MDR1 Polymorphisms and Modulation of ARV Drug Induced Hepatotoxicity

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Abstract

Background

Antiretroviral (ARV) regimen is used to manage the progression of HIV infection. *MDR1* Polymorphism is associated with the treatment outcome. Ethnic disparities have been observed in the distribution of *MDR1* genotypes. Hence we analyzed the association of *MDR1* polymorphism with the modulation of ARV associated hepatotoxicity. *MDR1* polymorphisms (1236 C/T, 3435 C/T) was genotyped in a total 165 HIV-infected individuals (34 ARV drug induced hepatotoxicity were labeled as cases, 131 those without hepatotoxicity were controls) and 155 healthy individuals by utilization of PCR-RFLP.

Results

Individuals with haplotype TC of *MDR1* were at greater risk for hepatotoxicity severity (OR=1.96, P=0.06). Haplotypes TT and CC were associated with a reduced risk of hepatotoxicity severity (OR= 0.16, P=0.006; OR= 0.46, P=0.06). *MDR1* 1236TT genotype was seen higher among ARV drug induced hepatotoxicity cases who consumed alcohol than non-users (28.6% versus 14.8%, OR=1.50). There was an increased prevalence of *MDR1* 1236TT genotype in ARV drug induced hepatotoxicity cases on nevirapine in contrast with efavirenz (21.7% versus 9.1%, OR=2.11). The incidence of *MDR1* 1236CT, 1236TT genotypes was found to be more in individuals those without hepatotoxicity on nevirapine as compared to efavirenz (43.7% versus 33.3%, OR=1.66; 12.6% versus 8.3%, OR=1.96). *MDR1* 1236TT genotype in presence of nevirapine and who consumed alcohol showed risk for severity of hepatotoxicity (40.0% versus 16.67%, OR=2.21).

Conclusion

Haplotype TC was associated with hepatotoxicity severity. *MDR1* 1236TT and 3435CT genotypes in presence of nevirapine and who consume alcohol showed higher risk for acquisition and severity of ARV associated hepatotoxicity.

1.0 Background

Antiretroviral (ARV) therapy is being extensively utilized for the management of HIV patients. The death rate among individuals with HIV is constantly increasing. Long-term efficacy and toxicity are the significant issues associated with ARV regimen, to worry about. Hepatotoxicity is an adverse outcome of ARV drugs in HIV-infected individuals [1, 2]. A higher occurrence of hepatotoxicity was seen with the utilization of nevirapine-based regimen than the efavirenz-based regimen [3]. The occurrence of ARV drug induced hepatotoxicity because of nevirapine was shown to be 3.19% [4]. In another investigation, the occurrence of grade 3 or 4 hepatotoxicity was 10.8% in the patients treated with efavirenz and 8.9% in

patients treated with nevirapine (5). *ABCB1* is one of the universal adenosine triphosphate (ATP)-binding cassette (ABC) genes, responsible for cell homeostasis [6–8].

ABCB1 is situated on chromosome 7q21. It is a part of the MDR subfamily [8]. *ABCB1* is expressed in a few tissues including epithelial cells of the blood-brain barrier [9, 10] and transports numerous drugs [11]. P-glycoprotein (P-gp), a transmembrane transporter protein, is encoded by the *ABCB1/MDR1* gene. P-gp is an ATP dependent efflux system that transports substances including drugs from intracellular to the extracellular matrix [12–14]. The variation in P-gp expression may vary its function, thus can affect the transport of drugs including nevirapine (NVP) [15]. The absorption and penetration of efavirenz (EFV) and NVP have evidently shown to be influenced by the P-gp expression [16]. Chelule et al., (2003) have reported the prevalence of *MDR1* 3435CC genotype to be 85.9% in Africans, 41.70% in Indians, and 35.7% in the whites of Kwazulu-Natal and South Africa, respectively. In the African population, the presence of the *MDR1* 3435CC genotype was found to be associated with an overexpression of P-glycoprotein, whereas, the patients with TT genotype demonstrated a lower expression of P-gp [17, 18]. Studies have reported significantly increased CD4 cell counts among HIV patients with *MDR1* 3435TT genotype [19, 20]. Haas et al., (2006) proposed that *MDR1* 3435C/T polymorphism was not linked to efavirenz exposure [19]. Salem et al., (2014) suggested that *MDR1* 3435C/T polymorphism had no impact on efavirenz clearance [21]. Zhu et al., (2013) proposed that polymorphisms of *MDR1* 3435T/C and 2677T/G were linked to the response of nevirapine treatment ($P=0.031$, $P=0.001$) and could help to predict the drug response in HIV patients [22]. *MDR1* 3435TT genotype among individuals treated with EFV or nelfinavir correlated with a more elevated level of CD4+ count than the CT/CC genotypes [23]. Leschziner et al., (2007) showed that *MDR1* 3435 TT genotype were linked with higher adverse outcomes of 3TC (Lamivudine) and NVP treatment than EFV treatment [24]. Ritchie et al. (2006) indicated a significantly reduced risk of hepatotoxicity with NNRTI-containing regimens in the presence of *MDR1* 3435C/T polymorphism [25]. The link between polymorphism of *MDR1* 3435C/T and nevirapine induced hepatotoxicity has also been documented by studies [25, 26].

Few studies have shown an association between *MDR1* polymorphism and adverse outcomes of ARV drugs whereas, other studies suggest no correlation. The results were not conclusive. Moreover, the association between *MDR1* polymorphism and ARV drug induced hepatotoxicity has not been reported from India. Thus, we have analyzed the association between *MDR1* (1236C/T and 3435C/T) polymorphisms and modulation of ARV drug induced hepatotoxicity.

