.02, P = 0.07).

The haplotypes frequency of IL-1RN/IL-1β polymorphism did not differ between HIV-infected individuals and healthy controls.

### 3.5. IL-1RN (VNTR) and IL-1β (-511C/T) Polymorphism and Environmental Factors

In the gene-environment interaction analysis, the effect of tobacco and alcohol usage in the HIV-infected individuals was analyzed with respect to IL-1RN (VNTR) and IL-1β (-511C/T) polymorphism. The frequency of IL-1β carriage -511TT genotype did not differ between tobacco using cases of severe hepatotoxicity and nonusers (42.9% versus 37.0%, OR = 0.75, 95% CI: 0.68–0.83, P = 0.78). However, the frequency of IL-1β carriage -511TT genotype was significantly higher in tobacco using HIV-infected individuals compared with nonusers (52.4% versus 29.1%, OR = 3.74, 95% CI: 0.97–15.57, P = 0.05).

**IL-1β-511C/T** and -511TT genotypes among alcohol users increased the risk of severe hepatotoxicity (42.9% versus 40.7%, OR = 1.64, 95% CI: 0.10–51.13, P = 0.84 and 42.9% versus 37.0%, OR = 1.80, 95% CI: 0.11–56.75, P = 0.90, resp.). Similarly, IL-1β-511CT and -511TT genotypes and alcohol usage showed increased risk to develop ARV-associated hepatotoxicity (48.8% versus 45.9%, OR = 2.29, 95% CI: 0.61–9.28, P = 0.27 and 41.9% versus 34.1%, OR = 2.64, 95% CI: 0.68–11.06, P = 0.19, resp.) (Table 6).

| Table 7: Frequency distribution of IL-1RN (VNTR) and IL-1β (-511C/T) genotypes in nevirapine and efavirenz using HIV-infected individuals with and without hepatotoxicity. |  |
|---|---|
| **Nevirapine usage and hepatotoxicity** |  |
| IL-1RN (VNTR) Genotypes | Nevirapine users in HIV-infected patients with hepatotoxicity N = 23 (%) | Nevirapine users in HIV-infected patients without hepatotoxicity N = 116 (%) | P value | OR (95% CI) |
| 1/1 | 11 (47.8%) | 53 (45.7%) | — | 1 (reference) |
| 1/2 | 7 (30.4%) | 48 (41.4%) | 0.48 | 0.69 (0.23–1.94) |
| 2/2 | 3 (13.0%) | 10 (8.6%) | 0.64 | 1.42 (0.33–6.20) |
| 1/3 | 2 (8.7%) | 1 (0.86%) | 0.09 | 8.79 (0.71–109.16) |
| **IL-1β (-511C/T) Genotypes** | Nevirapine users in HIV-infected patients with hepatotoxicity N = 23 (%) | Nevirapine users in HIV-infected patients without hepatotoxicity N = 116 (%) | P value | OR (95% CI) |
| CC | 4 (17.4%) | 18 (15.5%) | — | 1 (reference) |
| CT | 9 (39.1%) | 58 (50.0%) | 0.27 | 0.57 (0.21–1.56) |
| TT | 10 (43.5%) | 40 (34.48%) | 0.87 | 0.90 (0.24–3.33) |
| **Efavirenz usage and hepatotoxicity** |  |
| IL-1RN (VNTR) Genotypes | Efavirenz users in HIV-infected patients with hepatotoxicity N = 11 (%) | Efavirenz users in HIV-infected patients without hepatotoxicity N = 12 (%) | P value | OR (95% CI) |
| 1/1 | 8 (72.7%) | 7 (58.3%) | — | 1 (reference) |
| 1/2 | 3 (27.3%) | 3 (25.0%) | 0.78 | 0.75 (0.10–5.56) |
| **IL-1β (-511C/T) Genotypes** | Efavirenz users in HIV-infected patients with hepatotoxicity N = 11 (%) | Efavirenz users in HIV-infected patients without hepatotoxicity N = 12 (%) | P value | OR (95% CI) |
| CC | 3 (27.3%) | 3 (25.0%) | 1 | Reference |
| CT | 5 (45.5%) | 2 (16.7%) | 0.20 | 4.29 (0.47–39.40) |
| TT | 3 (27.3%) | 7 (58.3%) | 0.56 | 1.95 (0.20–19.29) |

N: number of subject participants; OR: odds ratio; CI: confidence interval. Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (1/1 genotype for IL-1RN) (CC genotype for IL-1β) with other genotypes.
regimen and a combination of the regimen with alcohol usage in the risk to develop ARV-associated hepatotoxicity and its severity.

