Analysis of MDR1 Polymorphisms Among HIV-Infected Individuals on ARV Therapy from Western India

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Abstract

Background: *MDR1* is involved in the transport of numerous drugs. Polymorphism of *MDR1* is linked with the treatment outcome. ARV regimen is being used to manage the progression of HIV infection. Ethnic disparities have been observed in the distribution of *MDR1* genotypes.

Methods: *MDR1* polymorphism (1236 C/T, 3435 C/T) was genotyped in 34 individuals with ARV-associated hepatotoxicity, 131 HIV-infected, and one-fifty-five healthy by utilization of PCR-RFLP.

Results: Haplotype TC exposed the greater risk for hepatotoxicity severity when compared between individuals with hepatotoxicity and HIV infected (OR=1.96, P=0.06). While haplotypes TT and CC bared a reduce risk for hepatotoxicity severity (OR= 0.16, P=0.006; OR= 0.46, P=0.06). Haplotype TT and CC displayed a decrease risk of hepatotoxicity severity while compared between individuals with hepatotoxicity and healthy (OR=0.09, P=0.003; OR=0.34, P=0.03). A higher occurrence of *MDR1* 1236TT genotype was seen among patients with hepatotoxicity who consumed alcohol (28.6% versus 14.8%, OR=1.50). In patients with hepatotoxicity taking nevirapine, there was an increased incidence of *MDR1* 1236TT genotype in contrast with efavirenz (21.7% versus 9.1%, OR=2.11). In HIV-infected people taking nevirapine, *MDR1* 1236CT, 1236TT genotypes found to be increased compared with efavirenz (43.7% versus 33.3%, OR=1.66; 12.6% versus 8.3%, OR=1.96). A higher occurrence of *MDR1* 1236TT genotype has happened in hepatotoxicity cases having both alcohol and nevirapine (40.0% versus 16.67%, OR=2.21).

Conclusion: *MDR1* haplotypes may have an influence on the severity of hepatotoxicity. Individual utilizing nevirapine and alcohol with *MDR1* 1236TT and 3435CT genotypes may have combined effect on vulnerability of severity of hepatotoxicity and progression of HIV infection

1. Background

Antiretroviral (ARV) regimen is being extensive utilized for the management of HIV patients, the death rate among individuals with HIV is constantly increasing. Long-term efficacy and toxicity are significant issues of worry with the ARV regimen. 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 shown occurrence of hepatotoxicity because of nevirapine was 3.19% [4]. In another investigation, the stated occurrence of grade 3 or 4 hepatotoxicity had 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 is responsible for cell homeostasis [6–8].

ABCB1 gene is situated on chromosome 7q21. It is a part of the MDR subfamily [8]. *ABCB1* protein is expressed in a few tissues including epithelial cells of the blood-brain barrier [9, 10] and has appeared to transport numerous drugs [11]. P-glycoprotein (P-GP), a transmembrane transporter protein, is encoded by the *MDR1*. P-gp system of ATP- subordinate efflux transports outside particles including drugs from

intracellular to the extracellular matrix [12–14]. The variation in P-gp expression may vary the function, thus affect the transport of nevirapine (NVP) [15]. The absorption and penetration of efavirenz (EFV) and NVP are impacted by the P-gp expression [16]. Chelule et al., (2003) have shown the commonness of *MDR1* 3435CC genotype had 85.9% in Africans, 41.70% in Indians, and 35.7% in whites of Kwazulu-Natal, South Africa, respectively. In the African population, the predominance of the *MDR1* 3435CC genotype showed overexpression of P-glycoprotein, while in patients with TT genotype it was demonstrated a lower expression of P-GP [17, 18]. Studies have been reported a significant increase of CD4 cell counts with *MDR1* 3435TT genotype in HIV patients [19, 20]. Haas et al., (2005) 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 was 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 was connected with a more elevated level of CD4 + count than the CT/CC genotypes [22]. Leschziner et al., (2007) recommended that *MDR1* 3435 TT genotype was linked with higher adverse outcome of 3TC and NVP treatment than EFV treatment [23]. Ritchie et al., (2006) indicated a significant reduced risk of hepatotoxicity with NNRTI-containing regimens in the presence of *MDR1* 3435C/T polymorphism [24]. The link between polymorphism of *MDR1* 3435C/T and nevirapine induced hepatotoxicity was documented by studies [24, 25].