2.0 Methods

2.1 Study design and population

The study was carried out at the National AIDS Research Institute (NARI), Pune. The institute harbors an ARV therapy clinic, approved in 2005, by the National AIDS Control Organization (NACO) and provides free HIV testing and antiretroviral drugs under full NACO ARV therapy roll out programme. This works in collaboration with the Pune Municipal Corporation (PMC). Thus the patients are from the PMC area. A

case control study was conducted. The study population consisted of a cohort of patients who tested HIV positive and were on ARV therapy from August 2014 to September 2017, in the NARI clinic. Patients start ARV therapy based on the WHO criteria for the ARV therapy initiation in the adults and adolescents. All the individuals in this study were on first line ARV regimen, NNRTIs (Non-nucleoside reverse transcriptase inhibitors) during the period of study. A subclassification of the study population was made on the basis of liver function test (LFT) and this subset of population was defined as the HIV-infected individuals with ARV drug induced hepatotoxicity were labeled as cases. The male patients with SGOT > 93.8 U/ml, Alkaline phosphatase > 550.8 U/ml, total bilirubin > 3.22 mg/ml and SGPT > 229.5 U/ml and female patients with SGOT > 163.2 U/ml, Alkaline phosphatase > 550.8 U/ml, total bilirubin > 3.22 mg/ml and SGPT > 173.4 U/ml were classified as case group possessing hepatotoxicity. Exclusion criteria: (1) patients with Tuberculosis, Hepatitis B and C infections (2) patients with immune reconstitution syndrome and untreated opportunistic infections (3) patients on other known hepatotoxic medications for the case group showing hepatotoxicity.

At the same time HIV-infected individuals those without hepatotoxicity were labeled as a control group was age matched and recruited. Control population for the HIV positive group, tested negative for TB, Hepatitis B and C and also was not from a similar family as the study cases. The HIV infected males and females with, SGOT < 32 U/ml, Alkaline phosphatase < 108 U/ml, total bilirubin < 1.24 mg/ml, and SGPT < 34 U/ml. For the purpose of analysis the independent variables were categorized as: variables related to HIV (CD4⁺ count to define the stage of HIV infection and NNRTI regimen for drug induced hepatotoxic status), variables related to habits and lifestyle (drinking and tobacco usage) and physiological variables like the status of the liver enzymes.

Clinical information was noted through the reviews of case records, questionnaires, and personal interviews. The ethical endorsement was taken from the Ethics Committee, National AIDS Research Institute, Pune, India (Reference number: August 28, 2013, EC/NARI/Genetic Susceptibility/13-14/146) and written consents were taken from every single qualified subject.

The stages of HIV infection were defined on the basis of CD4⁺ cell counts of the patients at the time of recruitment. Fluorescence-activated cell sorting (FACS) analysis was utilized to estimate the CD4⁺ count. CD4⁺ cell counts of < 200 cells/mm³, 201-350 cells/mm³, and > 350 cells/mm³ were considered as advanced, intermediate and early stages of HIV infection, respectively.

HBsAg and hepatitis C testing was completed by ELISA with the Ortho HCV ELISA test system.

With regard to the ARV therapy, Efavirenz and Nevirapine were the antiretrovirals administered. A questionnaire was utilized to record the usage of tobacco and alcohol.

2.2 Extraction of DNA

The collection of 2ml peripheral blood was done from all subjects and put at -70°C until DNA extraction. The DNA extraction was done from blood leukocytes pellets utilizing the QIAamp DNA Mini Kit according to the kit manual.

2.3 Genotyping

Restriction fragment length polymorphism (RFLP) analysis was utilized to genotype the *MDR1* (1236 C/T and 3435 C/T) polymorphism. Primers to amplify the *MDR1* (C1236T and C3435T) polymorphism were utilized as designated by the previous report [27]. PCR was performed in a volume of 20 μ l. The PCR conditions for amplification of *MDR1* C1236T and C3435T polymorphisms were used as described previously [28]. A thermocycler was utilized to complete all the reactions. PCR products were visualized by ethidium bromide staining. The PCR products of *MDR1* C1236T and C3435T polymorphism was digested by utilization of restriction enzymes *HaeIII* and *MboI* at 37°C for 16 hours separately. 10% polyacrylamide gel along with molecular weight markers was utilized to the genotype of *MDR1* C1236T and C3435T polymorphisms. The sequences and location of SNPs were employed for genotyping of *MDR1* C1236T and C3535T polymorphisms. Genotypes for *MDR1* C1236T were: 93bp and 87bp for CC, 87, 58, and 35bp for TT, and 93, 87, 58, and 35bp for CT; for *MDR1* C3435T: 130 and 76bp for CC, 206bp for TT and 206, 130, and 76bp for CT. Re-genotyping of 20% of the samples were done to check the disparities in genotyping by additional staff. The errors in genotyping were cross-verified by DNA sequencing of 10% of the samples.

2.4 Statistical examination

The variable, age was communicated as mean $\pm$ standard deviation (SD). Hardy-Weinberg equilibrium was examined by utilization of the Chi-Square goodness of fit test in healthy individuals. Fisher's exact test was utilized to analyze the genotype frequencies between groups. Logistic regression was utilized to compute the odds ratios (ORs) and 95% confidence interval (CI). SPSS (SPSS Inc) programming form 17.0 was utilized for statistical examination and the two-sided value was taken as a test of statistical significance. A p-value under ≤ 0.05 was considered for significance. SNPStats online software was utilized to compare the frequency of haplotypes among groups (26). Linkage disequilibrium (LD) was analyzed between both the loci by computing the relative LD value (D') as $D' = D_{ij} / D_{max}$ (28). The D_{ij} value was compared among various groups by the comparison of confidence intervals.

3.0 Results

Out of who tested HIV positive and were on ARV therapy from August 2014 to September 2017, in the NARI clinic. A total of 165 HIV positive individuals, on ARV therapy who visited NARI clinic from August 2014 to September 2017 were considered for the study. Out of these, 34 individuals showed ARV drug induced hepatotoxicity and constituted the first case group of HIV positive individuals. The remaining 131 individuals didn't show hepatotoxicity (by LFT) and formed the second study group of HIV positive individuals on ARV therapy without ARV drug induced hepatotoxicity. Control population consisted of 155 healthy individuals. The demographic profiles of the participants are outlined in Table 1.