*IL-1RN* 2/2 and 1/3 showed an elevated risk in cases of severe hepatotoxicity compared to without hepatotoxicity among nevirapine users (13.0% versus 8.6%, OR = 1.42, 95% CI: 0.33–6.20, 95% CI: 0.41–4.98, resp.) (Table 9). Similarly, *IL-1β* 2/2 and 1/3 genotypes were overrepresented among both nevirapine and alcohol using HIV-infected individuals compared with efavirenz users and alcohol nonusers (54.05% versus 49.37%, OR = 2.56, 95% CI: 0.59–12.68 and 37.84% versus 31.65%, OR = 2.84, 95% CI: 0.60–14.71, P = 0.24, resp.). The frequency of *IL-RN* 1/2 and *IL-1β*-511TT genotypes were higher in combined efavirenz and alcohol using HIV-infected individuals compared with efavirenz users and alcohol nonusers (33.33% versus 20.00, OR = 2.67, 95% CI: 0.09–129.92, P = 1.00 and 66.67% versus 50.00, OR = 4.00, 95% CI: 0.16–174.72, P = 0.68, resp.) (Table 9).

### 4. Discussion

This is the first study that simultaneously examines the impact of genetic polymorphisms in the two loci of IL-1 cluster to develop ARV-associated hepatotoxicity and its severity. The IL-1RA is an immunologic regulator that competes with other IL-1 family members (IL-1α and IL-1β) for the IL-1 receptor in the target cells and acting as its negative regulator with an anti-inflammatory effect [18, 43, 44]. In response to inflammation, proinflammatory cytokines (IL-1β and TNF-α) are produced to control infections [45, 46]. The proinflammatory response is downregulated by cytokine transforming growth factor-β (TGF-β, IL-10) and specifically in the case of IL-1, IL-1RA to terminate the immune response and limit the potential for immunopathology [18]. An interindividual variation in the IL-1 family of genes seen

---

**Table 8: Frequency distribution of *IL-1RN* (VNTR) and *IL-1β* (-511C/T) genotypes in NNRTI regimen using HIV-infected individuals with and without hepatotoxicity.**

| Genotypes | Nevirapine users N = 23 (%) | Efavirenz users N = 11 (%) | P value | OR (95% CI) |
|-----------|-----------------------------|-----------------------------|---------|-------------|
| **IL-1RN** (VNTR) | | | | |
| 1/1 | 11 (47.8%) | 8 (72.7%) | — | 1 (reference) |
| 1/2 | 7 (30.4%) | 3 (27.3%) | 0.81 | 1.70 (0.26–11.84) |
| **IL-1β** (-511C/T) | | | | |
| CC | 4 (17.4%) | 3 (27.3%) | — | 1 (reference) |
| CT | 9 (39.1%) | 5 (45.5%) | 0.87 | 1.35 (0.15–12.60) |
| TT | 10 (43.5%) | 3 (27.3%) | 0.68 | 2.50 (0.24–28.86) |

**HIV-infected individuals without hepatotoxicity**

| Genotypes | Nevirapine users N = 116 (%) | Efavirenz users N = 12 (%) | P value | OR (95% CI) |
|-----------|-----------------------------|-----------------------------|---------|-------------|
| **IL-1RN** (VNTR) | | | | |
| 1/1 | 53 (45.7%) | 7 (58.3%) | — | 1 (reference) |
| 1/2 | 48 (41.4%) | 3 (25.0%) | 0.46 | 2.11 (0.45–11.02) |
| 1/3 | 1 (50.0%) | 1 (50.0%) | 0.60 | 0.13 (0.00–5.53) |
| **IL-1β** (-511C/T) | | | | |
| CC | 18 (15.5%) | 3 (25.0%) | — | 1 (reference) |
| CT | 58 (50.0%) | 2 (16.7%) | 0.20 | 4.83 (0.59–45.70) |
| TT | 40 (34.48%) | 7 (58.3%) | 0.76 | 0.95 (0.17–4.83) |

**N:** number of subject participants; OR: odds ratio; CI: confidence interval. Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (1/1 genotype for *IL-1RN*) (CC genotype for *IL-1β*) with other genotypes.
not only in the susceptibility to HIV infection but also in the progression of HIV-1 [31, 35, 36]. These genetic variations may also influence the gene expression.