Few studies have shown a link between *MDR1* polymorphism and adverse outcome of ARV drug and other studies do not suggest the link. Moreover, the link between *MDR1* polymorphism and ARV-related hepatotoxicity has not been examined from India. Thus, we analyzed the relationship between *MDR1* (1236C/T and 3435C/T) polymorphism and hepatotoxicity induced by ARV regimen.

2.0 Methods

2.1 Subjects

A case-control design was undertaken between August 2014 to September 2017. The number of individuals with HIV infection experienced a liver function test (LFT) was one hundred sixty-five. Out of which, thirty-four individuals of hepatotoxicity and one thirty-one individual with no hepatotoxicity were affirmed and one hundred fifty-five healthy people age-matched were recruited. The patients were enlisted from the clinic of the National Institute of AIDS Research, Pune. Tuberculosis, Hepatitis B and C, immune reconstitution syndrome, untreated opportunistic infections, and other known hepatotoxic medications have been excluded from the hepatotoxicity case group. At the same time, one hundred fifty-five people (those from a similar family have been excluded) lacking diseases for example, TB, Hepatitis B, C, and HIV, have been recruited as controls. Clinical information was noted through the reviews of case records, questionnaires, and personal interviews. The status of the liver enzymes was assessed by the utilization of LFT. In patients with hepatotoxicity, males with SGOT > 93.8 U/ml, Alkaline phosphatase > 550.8 U/ml, total bilirubin > 3.22 mg /ml, SGPT > 229.5 U/ml and females with SGOT > 163.2 U/ml, Alkaline phosphatase > 550.8 U/ml, total bilirubin > 3.22 mg /ml, SGPT > 173.4 U/ml were considered. In HIV-

infected male and female controls, SGOT < 32 U/ml, Alkaline phosphatase < 108 U/ml, total bilirubin < 1.24 mg /ml, and SGPT < 34 U/ml were considered. FACS analysis was utilized to estimate the CD4 + count. The stages of patients were characterized based on current CD4 status. CD4 + cell count ranges from < 200 cells/mm³, 201–350 cells/mm³, and > 350 cells /mm³ were considered as advanced, intermediate, and early stage of HIV infection. HBsAg and hepatitis C testing was completed by ELISA with the Ortho HCV ELISA test system. A questionnaire was utilized to record the usage of tobacco and alcohol. 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 has been taken from every single qualified subject.

2.2 Extraction of DNA

The collection of 2 ml peripheral blood was done from all subjects and put away at – 70⁰C until DNA extraction. The DNA extraction has been completed from blood leukocytes pellets utilizing the QIAamp DNA Mini Kit according to the guidance of the company.

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 [26]. PCR was performed in a volume of 20 µl. The PCR conditions for amplification of *MDR1* C1236T and C3435T polymorphisms were used as described previously [27]. 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 polymorphism. The sequences and location of SNP were employed for genotyping of *MDR1* C1236T and C3535T polymorphisms. Genotypes were: for *MDR1* C1236T: 93 and 87 bp for CC, 87, 58, and 35 bp for TT, and 93, 87, 58, and 35 bp for CT, for *MDR1* C3435T: 130 and 76 bp for CC, 206 bp for TT and 206, 130, and 76 bp for CT. Additional staff of the laboratory was done re-genotyping in 20% samples to check the disparities in genotyping. The genotyping mistake was cross-verified by DNA sequencing in 10% of samples.

2.4 Statistical examination

The age variable was communicated as mean ± standard deviation (SD). Hardy-Weinberg equilibrium was examined by the utilization of Chi-Square Goodness - of - fit test in healthy people. 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 (25). 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 between various groups by the comparison of confidence intervals. 3.0

3.0 Results

An aggregate of 165 individuals of HIV infection incorporating thirty-four individuals with hepatotoxicity, one-thirty-one HIV-infected and one- fifty-five healthy were used to analysis the polymorphism of *MDR1*. The mean age $\pm$ SD of individuals with hepatotoxicity, HIV infected, and healthy have been 37.24 ± 3.29 , 40.27 ± 2.45 , and 37.25 ± 6.30 years. The demographic profiles of the participants are outlined in Table 1.