Table 1
Characteristics of the study populations

Subjects	ARV drug induced hepatotoxicity	Individuals those without hepatotoxicity y	Healthy controls
Number	N=34	N=131	N=155
Mean Age (Range)	37.24 ± 3.29	40.27 ± 2.45	37.25 ± 6.30
Females	16(47.05)	44(33.58)	40(25.80)
Males	18(52.94)	84(64.12)	112(72.25)
NNRTI Regimen			
Efavirenz N=23	11 (32.35)	12 (9.16)	Not applicable (NA)
Nevirapine N=142	23(67.64)	119 (90.83)	Not applicable
Alcohol habit			
Users N=51	7(20.58)	44(33.58)	0
Non users N=114	27(79.41)	87(66.41)	0
Tobacco habit			
User N=50	23(67.64)	27(20.61)	0
Non user N=115	11(32.35)	104(79.38)	0
CD4+ Status			
0-200 (N=95)	16(16.84)	79(83.16)	NA
201-350(N=50)	17(50)	33(25.19)	NA
>350 (N=20)	1(2.94)	19(14.50)	NA
Abbreviations: NNRTI, Non-nucleoside reverse-transcriptase inhibitors; NA, Not applicable;			
N, total number of subject participants; 0, Alcohol and tobacco status was not reported			

The mean age ± SD of HIV infected individuals those without hepatotoxicity, with ARV drug induced hepatotoxicity and healthy individuals were 40.27 ± 2.45, 37.24 ± 3.29, and 37.25 ± 6.30 years. Each of the study population and control group has been further characterized on the basis of the NNRTI regimen (Efavirenz or Navirapine), alcohol and tobacco usage (users or non-users) and the CD4+ status (to define

the stage of HIV infection). These parameters were used to categorize the study populations and the control group and the incidence of *MDR1* polymorphisms were analyzed in all the categories as shown in the Tables 6-9.

Table 6

Distribution of MDR1 (1236 C/T and 3435 C/T) genotypes among individuals with ARV drug induced hepatotoxicity and those without hepatotoxicity

Genotype MDR1 (1236 C/T)	Tobacco users N= 7 (%)	Tobacco non-users N= 27 (%)	P-Value	OR(95%CI)
Individuals with ARV drug induced hepatotoxicity				
CC	4 (57.1%)	12 (44.4%)	-	1 (Reference)
CT	1 (14.3%)	11 (40.7%)	0.30	0.28 (0.024 - 3.18)
TT	2 (28.6%)	4 (14.8%)	0.88	1.50 (0.13 - 17.35)
Genotype MDR1 (3435 C/T)	Tobacco users N= 7 (%)	Tobacco non-users N= 27 (%)	P-Value	OR(95%CI)
CC	3 (42.9%)	5 (18.5.0%)	-	1 (Reference)
CT	2 (28.6%)	12 (44.4%)	0.37	0.37 (0.042 - 3.25)
TT	2 (28.6%)	10 (37.0%)	0.59	0.53 (0.051 - 5.40)
Individuals those without hepatotoxicity				
Genotype MDR1 (1236 C/T)	Tobacco users N= 43 (%)	Tobacco non-user N= 88 (%)	P-Value	OR(95%CI)
CC	19 (44.2%)	40 (45.5%)	-	1 (Reference)
CT	19(44.2%)	37(42.0%)	0.44	1.39 (0.60 - 3.20)
TT	5 (11.6%)	11 (12.5%)	0.90	1.08 (0.31 - 3.75)
Genotype MDR1 (3435 C/T)	Tobacco users N= 43 (%)	Tobacco non-user N= 88 (%)	P-Value	OR(95%CI)
CC	7 (16.3%)	17 (19.3%)	-	1 (Reference)

N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for MDR1 1236 C/T and 3435 C/T) with other genotypes.

Genotype <i>MDR1</i> (1236 C/T)	Tobacco users N= 7 (%)	Tobacco non-users N= 27 (%)	<i>P</i> -Value	OR(95%CI)
CT	17 (39.5%)	33(37.5%)	0.74	0.80 (0.28 - 2.46)
TT	19 (44.2%)	38 (43.2%)	0.98	1.006 (0.43 - 2.32)
N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes.				

Table 7

Distribution of MDR1 (1236 C/T and 3435 C/T) genotypes among individuals with ARV drug induced hepatotoxicity and those without hepatotoxicity

Genotype MDR1 (1236 C/T)	Alcohol users N= 7 (%)	Alcohol non-users N= 27 (%)	P-Value	OR(95%CI)
Individuals with ARV drug induced hepatotoxicity				
CC	4(57.1%)	12 (44.4%)	-	1(Reference)
CT	1 (14.3%)	11 (40.7%)	0.33	0.29 (0.025 - 3.43)
TT	2 (28.6%)	4 (14.8%)	0.88	1.50 (0.13 - 17.35)
Genotype MDR1 (3435 C/T)	Alcohol users N= 7 (%)	Alcohol non-users N= 27 (%)	P-Value	OR(95%CI)
CC	3(42.9)	5 (18.5%)	-	1(Reference)
CT	3(42.9)	11 (40.7%)	0.63	0.60 (0.077 - 4.73)
TT	1(14.3)	11 (40.7%)	0.32	0.26 (0.018 - 3.76)
Individuals those without hepatotoxicity				
Genotype MDR1 (1236 C/T)	Alcohol users N= 44 (%)	Alcohol non-users N= 87 (%)	P-Value	OR(95%CI)
CC	23(52.3%)	36 (41.4%)	-	1(Reference)
CT	18 (40.9%)	38 (43.7%)	0.95	1.02 (0.45 - 2.35)
TT	3 (6.8%)	13 (14.9%)	0.20	0.40 (0.098 - 1.64)
Genotype MDR1 (3435 C/T)	Alcohol users N= 44 (%)	Alcohol non-users N= 87 (%)	P-Value	OR(95%CI)
CC	6 (13.6%)	18(20.7%)	-	1(Reference)
CT	22 (50.0%)	28 (32.2%)	0.12	2.47 (0.79 - 7.70)
TT	16 (36.4%)	41 (47.1%)	0.81	1.15 (0.37 - 3.59)
N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for MDR1 1236 C/T and 3435 C/T) with other genotypes.				