In the present study, the frequency of IL-1RN (VNTR) and IL-1β (-511C/T) genotypes in healthy controls was comparable with a study carried out by Kesarswani and Mittal from North India and differed from other reports available from North India [37, 47–49]. In the present study, IL-1RN (VNTR) and IL-1β-511C/T polymorphisms were neither associated with severe hepatotoxicity and nor its genotypes distribution differed significantly between HIV-infected individuals and healthy controls. IL-1RN allele 2 has been related to increased production of IL-1RA and IL-1β and decreased the production of IL-1α [43]. There are no differences in the IL-1β pattern in HIV-1-infected compared to noninfected monocytes [50–52]. The distribution of IL-1RA genotypes found to be similar in the HIV-seropositive women and HIV-seronegative women [30, 53]. A low prevalence of IL-1RN allele 2 in a Black African people and an African-American population has also been previously reported [54, 55].

Multivariate loci contribute to the disease susceptibility rather than the polymorphism on single locus which indicates that haplotype study is more important to estimate the effect of polymorphisms on the gene expression [56]. Therefore, haplotype analysis accurately provides information about the interaction among the adjacent genes. In our study, haplotypes 1T and 2C showed a higher risk of severe hepatotoxicity (OR = 1.41, P = 0.25 and OR = 1.67, P = 0.31, resp.). It was known that haplotype risk was equal to the summation of individual risks of its constituent alleles.

In the current study, we also analyzed the distribution of IL-1RN and IL-1B polymorphisms in HIV disease stages to modulate the risk of HIV disease advancement. The presence of IL-1B-511CT genotype increased the risk of disease advancement in patients with early and advanced HIV

---

**Table 9: Frequency distribution of IL-1RN (VNTR) and IL-1β (-511C/T) genotypes in combined NNRTI regimen and alcohol using HIV-infected individuals with and without hepatotoxicity.**

| Genotypes | Nevirapine + alcohol users N = 5 (%) | Nevirapine users + alcohol nonusers N = 18 (%) | P value | OR (95% CI) |
|-----------|-------------------------------------|-----------------------------------------------|---------|-------------|
| 1/1       | 4 (80%)                             | 7 (38.9%)                                     |         | 1 (reference) |
| 1/2       | 1 (20%)                             | 6 (33.3%)                                     | 0.63    | 0.29 (0.01–4.58) |

| Genotypes | Nevirapine + alcohol users N = 5 (%) | Nevirapine users + alcohol nonusers N = 18 (%) | P value | OR (95% CI) |
|-----------|-------------------------------------|-----------------------------------------------|---------|-------------|
| CC        | 1 (20%)                             | 3 (16.66%)                                    |         | 1 (reference) |
| CT        | 1 (20%)                             | 8 (44.44%)                                    | 0.84    | 0.38 (0.01–20.05) |
| TT        | 3 (60%)                             | 7 (38.88%)                                    | 0.63    | 1.29 (0.05–47.87) |

**HIV-infected patients without hepatotoxicity**

| Genotypes | Nevirapine + alcohol users N = 37 (%) | Nevirapine users + alcohol nonusers N = 79 (%) | P value | OR (95% CI) |
|-----------|-------------------------------------|-----------------------------------------------|---------|-------------|
| 1/1       | 18 (51.43%)                         | 35 (45.45%)                                   |         | 1 (reference) |
| 1/2       | 16 (45.71%)                         | 32 (41.56%)                                   | 0.88    | 0.97 (0.39–2.41) |
| 2/2       | 1 (2.86%)                           | 9 (11.69%)                                    | 0.25    | 0.22 (0.01–1.93) |
| 1/4       | 1 (2.70%)                           | 2 (2.53%)                                     | 0.54    | 0.97 (0.09–15.26) |

| Genotypes | Nevirapine + alcohol users N = 37 (%) | Nevirapine users + alcohol nonusers N = 79 (%) | P value | OR (95% CI) |
|-----------|-------------------------------------|-----------------------------------------------|---------|-------------|
| CC        | 3 (8.11%)                           | 15 (18.99%)                                   |         | 1 (reference) |
| CT        | 20 (54.05%)                          | 39 (49.37%)                                   | 0.26    | 2.56 (0.59–12.68) |
| TT        | 14 (37.84%)                          | 25 (31.65%)                                   | 0.24    | 2.84 (0.60–14.71) |