Table 1
Characteristics of individuals of ARV related hepatotoxicity, HIV infected and healthy controls

Subjects	Individual of ARV- related hepatotoxicity (Grade III and IV)	Individual of HIV infection	Healthy controls
Number	N = 34	N = 131	N = 155
Mean Age (Range)	35.14 ± 8.96	39.29 ± 1.34	36.75 ± 8.50
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			
User N = 51	7(20.58)	44(33.58)	0
Non user 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			

3.1 MDR1 polymorphism and ARV-associated hepatotoxicity

The incidence of polymorphism of *MDR1* in individuals of hepatotoxicity and HIV infected has appeared in Table 2. *MDR1* polymorphisms has not been distinct between sufferers with hepatotoxicity and HIV infected. In hepatotoxicity cases, the predominance of *MDR1* 1236TT genotype was increased (17.6% versus 12.2%, OR = 1.38, 95%CI: 0.45–4.12, P = 0.57). *MDR1* 3435TT genotype and T allele were underrepresented in hepatotoxicity cases when contrasted with HIV-infected people (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). *MDR1* polymorphism were not significantly varied between hepatotoxicity cases and healthy people.

Table 2
Incidence of *MDR1* (1236 C/T and 3435 C/T) genotypes/alleles in individuals of ARV related hepatotoxicity and HIV infected

Genotype <i>MDR1</i> (1236 C/T)	Individual of ARV- related hepatotoxicity N = 34 (%)	Individual of HIV infection 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	Individual of ARV- related hepatotoxicity N = 68 (%)	Individual of HIV infection 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 <i>MDR1</i> (3435 C/T)	Individual of ARV- related hepatotoxicity N = 34 (%)	Individual of HIV infection 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	Individual of ARV- related hepatotoxicity N = 68 (%)	Individual of HIV infection 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)
Genotype <i>MDR1</i> (1236 C/T)	Individual of ARV- related hepatotoxicity N = 34 (%)	Healthy control N = 155(%)	P- Value	OR(95%CI)

N, total number of Individual of ARV-related hepatotoxicity (34), HIV infection (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)	Individual of ARV- related hepatotoxicity N = 34 (%)	Individual of HIV infection N = 131 (%)	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)
MDR1 (1236 C/T) Allele	Individual of ARV- related hepatotoxicity N = 68 (%)	Healthy Control 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)	Individual of ARV- related hepatotoxicity N = 34 (%)	Healthy Control 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)
MDR1 (3435 C/T) Allele	Individual of ARV- related hepatotoxicity N = 68 (%)	Healthy Control 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 Individual of ARV-related hepatotoxicity (34), HIV infection (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 in HIV-infected people

The occurrence of polymorphisms of *MDR1* (1236C/T, 3435C/T) in people with HIV infection and healthy is introduced in Table 3. The deviation from Hardy- Weinberg equilibrium in healthy people was

investigated for *MDR1* polymorphism, we found that it was followed ($P = 0.36, 0.13$). The distribution of *MDR1* polymorphism was alike between HIV-infected and healthy people. HIV-infected people 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

Incidence of *MDR1* (1236 C/T and 3435 C/T) genotypes/alleles in individual of HIV infection and healthy controls.

Genotype <i>MDR1</i> (1236 C/T)	Individual of HIV infection 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)
MDR1 (1236 C/T) Allele	Individual of HIV infection 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)	Individual of HIV infection 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)
MDR1 (3435 C/T) Allele	Individual of HIV infection N = 262 (%)	Healthy controls N = 310	P- Value	OR(95%CI)
C	98 (37.40%)	135 (43.54%)	-	1(Reference)

N, total number of individual of HIV infection (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)	Individual of HIV infection N = 131 (%)	Healthy controls N = 155(%)	<i>P</i> - Value	OR(95%CI)
T	164 (62.59%)	175 (56.45%)	0.41	1.18 (0.80– 1.74)
N, total number of individual of HIV infection (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 likewise investigated the occurrence of *MDR1* haplotypes among people of hepatotoxicity, HIV-infected, and healthy people. Haplotype CT (1236*C/3435*T) was considered as a reference. Haplotype occurrence has appeared in Table 4. The incidence of TT haplotype (1236*T/3435*T) had reduced in individuals with hepatotoxicity than HIV-infected (0.05% versus 0.22%, OR = 0.16, 95%CI: 0.04–0.059, P = 0.0065). However, in a similar investigation, the incidence of TC haplotype (1236*T/3435*C) was increased in individuals with hepatotoxicity than HIV-infected (0.30% versus 0.11%, OR = 1.96, 95%CI: 0.98–3.94, P = 0.06). The commonness of CC (1236*C/3435*C) and TT (1236*T/3435*T) haplotypes was reduced in peoples with hepatotoxicity than healthy (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). The incidence of TC (1236*T/3435*C) haplotype was predominantly higher in patients with hepatotoxicity when contrasted with healthy (0.30% versus 0.13%, OR = 1.94, 95%CI: 0.87–4.37, P = 0.11). The incidence of *MDR1* haplotypes was not distinguished between individuals of HIV contamination and healthy.