Table 8

Distribution of MDR1 (1236 C/T and 3435 C/T) genotypes among individuals with ARV drug induced hepatotoxicity and those without hepatotoxicity

Individuals those without hepatotoxicity				
Genotype MDR1 (1236 C/T)	Individuals those without hepatotoxicity taking nevirapine N= 142 (%)	Individuals those without hepatotoxicity taking efavirenz N= 23 (%)	P-Value	OR(95%CI)
CC	63 (44.4)	12 (52.2)	1	Reference
CT	59 (41.5)	9 (39.1)	0.56	1.33 (0.51-3.47)
TT	20 (14.1)	2 (8.7)	0.42	1.93 (0.39-9.45)
Genotype MDR1 (3435 C/T)	Individuals those without hepatotoxicity taking nevirapine N= 142 (%)	Individuals those without hepatotoxicity taking efavirenz N= 23 (%)	P-Value	OR(95%CI)
*/sCC	31 (21.8)	1 (4.3)	1	Reference
CT	52 (36.6)	12 (52.2)	0.063	0.14 (0.017-1.11)
TT	59 (41.5)	10 (43.5)	0.14	0.20 (0.025 - 1.68)
Individuals with ARV drug induced hepatotoxicity				
Genotype MDR1 (1236 C/T)	Individuals with ARV drug induced hepatotoxicity taking Nevirapine N= 23 (%)	Individuals with ARV drug induced hepatotoxicity taking Efavirenz N= 11 (%)	P-Value	OR(95%CI)
CC	11 (47.8%)	5 (45.5%)	-	1(Reference)
CT	7 (30.4%)	5 (45.5%)	0.64	0.69 (0.14 - 3.35)
TT	5 (21.7%)	1 (9.1%)	0.55	2.11 (0.18 - 24.66)
Genotype MDR1 (3435 C/T)	Individuals with ARV drug induced hepatotoxicity taking Nevirapine N= 23 (%)	Individuals with ARV drug induced hepatotoxicity taking Efavirenz N= 11 (%)	P-Value	OR(95%CI)
NS, not significant. N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes.				

Individuals those without hepatotoxicity				
CC	7 (30.4%)	1 (9.1%)	-	1(Reference)
CT	8 (34.8%)	6 (54.5%)	0.18	0.19 (0.017 - 2.11)
TT	8 (34.8%)	4 (36.4%)	0.43	0.37 (0.030 - 4.49)
Individuals those without hepatotoxicity				
Genotype MDR1 (1236 C/T)	Individuals those without hepatotoxicity taking Nevirapine N= 119 (%)	Individuals those without hepatotoxicity taking Efavirenz N= 12 (%)	P-Value	OR(95%CI)
CC	52(43.7%)	7 (58.3%)	-	1(Reference)
CT	52 (43.7%)	4 (33.3%)	0.45	1.66 (0.44 - 6.24)
TT	15 (12.6%)	1 (8.3%)	0.55	1.96 (0.22 - 17.42)
Genotype MDR1 (3435 C/T)	Individuals those without hepatotoxicity taking Nevirapine N= 119 (%)	Individuals those without hepatotoxicity taking Efavirenz N= 12 (%)	P-Value	OR(95%CI)
CC	24 (20.2%)	0 (0.0%)	NS	-
CT	44 (37.0%)	6 (50.0%)	-	1(Reference)
TT	51 (42.9%)	6(50.0%)	0.81	1.16 (0.34- 3.12)
NS, not significant. N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes.				

Table 9

Incidence of MDR1 (1236 C/T and 3435 C/T) genotypes among HIV infected individuals with and without ARV induced hepatotoxicity (subclassified as alcohol and NNRTIs users and non-users)

Genotype <i>MDR1</i> (1236 C/T)	Alcohol+ Nevirapine users N= 5 (%)	Nevirapine users+ Alcohol non-user N= 18 (%)	P- Value	OR(95%CI)
Individuals with ARV drug induced hepatotoxicity				
CC	3 (60.0%)	8 (44.44%)	-	1(Reference)
CT	0 (0.0%)	7 (38.89%)	NS	-
TT	2 (40.0%)	3 (16.67%)	0.55	2.21 (0.17 - 29.21)
Genotype <i>MDR1</i> (3435 C/T)	Alcohol+ Nevirapine users N= 5 (%)	Nevirapine users+ Alcohol non-user N= 18 (%)	P- Value	OR(95%CI)
CC	3 (60.0%)	4 (22.22%)	-	1(Reference)
CT	1(20.0%)	7 (38.39%)	0.38	0.27 (0.015 - 5.01)
TT	1 (20.0%)	7 (38.39%)	0.75	0.61 (0.031 - 12.05)
Genotype <i>MDR1</i> (1236 C/T)	Alcohol+ Efavirenz users N= 2(%)	Efavirenz users+ Alcohol non- users N= 9(%)	P- Value	OR(95%CI)
CC	1 (50.0%)	4 (44.44%)	-	1(Reference)
CT	1 (50.0%)	4 (44.44%)	0.85	0.73 (0.028 - 18.97)
TT	0 (0.0%)	1 (11.12%)	NS	-
Genotype <i>MDR1</i> (3435 C/T)	Alcohol+ Efavirenz users N= 2(%)	Efavirenz users+ Alcohol non- users N= 9(%)	P- Value	OR(95%CI)
CC	0 (0.0%)	1 (11.12%)	NS	-
CT	2 (100%)	4 (44.44%)	-	1(Reference)

NS, not significant. N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *MDR1* 1236 C/T and 3435 C/T) with other genotypes.

Genotype <i>MDR1</i> (1236 C/T)	Alcohol+ Nevirapine users N= 5 (%)	Nevirapine users+ Alcohol non-user N= 18 (%)	P- Value	OR(95%CI)
TT	0 (0.0%)	4 (44.44%)	NS	-
Individuals those without hepatotoxicity				
Genotype <i>MDR1</i> (1236 C/T)	Alcohol+ Nevirapine users N= 38 (%)	Nevirapine users +Alcohol non-users N= 81 (%)	P- Value	OR(95%CI)
CC	18 (47.37%)	34 (41.98%)	-	1(Reference)
CT	17 (44.74%)	35 (43.20%)	0.68	1.20 (0.50 - 2.89)
TT	3 (7.89%)	12 (14.82%)	0.40	0.54 (0.13 - 2.26)
Genotype <i>MDR1</i> (3435 C/T)	Alcohol+ Nevirapine users N= 38 (%)	Nevirapine users+ Alcohol non-users N= 81 (%)	P- Value	OR(95%CI)
CC	6 (15.78%)	18 (22.22%)	-	1(Reference)
CT	17 (44.74%)	27 (33.33%)	0.23	2.04 (0.64 - 6.53)
TT	15 (39.47%)	36 (44.45%)	0.74	1.22 (0.39 - 3.84)
Genotype <i>MDR1</i> (1236 C/T)	Alcohol+ Efavirenz users N= 6(%)	Efavirenz users+ Alcohol non- user N= 6(%)	P- Value	OR(95%CI)
CC	5 (83.33%)	2 (33.33%)	-	1(Reference)
CT	1 (16.67%)	3 (50.0%)	0.16	0.13 (0.008 - 2.18)
TT	0 (0.0%)	1 (16.67%)	NS	-
Genotype <i>MDR1</i> (3435 C/T)	Alcohol+ Efavirenz users N= 6(%)	Efavirenz users+ Alcohol non- user N= 6(%)	P- Value	OR(95%CI)
CC	0 (0.0%)	0	NS	-

NS, not significant. N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *MDR1* 1236 C/T and 3435 C/T) with other genotypes.