| Genotypes | Efavirenz + alcohol users N = 6 (%) | Efavirenz users + alcohol nonusers N = 6 (%) | P value | OR (95% CI) |
|-----------|------------------------------------|-----------------------------------------------|---------|-------------|
| 1/1       | 3 (50.00%)                          | 4 (80.00%)                                    |         | 1 (reference) |
| 1/2       | 2 (33.33%)                          | 1 (20.00%)                                    | 1.00    | 2.67 (0.09–129.92) |

| Genotypes | Efavirenz + alcohol users N = 6 (%) | Efavirenz users + alcohol nonusers N = 6 (%) | P value | OR (95% CI) |
|-----------|------------------------------------|-----------------------------------------------|---------|-------------|
| CC        | 1 (16.67%)                          | 2 (33.33%)                                    |         | 1 (reference) |
| CT        | 1 (16.67%)                          | 1 (16.67%)                                    | 0.57    | 0.50 (0.00–62.81) |
| TT        | 4 (66.67%)                          | 3 (50.00%)                                    | 0.68    | 4.00 (0.16–174.72) |

N: number of subject participants; OR: odds ratio; CI: confidence interval. Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (1/1 genotype for IL-1RN) (CC genotype for IL-1β) with other genotypes/alleles.
disease stages compared to healthy controls (OR = 2.66, P = 0.25; OR = 1.46, P = 0.44).

Studies suggested that the gene-environment interaction determines the etiology of the disease [57, 58]. However, the selection criteria for case-control association studies with the environmental influences and controls must be matched with cases in the population. Otherwise, it can lead to false interactions [59]. In such cases, the case-only method may be used to study gene-environment interaction under the assumption of the independence between exposure and genotype in the population [60, 61]. Therefore, we adopted the case-only study design to describe the influence of the risk in the development of ARV-associated hepatotoxicity and its severity with tobacco, alcohol, and drug usage. A study reported that heavy alcohol consumption has a negative impact on the CD4 cell count in the HIV-infected people who are not having combinational antiretroviral therapy [62]. A study conducted in HIV-infected population investigated that a decreased response to ART in smoking HIV-infected women. In the present study, IL-1β carriage-511TT genotype among alcohol users increased the risk in cases of severe hepatotoxicity (OR = 1.80, P = 0.90). Similarly, IL-1β-511TT genotype among alcohol users increased the risk for the development ARV-associated hepatotoxicity in HIV-infected individuals (OR = 2.64, P = 0.19). It supports the idea that individuals with IL-1β-511TT genotype are more prone to alcohol-related hepatotoxicity.

The IL-1β carriage -511TT genotype among nevirapine users was likely to be associated with cases of severe hepatotoxicity (43.5% versus 27.3%, OR = 2.50, P = 0.68). Also, IL-1β-511CT genotype among nevirapine using HIV-infected individuals enhanced the risk for the development of ARV-associated hepatotoxicity (OR = 4.83, P = 0.20). IL-1β-511TT genotype among combined nevirapine and alcohol users increased the risk for the development of ARV-associated hepatotoxicity in HIV-infected individuals (OR = 2.84, P = 0.24). It suggested that individuals with IL-1β-511TT genotype are likely to be at risk of alcohol- and drug-induced hepatotoxicity.

5. Conclusion

IL-1β-511TT genotype may influence the development of ARV-associated hepatotoxicity and its severity in alcohol and nevirapine users independently and in combination. The present study suggested that IL-RN (VNTR) and IL-1β-511C/T polymorphisms should be explored simultaneously further in cases of severe hepatotoxicity in larger sample size with other population. Also, further studies of other inflammatory response related gene (TNF-α, IL-2, IL-10, and TGF-β) are required to understand the termination of immune response and limit the potential for immunopathology. IL-1β polymorphism may reflect the host immune status as well as the control over natural disease and may also affect the virological response to antiviral treatment. Genotyping of IL-1RN (VNTR) and IL-1β 511C/T polymorphism may be beneficial to predict the immune defense against infection and a prolonged Th1 cell-mediated immune response.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and comparable ethical standards.