Table 4

Incidence of *MDR1* haplotypes (1236C/T and 3435C/T) in individual of ARV- related hepatotoxicity, HIV infection and healthy controls

Haplotype <i>MDR1</i> (1236 C/T and 3435 C/T)	Individual of ARV- related hepatotoxicity (N = 68)	Individual of HIV infection (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 <i>MDR1</i> (1236 C/T and 3435 C/T)	Individual of ARV- related 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 <i>MDR1</i> (1236 C/T and 3435 C/T)	Individual of HIV infection (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 HIV- patients with hepatotoxicity (68), 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)				

Table 5
Occurance of *MDR1* (1236 C/T and 3435 C/T) genotypes in different HIV stages of HIV and healthy controls

Genotype <i>MDR1</i> (1236 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	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.							

3.4 *MDR1* polymorphisms and stages of HIV-1

The incidence of *MDR1* polymorphism between stages of HIV infection and healthy people was examined as outlined in table-5. A reduced frequency of *MDR1* 1236TT genotype was found in individuals with an intermediate stage of HIV than healthy (24.2% versus 41.94%, OR = 0.43, P = 0.09). The incidence of *MDR1* 3435CT and 3435TT genotypes did not vary between individuals of stages of HIV infection and healthy.

3.5 Gene-environment interaction

The dispersion of *MDR1* polymorphism between individuals with hepatotoxicity and HIV infected utilizing tobacco, alcohol, and NNRTI regimen and nonusers appeared in Tables 6–9. The occurrence of polymorphisms of *MDR1* (1236C/T and 3435C/T) was not varied between tobacco utilizing people with hepatotoxicity, HIV infected versus nonusers. *MDR1* 1236TT genotype was overrepresented in tobacco utilizing hepatotoxicity patients than nonusers (28.6% versus 14.8%) (Table-6). The incidence of

*MDR1*1236TT genotype was higher in alcohol devouring people with hepatotoxicity than nonusers (28.6% versus 14.8%, OR = 1.50, 95% CI: 0.13–17.35, P = 0.88). Moreover, an increased incidence of 3435CT genotype of *MDR1* was observed in HIV-infected people who consumed alcohol than nonusers (50.0% versus 32.2%, OR = 2.47, 95%CI: 0.79–7.70, P = 0.12) (Table-7). The genotype 1236TT of *MDR1* was greater in nevirapine taking HIV-contaminated individuals than efavirenz users (14.1% versus 8.7%, OR = 1.93, 95%CI: 0.39–9.45, P = 0.42). *MDR1* 1236TT genotype distribution was increased in nevirapine taking patients with hepatotoxicity than efavirenz users (21.7% versus 9.1%, OR = 2.11, 95%CI: 0.18–24.66, P = 0.55). The occurrence of *MDR1* 1236CT 1236TT genotypes was higher in HIV-infected people taking 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). In both alcohol and nevirapine taking hepatotoxicity patients, the dispersion of *MDR1* 1236TT genotype appeared greater when contrasted to alcohol nonusers and nevirapine users (40.0% versus 16.67%, OR = 2.21, 95%CI: 0.17–29.21, P = 0.55).

Table 6

Incidence of MDR1 (1236 C/T and 3435 C/T) genotypes in individuals of ARV related hepatotoxicity and HIV infected utilizing tobacco and non-users

Genotype <i>MDR1</i> (1236 C/T)	Tobacco users N = 7 (%)	Tobacco non- users N = 27 (%)	P- Value	OR(95%CI)
Individuals of ARV related 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 <i>MDR1</i> (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 of HIV infection				
Genotype <i>MDR1</i> (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 <i>MDR1</i> (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

Frequency distribution of MDR1 (1236 C/T and 3435 C/T) genotypes in hepatotoxicity and HIV patients with alcohol users and non-users

Genotype <i>MDR1</i> (1236 C/T)	Alcohol users N = 7 (%)	Alcohol non-users N = 27 (%)	P- Value	OR(95%CI)
Individuals of ARV related 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 <i>MDR1</i> (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 of HIV infection				
Genotype <i>MDR1</i> (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 <i>MDR1</i> (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 <i>MDR1</i> 1236 C/T and 3435 C/T) with other genotypes.				