Genotype <i>MDR1</i> (1236 C/T)	Alcohol+ Nevirapine users N= 5 (%)	Nevirapine users+ Alcohol non-user N= 18 (%)	P- Value	OR(95%CI)
CT	5 (83.33%)	1 (16.67%)	-	1 (Reference)
TT	1 (16.67%)	5 (83.33%)	0.04	0.04 (0.002 - 0.83)
NS, not significant. N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes.				

3.1 *MDR1* polymorphisms and ARV drug induced hepatotoxicity and control population

The incidence of *MDR1* polymorphisms in the two study populations is shown in Table 2. *MDR1* polymorphisms were not found to be distinct between ARV drug induced hepatotoxicity cases and those without hepatotoxicity. Although, among the ARV drug induced hepatotoxicity cases, the predominance of *MDR1* 1236TT genotype was more as compared to those without hepatotoxicity (17.6% versus 12.2%, OR=1.38, 95%CI: 0.45-4.12, P=0.57). Whereas, *MDR1* 3435TT genotype and T allele were underrepresented in the ARV drug induced hepatotoxicity cases as compared to those without hepatotoxicity (35.3% versus 43.5%, OR=0.56, 95%CI: 0.20- 1.59, P=0.28 and 55.88% versus 62.59%, OR=0.65, P=0.13). Also, *MDR1* polymorphisms were not significantly different between ARV drug induced hepatotoxicity and the healthy controls.

Table 2

Distribution of **MDR1 (1236 C/T and 3435 C/T) polymorphisms among** individuals with ARV drug induced hepatotoxicity **and** those without hepatotoxicity

Genotype MDR1 (1236 C/T)	Individuals with ARV drug induced hepatotoxicity N= 34 (%)	Individuals without hepatotoxicity N=131 (%)	P- Value	OR(95%CI)
CC	16 (47.1%)	59 (45.0%)		1(Reference)
CT	12 (35.3%)	56 (42.7%)	0.37	0.68 (0.29-1.60)
TT	6 (17.6%)	16 (12.2%)	0.57	1.37 (0.45-4.12)
CT+TT	18(52.94)	72(54.96)	0.63	0.82 (0.38-1.79)
MDR1 (1236 C/T) Allele	Individuals with ARV drug induced hepatotoxicity N= 68 (%)	Individuals without hepatotoxicity N= 262 (%)	P- Value	OR(95%CI)
C	44 (64.71%)	174 (66.41%)		1(Reference)
T	24 (35.29%)	88 (33.59%)	0.91	1.03 (0.59-1.82)
Genotype MDR1 (3435 C/T)	Individuals with ARV drug induced hepatotoxicity N= 34 (%)	Individuals without hepatotoxicity N=131 (%)	P- Value	OR(95%CI)
CC	8 (23.5%)	24 (18.3%)	-	1(Reference)
CT	14 (41.2%)	50 (38.2%)	0.70	0.82 (0.30-2.25)
TT	12 (35.3%)	57 (43.5%)	0.28	0.56 (0.20-1.59)
CT+TT	26(74.47%)	107(81.67%)	0.42	0.68 (0.27-1.71)
MDR1 (3435 C/T) Allele	Individuals with ARV drug induced hepatotoxicity N= 68 (%)	Individuals without hepatotoxicity N= 262 (%)	P- Value	OR(95%CI)
C	30 (44.12%)	98 (37.40%)	-	1(Reference)
T	38 (55.88%)	164 (62.59%)	0.13	0.65 (0.37-1.14)

N, total number of individuals with ARV drug induced hepatotoxicity (34), those without hepatotoxicity (131) and healthy controls (155). OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *MDR1* 1236 C/T and 3435 C/T) with other genotypes/alleles.

Genotype <i>MDR1</i> (1236 C/T)	Individuals with ARV drug induced hepatotoxicity N= 34 (%)	Individuals without hepatotoxicity N=131 (%)	P- Value	OR(95%CI)
Genotype <i>MDR1</i> (1236 C/T)	Individuals with ARV drug induced hepatotoxicity N= 34 (%)	Healthy controls N= 155(%)	P- Value	OR(95%CI)
CC	16 (47.1%)	69 (44.52%)		1 (Reference)
CT	12 (35.3%)	65 (41.94%)	0.45	0.70 (0.27- 1.79)
TT	6 (17.6%)	21 (13.54%)	0.98	0.99 (0.29- 3.38)
CT+TT	18(52.94)	86(55.48)	0.55	0.77 (0.33- 1.82)
<i>MDR1</i> (1236 C/T) Allele	Individuals with ARV drug induced hepatotoxicity N=68	Healthy Controls N= 310	P- Value	OR(95%CI)
C	44 (64.71%)	203 (65.48%)	-	1(Reference)
T	24 (35.29%)	107 (34.52%)	0.77	0.91 (0.49- 1.71)
Genotype <i>MDR1</i> (3435 C/T)	Individuals with ARV drug induced hepatotoxicity N= 34 (%)	Healthy Controls N= 155	P- Value	OR(95%CI)
CC	8 (23.5%)	34 (21.94%)	-	1(Reference)
CT	14 (41.2%)	67 (43.23%)	0.24	0.52 (0.17- 1.58)
TT	12 (35.3%)	54 (34.83%)	0.28	0.53 (0.16- 1.69)
CT+TT	26(76.47)	121(78.06)	0.22	0.52 (0.19- 1.46)
<i>MDR1</i> (3435 C/T) Allele	Individuals with ARV drug induced hepatotoxicity N= 68 (%)	Healthy Controls N= 310	P- Value	OR(95%CI)
C	30 (44.12%)	135 (43.54%)	-	1(Reference)
T	38 (55.88%)	175 (56.45%)	0.39	0.76 (0.41- 1.41)
N, total number of individuals with ARV drug induced hepatotoxicity (34), those without hepatotoxicity (131) and healthy controls (155). OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes/alleles.				