Consent

Informed consent was obtained from all individual participants included in the study. (ICF version 1.0 dated 18 April 2011).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors’ Contributions

HariOm Singh was tasked with the overall supervision and writing of the manuscript. Dharmesh Amani performed the experiments and analysis of data. Vijay Nema was tasked with manuscript review. Manisha V. Ghate was tasked with the recruitment of subject participants. R. R. Gangakhedkar was tasked with proposal development, clinical support, and manuscript review.

Acknowledgments

The authors gratefully acknowledge the clinic in-charge Dr. Seema Sahay for the arrangement of community staff for the recruitment of healthy control; Mansa Angadi, Shradha Bapat, and Jyoti Pawar for filling the clinical research pro forma; Tuman Katendra, Jai Mane, and Ayesha for counseling of subject participants; and sisters Ujjawala Ghule and Sunita for the collection of blood sample. The study was supported by a research grant from NARI-Indian Council of Medical Research (ICMR) India.

References

[1] M. E. O’Brien, R. A. Clark, C. L. Besch, L. Myers, and P. Kissinger, “Patterns and correlates of discontinuation of the initial HAART regimen in an urban outpatient cohort,” Journal of Acquired Immune Deficiency Syndromes, vol. 34, no. 4, pp. 407–414, 2003.

[2] R. B. Van Dyke, L. Wang, P. L. Williams, and Pediatric AIDS Clinical Trials Group 219C Team, “Toxicities associated with dual nucleoside reverse-transcriptase inhibitor regimens in HIV-infected children,” The Journal of Infectious Diseases, vol. 198, no. 11, pp. 1599–1608, 2008.

[3] B. McGovern, “Hepatic safety and HAART,” Journal of the International Association of Physicians in AIDS Care, vol. 3, Supplement 2, pp. S24–S40, 2004.

[4] V. Racanelli and B. Rehermann, “The liver as an immunological organ,” Hepatology, vol. 43, no. S1, pp. S54–S62, 2006.

[5] N. Kaplowitz, “Biochemical and cellular mechanisms of toxic liver injury,” Seminars in Liver Disease, vol. 22, no. 2, pp. 137–144, 2002.
Mediators of Inflammation

[6] Y. Masubuchi, S. Sugiyama, and T. Horie, "Th1/Th2 cytokine balance as a determinant of acetaminophen-induced liver injury," Chemico-Biological Interactions, vol. 179, no. 2-3, pp. 273–279, 2009.

[7] R. L. Reisler, S. Servoss, J. Robbins, G. Theodore, D. Murphy, and R. Chung, "Incidence of hepatoencephalopathy and mortality in 21 adult antiretroviral treatment trials," in The 1st IAS Conference on HIV Pathogenesis and Treatment, Buenos Aires, Argentina, 2001.

[8] O. M. Minzi, H. Irunde, and C. Moshiro, "HIV patients presenting common adverse drug events caused by highly active antiretroviral therapy in Tanzania," Tanzania Journal of Health Research, vol. 11, no. 1, pp. 5–10, 2009.

[9] M. Nagpal, V. Tayal, S. Kumar, and U. Gupta, "Adverse drug reactions to antiretroviral therapy in AIDS patients at a tertiary care hospital in India: a prospective observational study," Indian Journal of Medical Sciences, vol. 64, no. 6, pp. 245–252, 2010.

[10] E. T. Morgan, "Regulation of cytochromes P450 during inflammation and infection," Drug Metabolism Reviews, vol. 29, no. 4, pp. 1129–1188, 2008.

[11] A. E. Atitken, T. A. Richardson, and E. T. Morgan, "Regulation of drug-metabolizing enzymes and transporters in inflammation," Annual Review of Pharmacology & Toxicology, vol. 46, no. 1, pp. 123–149, 2006.

[12] E. T. Morgan, "Regulation of cytochrome p450 by inflammatory mediators: why and how?", Drug Metabolism & Disposition, vol. 29, no. 3, pp. 207–212, 2001.

[13] D. Golettì, A. L. Kinter, E. C. Hardy, G. Poli, and A. S. Fauci, "Modulation of endogenous IL-1 beta and IL-1 receptor antagonist results in opposing effects on HIV expression in chronically infected monocytes," Journal of Immunology, vol. 156, no. 9, pp. 3501–3508, 1996.