Table 8

Incidence of MDR1 (1236 C/T and 3435 C/T) genotypes in individuals of ARV- related hepatotoxicity and HIV infected with NNRTIs regimen users and non-users

Genotype MDR1 (1236 C/T)	Total Individuals of HIV infection with nevirapine users N = 142 (%)	Total Individuals of HIV infection with efavirenz users 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)	Total Individuals of HIV infection with with nevirapine users N = 142 (%)	Total Individuals of HIV infection with with efavirenz users N = 23 (%)	P- Value	OR(95%CI)
CC	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 of ARV- related hepatotoxicity				
Genotype MDR1 (1236 C/T)	Nevirapine users N = 23 (%)	Efavirenz users 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)

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)	Total Individuals of HIV infection with nevirapine users N = 142 (%)	Total Individuals of HIV infection with efavirenz users N = 23 (%)	P- Value	OR(95%CI)
Genotype <i>MDR1</i> (3435 C/T)	Nevirapine users N = 23 (%)	Efavirenz users N = 11 (%)	P- Value	OR(95%CI)
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 of HIV infection				
Genotype <i>MDR1</i> (1236 C/T)	Nevirapine users N = 119 (%)	Efavirenz users 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 <i>MDR1</i> (3435 C/T)	Nevirapine users N = 119 (%)	Efavirenz users 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 in individuals of ARV- related hepatotoxicity and HIV infected with alcohol and NNRTI regimen 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 of ARV- related 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 of HIV infected				
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.				

The incidence of 3435CT genotype of *MDR1* was greater in nevirapine and alcohol consumed HIV-infected individuals than nevirapine users and alcohol nonusers (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 relationship between *MDR1* polymorphism and ARV-related hepatotoxicity from Western India. *MDR1* encodes for the ATP- dependent membrane efflux transporter (14). The genetic variants that impact patients drugs are substrates of P-glycoproteins. The occurrence of *MDR1* polymorphism changes from the population to the population [17]. We analyzed the *MDR1* genotypes and haplotypes between individuals infected with HIV and hepatotoxicity.

The occurrence of *MDR1* 3435C/T polymorphism in our healthy people was identical to the investigations done in European, North India, Turkish, and Asian populations [29–34] and contrasted with the examinations did in Chinese, Iranian and Thailand populations [26, 32, 35]. The genotypic dispersal of *MDR1* 1236C/T polymorphism in our healthy people was almost alike to the investigation announced from North India [34], however, contrasted with the examinations shown from Indians, Mexicans, Chinese and South Africans populations [18, 30, 37, 38]. We have done the genotype-phenotype analysis and found that the *MDR1*1236TT genotype was displayed a hazard for hepatotoxicity severity (OR = 1.37, P = 0.57). However, due to the small sample size in the hepatotoxicity group, the risk could not reach statistical significance. The low phenotypic expression is dictated by common polymorphisms inside P-gp. People with 3435TT genotype were connected with lower levels of P-gp than CC and CT genotype. *MDR1* 3435C/T polymorphism was related with the reduced risk of NNRTI-induced liver toxicity [24].

We have examined the relationship of gene-gene interaction to understand the additive impact of *MDR1* polymorphism on ARV-related hepatotoxicity. The gene-gene collaboration had a greater effect on gene expression than a single gene [39]. In our investigation, haplotype TC was shown with a greater risk for the severity of hepatotoxicity (OR = 1.96, P = 0.06). While, haplotypes TT and CC were linked with reduced risk of severity of 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). It is likely that individuals with haplotype TC may have prone to the severity of hepatotoxicity, whereas haplotypes TT and CC may have reduced risk for hepatotoxicity severity.