3.2 MDR1 polymorphism and HIV+ those without ARV drug induced hepatotoxicity

The occurrence of *MDR1* polymorphisms (1236C/T, 3435C/T) in those without hepatotoxicity versus the healthy population is tabulated in Table 3. The healthy population followed the deviation from Hardy-Weinberg equilibrium ($P=0.36$, 0.13). The distribution of *MDR1* polymorphism was almost alike between those without hepatotoxicity and healthy controls. Although, individuals those without hepatotoxicity had more occurrence of *MDR1* 3435TT genotype than healthy people (43.5% versus 34.83%, OR=1.24, 95%CI: 0.59-2.61, $P=0.57$). The dispersion of other genotypes and alleles of *MDR1* polymorphisms were comparable between both groups.

Table 3

Distribution of *MDR1* (1236 C/T and 3435 C/T) polymorphism in individuals those without hepatotoxicity versus healthy controls.

Genotype <i>MDR1</i> (1236 C/T)	Individuals those without hepatotoxicity N=131 (%)	Healthy controls N= 155(%)	P- Value	OR(95%CI)
CC	59 (45.0%)	69 (44.52%)	-	1(Reference)
CT	56 (42.7%)	65 (41.94%)	0.65	0.87 (0.48- 1.59)
TT	16 (12.2%)	21 (13.54%)	0.39	0.69 (0.29- 1.61)
CT+TT	72(54.96)	86(55.48)	0.49	0.82 (0.47- 1.43)
<i>MDR1</i> (1236 C/T) Allele	Individuals those without hepatotoxicity N= 262 (%)	Healthy controls N= 310	P- Value	OR(95%CI)
C	174 (66.41%)	203 (65.48%)	-	1(Reference)
T	88 (33.59%)	107 (34.52%)	0.37	0.83 (0.55- 1.25)
Genotype <i>MDR1</i> (3435 C/T)	Individuals those without hepatotoxicity N=131 (%)	Healthy controls N= 155	P- Value	OR(95%CI)
CC	24 (18.3%)	34 (21.94%)	-	1(Reference)
CT	50 (38.2%)	67 (43.23%)	0.53	0.79 (0.37- 1.66)
TT	57 (43.5%)	54 (34.83%)	0.57	1.24 (0.59- 2.61)
CT+TT	107(81.67)	121(78.06)	0.97	0.99 (0.50- 1.94)
<i>MDR1</i> (3435 C/T) Allele	Individuals those without hepatotoxicity N= 262 (%)	Healthy controls N= 310	P- Value	OR(95%CI)

N, total number of individuals those without hepatotoxicity (131) and healthy controls (155). OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *MDR1* 1236 C/T and 3435 C/T) with other genotypes/alleles.

Genotype <i>MDR1</i> (1236 C/T)	Individuals those without hepatotoxicity N=131 (%)	Healthy controls N= 155(%)	<i>P</i> - Value	OR(95%CI)
C	98 (37.40%)	135 (43.54%)	-	1(Reference)
T	164 (62.59%)	175 (56.45%)	0.41	1.18 (0.80- 1.74)
N, total number of individuals those without hepatotoxicity (131) and healthy controls (155). OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes/alleles.				

3.3 Haplotypes distribution

We have also analyzed the *MDR1* haplotypes among the two study groups and the healthy controls, as shown in Table 4. Haplotype CT (1236*C/3435*T) was considered as a reference. The incidence of TT haplotype (1236*T/3435*T) has been found to be significantly lesser among ARV drug induced hepatotoxicity cases than those without hepatotoxicity (0.05% versus 0.22%, OR=0.16, 95%CI: 0.04-0.059, P=0.0065), whereas the incidence of TC haplotype (1236*T/3435*C) was significantly more in ARV drug induced hepatotoxicity cases than those without hepatotoxicity (0.30% versus 0.11%, OR=1.96, 95%CI: 0.98-3.94, P=0.06). This suggests that haplotype TC was likely to be associated with increased risk for severity of hepatotoxicity because of synergistic effect of gene-gene interaction. The occurrence of CC (1236*C/3435*C) and TT (1236*T/3435*T) haplotypes was lesser among ARV drug induced hepatotoxicity cases than healthy controls (0.14% versus 0.30%, OR=0.34, 95%CI: 0.12- 0.94, P=0.039, 0.05% versus 0.22%, OR=0.09, 95%CI: 0.02-0.44, P=0.0032). Whereas, the incidence of TC (1236*T/3435*C) haplotype was predominantly higher in ARV drug induced hepatotoxicity cases compared to the healthy controls (0.30% versus 0.13%, OR=1.94, 95%CI: 0. 87-4.37, P=0.11). The incidence of *MDR1* haplotypes among those without hepatotoxicity individuals was not significantly different from the healthy population.