[14] E. V. Granowitz, B. M. Saget, M. Z. Wang, C. A. Dinarello, and P. R. Skolnick, "Interleukin 1 induces HIV-1 expression in chronically infected U1 cells: blockade by interleukin 1 receptor antagonist and tumor necrosis factor binding protein type 1," Molecular Medicine, vol. 1, no. 6, pp. 667–677, 1995.

[15] G. Poli, A. L. Kinter, and A. S. Fauci, "Interleukin 1 induces expression of the human immunodeficiency virus alone and in synergy with interleukin 6 in chronically infected U1 cells: inhibition of inductive effects by the interleukin 1 receptor antagonist," Proceedings of the National Academy of Sciences of the United States of America, vol. 91, no. 1, pp. 108–112, 1994.

[16] H. M. Sadeghi, L. Weiss, M. D. Kazatchkine, and N. Haefnner-Cavaillon, "Antiretroviral therapy suppresses the constitutive production of interleukin-1 associated with human immunodeficiency virus infection," The Journal of Infectious Diseases, vol. 172, no. 2, pp. 547–550, 1995.

[17] D. E. Smith, B. R. Renshaw, R. R. Ketchum, M. Kubin, K. E. Garka, and J. E. Sims, "Four new members expand the interleukin-1 superfamily," The Journal of Biological Chemistry, vol. 275, no. 2, pp. 1169–1175, 2000.

[18] W. P. Arend, M. Malysak, C. J. Guthridge, and C. Gabay, "Interleukin-1 receptor antagonist: role in biology," Annual Review of Immunology, vol. 16, no. 1, pp. 27–55, 1998.

[19] C. A. Dinarello, "Biologic basis for interleukin-1 deficiency," Blood, vol. 87, no. 6, pp. 2095–2147, 1996.

[20] S. P. Eisenberg, M. T. Brewer, E. Verderber, P. Heimdal, B. J. Brandhuber, and R. C. Thompson, "Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: evolution of a cytokine control mechanism," Proceedings of the National Academy of Sciences of the United States of America, vol. 88, no. 12, pp. 5232–5236, 1991.

[21] N. Iizuka, S. Hazama, K. Hirose et al., "Interleukin-1 receptor antagonist mRNA expression and the progression of gastric carcinoma," Cancer Letters, vol. 142, no. 2, pp. 179–184, 1999.

[22] M. J. H. Nicklin, A. Weith, and G. W. Duff, "A physical map of the region encompassing the human interleukin-1α, interleukin-1β, and interleukin-1 receptor antagonist genes," Genomics, vol. 19, no. 2, pp. 382–384, 1994.

[23] R. J. Wilkinson, P. Patel, M. Llewellyn et al., "Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1β gene on tuberculosis," The Journal of Experimental Medicine, vol. 189, no. 12, pp. 1863–1874, 1999.

[24] C. A. Dinarello, "Interleukin-1β and the autoinflammatory diseases," The New England Journal of Medicine, vol. 360, no. 23, pp. 2467–2470, 2009.

[25] C. A. Dinarello, "Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist," International Reviews of Immunology, vol. 16, no. 5-6, pp. 457–499, 2009.

[26] J. K. Tarlow, A. I. Blakemore, A. Lennard et al., "Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat," Human Genetics, vol. 91, no. 4, pp. 403–404, 1993.

[27] J. Xue, L. Mou, X. Lu, and H. Wu, "No association of interleukin-1 receptor antagonist VNTR polymorphism and rheumatoid arthritis susceptibility: a meta analysis," Clinical & Experimental Rheumatology, vol. 28, no. 5, pp. 654–660, 2010.

[28] K. W. McIntyre, G. J. Stepan, K. D. Kolinsky et al., "Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody," The Journal of Experimental Medicine, vol. 173, no. 4, pp. 931–939, 1991.

[29] E. Fischer, K. van Zee, M. A. Marano et al., "Interleukin-1 receptor antagonist circulates in experimental inflammation and in human disease," Blood, vol. 79, no. 9, pp. 2196–2200, 1992.

[30] S. S. Witkin, I. M. Linhares, S. Gerber, M. E. Caetano, and A. C. Segurado, "Interleukin-1 receptor antagonist gene polymorphism and circulating levels of human immunodeficiency virus type 1 RNA in Brazilian women," Journal of Virology, vol. 75, no. 13, pp. 6242–6244, 2001.