Likewise, we analyzed the relationship between the *MDR1* genotype and the stage of HIV infection. In our investigation, the prevalence of *MDR1* genotypes did not significantly vary between people of various stages of HIV and healthy. *MDR1* 1236CT, 1236TT, and 3435CT genotypes were correlated with the HIV disease progression, however, these polymorphisms did not regulate the susceptibility of HIV-1 [40]. Following the treatment of a half year, patients with 3435 TT genotype had raised the CD4 + count [22].

Analysis of the relationship of gene environment was done to take a look at the impact on the etiology of the disease [41, 42]. We had selected a case-only analysis. We did not examine the case-control in light of the fact that in the case-control investigation, cases must be coordinated with the controls in the population [43]. Hence, we utilized the case-only analysis. HIV patient's naïve ART utilizing alcohol has demonstrated a reduction in CD4 + cell count [44]. In women with HIV disease and tobacco utilization, ART response was seen to be diminished [45]. In our examination, the patients of hepatotoxicity utilizing alcohol with *MDR1*1236TT genotype exposed a hazard for severity of hepatotoxicity (OR = 1.50, P = 0.88), while individuals of HIV contamination using alcohol with 3435CT genotype were at higher danger of HIV disease progression (OR = 2.47, P = 0.12). In hepatotoxic patients utilizing nevirapine and *MDR1*, 1236TT genotype demonstrated a higher threat for the hepatotoxicity severity (OR = 2.11, P = 0.55).

Individuals with HIV infection and utilizing nevirapine with *MDR1* 1236CT, 1236TT genotypes exposed a risk for the progression of HIV infection (OR = 1.66, P = 0.45; OR = 1.96, P = 0.55). In people with hepatotoxicity who took both alcohol and nevirapine, *MDR1* 1236TT genotype exposed the higher vulnerability for hepatotoxicity severity (OR = 2.21, P = 0.55). In people having an infection of HIV with *MDR1* 3435CT genotype and taking both nevirapine and alcohol showed a vulnerability for the progression of HIV infection (OR = 2.04, P = 0.23). This suggests that individuals with *MDR1* 1236TT and 3435CT genotypes having HIV infection or ARV-related hepatotoxicity have an additive effect on vulnerability of hepatotoxicity severity and progression of HIV disease. Individuals with 3435 T allele having infection of HIV taking nevirapine were connected with the reduced risk of hepatotoxicity [25]. People with *MDR1* 1236T and 1235T alleles were related to diminished plasma NNRTI concentration influencing the virological response to HAART [21]. Haas et al., (2005) recommended no significant relationship between *ABCB1* variations and plasma EFV concentrations [19].

This work has a few limit points, it can just assess association and not indicate causation. In the beginning, the present investigation was planned for a 1:4 proportion of case controls. However, we were not able to complete to select a similar proportion of controls. Though, we recruited a 1:3 proportion which may be sufficient.

Conclusions

MDR1 haplotypes may have impact on hepatotoxicity severity. Individual with *MDR1* 1236TT and 3435CT genotypes in presence of alcohol and nevirapine had an additive effect for vulnerability of

severity of hepatotoxicity and progression of HIV disease.

MDR1 is associated with drug clearance. *MDR1* expression differed in response to NVP and EFV administration. Hence, further examination of the relationship between *MDR1* polymorphism and plasma drug concentration would be done with a bigger sample size in different populations. In addition, the correlation of polymorphisms of other drug transporter genes with plasma drug levels is required to comprehend the effect of genetic variants on treatment effect.

Declarations

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Availability of data and material: On request by email to Corresponding Author

Abbreviation:

HIV, Human immunodeficiency virus; MDR-1; Multidrug-resistant-1, ABCB1, ATP binding cassette subfamily B member 1; NNRTIs, Non-nucleoside reverse transcriptase inhibitors; ARV, Antiretroviral; *ART*, Antiretroviral therapy; NVP, Nevirapine; EFV, Efavirenz; SNP, Single nucleotide polymorphism; PCR-RFLP, Polymerase chain reaction- Restriction fragment length polymorphisms; DILI, drug-induced liver injury; ADR, Adverse drug reaction; LFT, Liver function test; SGOT, Serum glutamic oxaloacetic transaminase; SGPT, Serum glutamic pyruvic transaminase; ELISA, Enzyme-linked immunosorbent assay; ABC, ATP-binding cassette

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Author's contribution

HS: Overall supervision

DS: Experimental work

VC: Manuscript writing

TN D: Clinical input and Critical Review of manuscript

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