Table 4

Distribution of MDR1 haplotypes (1236C/T and 3435C/T) in HIV infected individuals with and without ARV associated hepatotoxicity and healthy controls

Haplotype <i>MDR1</i> (1236 C/T and 3435 C/T)	individuals with ARV drug induced hepatotoxicity y (N = 68)	individuals those without hepatotoxicity (N = 262)	<i>P</i> -Value	OR(95%CI)
CT	0.51	0.41	-	1(Reference)
CC	0.14	0.26	0.062	0.46 (0.20-1.03)
TT	0.05	0.22	0.006	0.16(0.04-0.59)
TC	0.30	0.11	0.06	1.96 (0.98-3.94)
Haplotype MDR1 (1236 C/T and 3435 C/T)	Individuals with ARV drug induced hepatotoxicity (N = 68)	Healthy controls (N=310)	<i>P</i> -Value	OR(95%CI)
CT	0.51	0.35	-	1(Reference)
CC	0.14	0.30	0.03	0.34 (0.12-0.94)
TT	0.05	0.22	0.003	0.09 (0.02-0.44)
TC	0.30	0.13	0.11	1.94 (0.87-4.37)
Haplotype MDR1 (1236 C/T and 3435 C/T)	Individuals those without hepatotoxicity (N= 262)	Healthy controls (N=310)	<i>P</i> -Value	OR(95%CI)
CT	0.41	0.35	-	1(Reference)
CC	0.26	0.30	0.31	0.77 (0.46-1.28)
TT	0.22	0.22	0.31	0.75 (0.43-1.31)
TC	0.11	0.13	0.43	0.77 (0.40-1.48)
N, total number of allele in individuals with ARV drug induced hepatotoxicity (68), those without hepatotoxicity (262) and healthy controls (310). OR and 95% CIs were derived from logistic regression model comparing the haplotype CT with other haplotypes. Significant <i>P</i> values are shown in bold (<i>P</i> <0.05)				

3.4 MDR1 polymorphisms and stages of HIV

The incidence of *MDR1* polymorphism among individuals in different stages of HIV infection and the healthy controls was also studied as outlined in Table 5. A reduced frequency of *MDR1* 1236TT genotype was found among individuals intermediate stage of HIV infection than healthy control population (24.2% versus 41.94%, OR=0.43, P=0.09). The incidence of *MDR1* 3435CT and 3435TT genotypes did not vary among the individuals in different stages of HIV infection and healthy population.

Table 5

Distribution of *MDR1* (1236 C/T and 3435 C/T) genotypes among individuals in different stages of HIV infection versus healthy controls

Genotype <i>MDR1</i> (1236 C/T)	Healthy controls N=155 (%)	Early stage of HIV infection		Intermediate stage of HIV infection		Advanced stage of HIV infection	
		N=19 (%)	OR (P)	N= 33 (%)	OR (P)	N= 79 (%)	OR (P)
CC	69 (44.52%)	7 (36.8%)	1 (Reference)	18 (54.5%)	1 (Reference)	34 (43.0%)	1 (Reference)
CT	65 (41.94%)	10 (52.6%)	1.67 (0.36)	8 (24.2%)	0.43 (0.09)	38 (48.1%)	1.03 (0.93)
TT	21 (13.54%)	2 (10.5%)	0.77 (0.77)	7 (21.2%)	0.93 (0.90)	7 (8.9%)	0.58 (0.32)
Genotype <i>MDR1</i> (3435 C/T)	Healthy controls N=155 (%)	Early stage of HIV		Intermediate stage of HIV		Advanced stage of HIV	
		N= 19 (%)	OR (P)	N= 33 (%)	OR (P)	N= 79 (%)	OR (P)
CC	34 (21.94%)	4 (21.1%)	1 (Reference)	5 (15.2%)	1 (Reference)	15 (19.0%)	1 (Reference)
CT	67 (43.23%)	6 (31.6%)	0.41 (0.23)	12 (36.4%)	0.80 (0.72)	32 (40.5%)	0.83 (0.66)
TT	54 (34.83%)	9 (47.4%)	0.93 (0.92)	16 (48.5%)	1.56 (0.45)	32 (40.5%)	1.11 (0.81)
N= number of subjects, (%) = frequency of subjects, OR and 95% CIs were derived from logistic regression model comparing the homozygous wild-type genotype/allele (CC genotype and C allele for <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes.							

3.5 Gene-environment interaction

The distribution of *MDR1* polymorphisms among ARV drug induced hepatotoxicity cases and those without hepatotoxicity and the healthy control group was analyzed by categorizing them on the basis of tobacco and alcohol consumption and NNRTI regimen as shown in Tables 6-9. The occurrence of polymorphisms of *MDR1* (1236C/T and 3435C/T) was not different among the individual consuming tobacco in both the study populations and the healthy control group. *MDR1* 1236TT genotype was overrepresented among the tobacco consumers than the non-consumers in the ARV drug induced hepatotoxicity group (28.6% versus 14.8%) (Table-6). Also its occurrence was higher among the alcohol

consumers than the non-consumers in the ARV drug induced hepatotoxicity group (28.6% versus 14.8%, OR=1.50, 95% CI: 0.13 - 17.35, P=0.88). An increased incidence of 3435CT genotype of *MDR1* was observed among the alcohol consumers than the nonusers in the individuals those without hepatotoxicity (50.0% versus 32.2%, OR=2.47, 95%CI: 0.79 -7.70, P=0.12) (Table-7). The occurrence of genotype 1236TT of *MDR1* was greater in nevirapine taking individuals those without hepatotoxicity than efavirenz users (14.1% versus 8.7%, OR=1.93, 95%CI: 0.39- 9.45, P=0.42). Also it was greater in individuals on nevirapine with ARV drug induced hepatotoxicity than efavirenz users (21.7% versus 9.1%, OR= 2.11, 95%CI:0.18- 24.66, P=0.55). This suggest that individuals with *MDR1*1236TT genotype along with nevirapine usage may increase risk for severity of hepatotoxicity because of combined effect. The occurrence of *MDR1* 1236CT and 1236TT genotypes were also higher in individuals those without hepatotoxicity on nevirapine than efavirenz users (43.7% versus 33.3%, OR= 1.66, 95%CI: 0.44 - 6.24, P=0.45 and 12.6% versus 8.3%, OR=1.96, 95%CI: 0.22 - 17.42, P=0.55) (Table-8). Among the ARV drug induced hepatotoxicity cases, who were on nevirapine and consumed alcohol, the dispersion of *MDR1* 1236TT genotype was greater when contrasted to the alcohol nonusers on nevirapine (40.0% versus 16.67%, OR= 2.21, 95%CI: 0.17-29.21, P=0.55). This suggest that individuals with *MDR1* 1236TT genotype taking nevirapine and alcohol may increase the risk for severity of hepatotoxicity because of combined effect. The incidence of 3435CT genotype of *MDR1* was greater among the alcohol consumers on nevirapine than alcohol non-consumers on nevirapine, in individuals those without hepatotoxicity (44.74% versus 33.33%, OR= 2.04, 95%CI: 0.64 - 6.53, P=0.23) (Table 9).