[31] E. D. Carrol, A. Payton, D. Payne et al., "The IL1RN promoter rs4251961 correlates with IL-1 receptor antagonist concentrations in human infection and is differentially regulated by GATA-1," Journal of Immunology, vol. 186, no. 4, pp. 2329–2335, 2011.

[32] S. Hartland, J. L. Newton, S. M. Griffin, and P. T. Donaldson, "A functional polymorphism in the interleukin-1 receptor-1 gene is associated with increased risk of Helicobacter pylori infection but not with gastric cancer," Digestive Diseases & Sciences, vol. 49, no. 9, pp. 1545–1550, 2004.

[33] P. Pociot, J. Molvig, L. Wogensen, H. Worsaae, and J. Nerup, "Four new members expand the interleukin-1 superfamily," The Journal of Biological Chemistry, vol. 275, no. 2, pp. 1169–1175, 2000.

[34] F. di Giovine, E. Takhsh, A. I. F. Blakemore, and G. W. Duff, "Single base polymorphism at +511 in the human interleukin-
1β gene (IL1β),” Human Molecular Genetics, vol. 1, no. 6, p. 450, 1992.

[35] V. Asensi, C. Rego, A. H. Montes et al., “IL-1β (+3954C/T) polymorphism could protect human immunodeficiency virus (HIV)-infected patients on highly active antiretroviral treatment (HAART) against lipodystrophic syndrome,” Genetics in Medicine, vol. 10, no. 3, pp. 215–223, 2008.

[36] L. A. Pemberton, E. Stone, P. Price, F. van Bockxmeer, and B. J. Brew, "The relationship between ApoE, TNFA, IL1a, IL1b and IL12b genes and HIV-1-associated dementia," HIV Medicine, vol. 9, no. 8, pp. 677–680, 2008.

[37] H. Singh, R. Sachan, H. Goel, and B. Mittal, "Genetic variants of interleukin-1RN and interleukin-1β genes and risk of cervical cancer," BJOG: An International Journal of Obstetrics & Gynaecology, vol. 115, no. 5, pp. 633–638, 2008.

[38] R. Rad, C. Prinz, B. Neu et al., "Synergistic effect of Helicobacter pylori virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa," The Journal of Infectious Diseases, vol. 188, no. 2, pp. 272–281, 2003.

[39] Y. Y. Shi and L. He, “SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci,” Cell Research, vol. 15, no. 2, pp. 97–98, 2005.

[40] Z. Li, Z. Zhang, Z. He et al., “A partition-igation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn),” Cell Research, vol. 19, no. 4, pp. 519–523, 2009.

[41] J. Shen, Z. Li, J. Chen, Z. Song, Z. Zhou, and Y. Shi, “SHEsisPlus, a toolkit for genetic studies on polyploid species,” Scientific Reports, vol. 6, no. 1, article 24095, 2016.

[42] A. Cox, N. J. Camp, M. J. H. Nicklin, F. S. di Giovine, and G. W. Duff, “An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers,” American Journal of Human Genetics, vol. 62, no. 5, pp. 1180–1188, 1998.

[43] M. Hurme and S. Santtila, "IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1β genes," European Journal of Immunology, vol. 28, no. 8, pp. 2598–2602, 1998.

[44] E. V. Granowitz, B. D. Clark, J. Mancilla, and C. A. Dinarello, "Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor," The Journal of Biological Chemistry, vol. 266, no. 22, pp. 14147–14150, 1991.

[45] V. Kindler, A. P. Sappino, G. E. Grau, P. F. Piguet, and P. Vassalli, "The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection," Cell, vol. 56, no. 5, pp. 731–740, 1989.

[46] K. Sugisaki, A. M. Dannenberg Jr, Y. Abe et al., “Nonspecific and immune-specific up-regulation of cytokines in rabbit dermal tuberculous (BCG) lesions,” Journal of Leukocyte Biology, vol. 63, no. 4, pp. 440–450, 1998.

[47] N. Moorchung, A. N. Srivastava, N. K. Gupta, U. C. Ghoshal, B. R. Achyut, and B. Mittal, "Cytokine gene polymorphisms and the pathology of chronic gastritis," Singapore Medical Journal, vol. 48, no. 5, pp. 447–454, 2007.

[48] P. Kesarwani and R. D. Mittal, "Association of pro/anti-inflammatory cytokine gene polymorphisms with benign prostate hyperplasia risk," Indian Journal of Clinical Biochemistry, vol. 25, no. 4, pp. 342–348, 2010.

[49] B. R. Achyut, A. Srivastava, S. Bhattacharya, and B. Mittal, “Genetic association of interleukin-1β (−511C/T) and interleukin-1 receptor antagonist (86 bp repeat) polymorphisms with type 2 diabetes mellitus in North Indians,” Clinica Chimica Acta, vol. 377, no. 1–2, pp. 163–169, 2007.

[50] W. Glienke, R. Esser, H. von Briesen et al., “Cytokine expression of HIV-infected monocytes/macrophages at the single-cell level,” Research in Virology, vol. 145, no. 3–4, pp. 193–197, 1994.

[51] M. S. Meltzer, L. Baca, J. A. Turpin et al., “Regulation of cytokine and viral gene expression in monocytes infected with the human immunodeficiency virus,” Pathobiology, vol. 59, no. 4, pp. 209–213, 1991.

[52] H. E. Gendelman, R. M. Friedman, S. Joe et al., “A selective defect of interferon alpha production in human immunodeficiency virus-infected monocytes,” The Journal of Experimental Medicine, vol. 172, no. 5, pp. 1433–1442, 1990.

[53] J. Jeremias, P. Giraldo, S. Durrant, A. Ribeiro-Filho, and S. S. Witkin, “Relationship between Ureaplasma urealyticum vaginal colonization and polymorphism in the interleukin-1 receptor antagonist gene,” The Journal of Infectious Diseases, vol. 180, no. 3, pp. 912–914, 1999.

[54] R. Bellamy, D. Kwiatkowski, and A. V. S. Hill, “Absence of an association between intercellular adhesion molecule 1, complement receptor 1 and interleukin 1 receptor antagonist gene polymorphisms and severe malaria in a West African population,” Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 92, no. 3, pp. 312–316, 1998.

[55] L. G. Rider, C. M. Artlett, C. B. Foster et al., “Polymorphisms in the IL-1 receptor antagonist gene VNTR are possible risk factors for juvenile idiopathic inflammatory myopathies,” Clinical & Experimental Immunology, vol. 121, no. 1, pp. 47–52, 2000.

[56] L. J. Palmer and L. R. Cardon, “Shaking the tree: mapping complex disease genes with linkage disequilibrium,” Lancet, vol. 366, no. 9492, pp. 1223–1234, 2005.

[57] Y. Deng, B. Newman, M. P. Dunne, P. A. Silburn, and G. D. Mellick, “Case-only study of interactions between genetic polymorphisms of GSTM1, PI, T1 and Z1 and smoking in Parkinson’s disease,” Neuroscience Letters, vol. 366, no. 3, pp. 326–331, 2004.

[58] D. J. Hunter, S. E. Hankinson, H. Hough et al., “A prospective study of NAT2 acetylation genotype, cigarette smoking, and risk of breast cancer,” Carcinogenesis, vol. 18, no. 11, pp. 2127–2132, 1997.

[59] S. Greenland, “The effect of misclassification in the presence of covariates,” American Journal of Epidemiology, vol. 112, no. 4, pp. 564–569, 1980.

[60] W. W. Piegorsch, C. R. Weinberg, and J. A. Taylor, “Non-hierarchical logistic models and case-only designs for assessing susceptibility in population-based case-control studies,” Statistics in Medicine, vol. 13, no. 2, pp. 153–162, 1994.

[61] Q. Yang, M. J. Khoury, F. Sun, and W. D. Flanders, “A selective defect of interferon alpha production in human immunodeficiency virus-infected monocytes,” The Journal of Experimental Medicine, vol. 172, no. 5, pp. 1433–1442, 1990.

[62] N. Moorchung, A. N. Srivastava, N. K. Gupta, U. C. Ghoshal, B. R. Achyut, and B. Mittal, "Cytokine gene polymorphisms and the pathology of chronic gastritis," Singapore Medical Journal, vol. 48, no. 5, pp. 447–454, 2007.

[63] P. Kesarwani and R. D. Mittal, "Association of pro/anti-inflammatory cytokine gene polymorphisms with benign prostate hyperplasia risk," Indian Journal of Clinical Biochemistry, vol. 25, no. 4, pp. 342–348, 2010.