4.0 Discussion

We analyzed the association between *MDR1* polymorphism and ARV drug induced hepatotoxicity. *MDR1* encodes for the ATP- dependent membrane efflux transporter (14). P-glycoproteins are substrate for the genetic variant that impact patient drugs. The occurrence of *MDR1* polymorphism changes from population to population [17]. We analyzed the *MDR1* genotypes and haplotypes among the individuals with ARV drug induced hepatotoxicity and those without hepatotoxicity.

We found that the occurrence of *MDR1* 3435C/T polymorphism in our healthy control population is identical to the investigations done in European, North Indian, Turkish, and Asian populations [30–35] and contrasted with the similar studies done in the Chinese, Iranian and Thailand populations [27, 33, 36]. Also, the genotypic dispersal of *MDR1* 1236C/T polymorphism in our healthy individuals was almost alike to the similar investigations in the North Indian population [35]. However, it contrasted from the Mexican, Chinese and South African populations [18, 31, 38, 39]. We have done a genotype-phenotype analysis and found that the individuals with *MDR1*1236TT genotype were at increased risk for severity of hepatotoxicity (OR=1.37, P=0.57). However, due to the small number of ARV drug induced hepatotoxicity cases, the risk could not reach statistical significance. The low phenotypic expression was linked with polymorphisms in P-gp. People with 3435TT genotype were found to have lower levels of P-gp than CC and CT genotypes. Also, *MDR1* 3435C/T polymorphism was associated with the reduced risk of NNRTI-induced liver toxicity [25].

We also studied the gene-gene interactions to understand the synergistic impact of *MDR1* polymorphism on ARV drug induced hepatotoxicity. The gene-gene interactions are known to have greater effects on gene expression than a single gene [40]. In our investigation, individuals possessing haplotype TC were more prone to a severe ARV drug induced hepatotoxicity (OR=1.96, P=0.06); and the ones with TT and CC haplotypes may have reduced risk for acquiring of ARV drug induced hepatotoxicity OR=0.16, P=0.006; OR=0.46, P=0.06; OR=0.09, P=0.003; OR=0.34, P=0.03).

Likewise, we analyzed the association between the *MDR1* genotypes and the stage of HIV infection. In our investigation, the incidence of *MDR1* genotypes did not vary significantly among individuals of various stages of HIV infection as well as the healthy population. *MDR1* 1236CT, 1236TT, and 3435CT genotypes have been shown to be correlated with the HIV disease progression, haven't been found to regulate the susceptibility to HIV-1 infection [41]. Additionally, the patients with 3435 TT genotype had an increase in the CD4+ counts, following a treatment for half an year [23].

An analysis of the gene-environment interaction helps to know its impact on the disease etiology [42, 43]. We did a case-only analysis to study the gene-environment interaction. We did not rather go for a case-control analysis in light of the fact that a case-control investigation, requires a coordination of cases with the control population [44]. HIV infected individuals who are naïve to ARV therapy and consume alcohol have been found to have a reduction in the CD4+ cell count [45]. Also, in the HIV infected women who consume tobacco, a diminished response to ARV therapy has been observed [46]. In our study, in ARV drug induced hepatotoxicity cases, who consume alcohol, *MDR1*1236TT genotype exposed a risk for severe hepatotoxicity (OR=1.50, P=0.88). Among the individuals those without hepatotoxicity and used alcohol, the incidence of 3435CT genotype posed a higher risk of acquisition of ARV drug induced hepatotoxicity (OR=2.47, P=0.12) because of combined effect of gene and environment. Among the ARV drug induced hepatotoxicity cases on nevirapine, presence of *MDR1* 1236TT genotype was likely to be associated with the higher risk of severe hepatotoxicity (OR= 2.11, P=0.55). Whereas among individuals those without hepatotoxicity on nevirapine, *MDR1* 1236CT, 1236TT genotypes exposed a higher risk of acquisition of ARV drug induced hepatotoxicity (OR=1.66, P=0.45; OR=1.96, P=0.55). In individuals with ARV drug induced hepatotoxicity on nevirapine who consume, *MDR1* 1236TT genotype exposed a higher susceptibility to severe hepatotoxicity (OR=2.21, P=0.55) because of combined effect of gene and environment.

In the individuals those without hepatotoxicity, who consume alcohol, *MDR1* 3435CT genotype showed a susceptibility to acquisition of ARV drug induced hepatotoxicity (OR=2.04, P=0.23). This suggests that individuals with *MDR1* 1236TT and 3435CT genotypes with or without ARV drug induced hepatotoxicity may have a combined effect on acquisition and severity of hepatotoxicity. Also, individuals on nevirapine, possessing 3435T allele has a reduced risk of hepatotoxicity [26]. Individuals with *MDR1* 1236T and 1235T alleles have been associated with a diminished plasma NNRTI concentration, influencing the virological response to HAART [21]. Haas et al., (2005) have not found any significant association between *ABCB1* variations and plasma EFV concentrations [19].

The fact that this work can just assess the association and does not indicate causation, is one of the limiting points of the study. Also, the present investigation was planned to constitute a 1:4 proportion of cases versus controls but this couldn't be accomplished and we recruited them in 1:3 proportion which may be sufficient.

Conclusions

MDR1 haplotypes have an impact on the severity of ARV drug induced hepatotoxicity. In the individuals on nevirapine who consume alcohol, *MDR1* 1236TT and 3435CT genotypes, had a combined effect on acquisition and severity of hepatotoxicity.

MDR1 is associated with drug clearance. As *MDR1* expression influences the response to NVP and EFV regimen. Therefore, further association studies between *MDR1* polymorphism and plasma drug concentration would be done with a larger sample size. In addition, the association of polymorphisms of other drug transporter genes with plasma drug levels is required to comprehend the effect of genetic variants on treatment effect.

Declarations

Ethical Approval and Consent to participate: Study is approved from Institutional Ethics committee (IEC): NARI/EC/ICF version 1.0, dated 28 August 2013. Consent was taken from all the participants

Consent for publication: Written informed consent was obtained from the patient for publication of this report. A copy of the written consent is available for review by the Editor-in-Chief of this journal."

Availability of supporting data: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions:

HariOm Singh: Overall supervision

Dharmesh Samani: Experimental work

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