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

HariOm Singh (hariomsgpgims@gmail.com)
National AIDS Research Institute
Dharmesh Samani
National AIDS Research Institute
Vijay Chauware
National AIDS Research Institute
T.N Dhole
Sanjay Gandhi Post Graduate Institute of Medical Sciences

Research article

Keywords: ARV-associated hepatotoxicity, Genetic predisposition, NNRTI regimen, Multidrug-resistant-1, HIV Infected Individuals

Posted Date: November 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-100712/v1

Abstract

Background: The multidrug resistance protein, MDR1 is involved in the transport of numerous drugs. Polymorphism of *MDR1* is linked with the treatment outcome. Antiretroviral (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* polymorphisms (1236 C/T, 3435 C/T) was genotyped in 34 HIV-infected individuals with ARV-associated hepatotoxicity, 131 HIV-infected individuals without ARV-associated hepatotoxicity, and one-fifty-five healthy individuals by utilization of PCR-RFLP.

Results: The incidence of haplotype TC of *MDR1* was found to be more in HIV infected individuals with hepatotoxicity than the non-hepatotoxic ones, thus indicating a greater risk for hepatotoxicity severity (OR=1.96, P=0.06). Whereas the haplotypes TT and CC were found to be linked with a reduced risk for hepatotoxicity severity (OR= 0.16, P=0.006; OR= 0.46, P=0.06). 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 on 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 on nevirapine, the incidence of *MDR1* 1236CT, 1236TT genotypes was found to be more as compared to the ones on efavirenz (43.7% versus 33.3%, OR=1.66; 12.6% versus 8.3%, OR=1.96). Also a higher occurrence of *MDR1* 1236TT genotype was found in the hepatotoxicity cases on nevirapine, who consume alcohol as compared to the alcohol nonusers on nevirapine (40.0% versus 16.67%, OR=2.21).

Conclusion: Haplotype TC was associated with the increased severity of hepatotoxicity because of synergistic effect. In the HIV infected individuals on nevirapine who consume alcohol, the presence of *MDR1* 1236TT and 3435CT genotypes may have a combined effect on vulnerability to hepatotoxicity severity and progression of HIV infection.

1. 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 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. 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. proposed that *MDR1* 3435C/T polymorphism was not linked to efavirenz exposure [19]. Salem et al. suggested that *MDR1* 3435C/T polymorphism had no impact on efavirenz clearance [21]. Zhu et al. 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 [22]. Leschziner et al. showed that *MDR1* 3435 TT genotype were linked with higher adverse outcomes of 3TC (Lamivudine) and NVP treatment than EFV treatment [23]. Ritchie et al. indicated a significantly 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 has also been documented by studies [24, 25].

Few studies have shown a link between *MDR1* polymorphism and adverse outcomes of ARV drugs whereas, other studies suggest no correlation. Moreover, the link between *MDR1* polymorphism and ARV-related hepatotoxicity has not been reported from India. Thus, we have analyzed the relationship between *MDR1* (1236C/T and 3435C/T) polymorphisms and hepatotoxicity induced by ARV regimen.

2. 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 positive individuals on ARV therapy showing hepatotoxicity. The male patients with SGOT>93.8 U/ml, Alkaline phosphatase

>550.8 U/ml, total bilirubin >3. 22mg /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. 22mg /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 control group was age matched and recruited. Control population for the HIV positive group, tested negative for HIV, 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. 24mg /ml, and SGPT<34 U/ml were considered as the control population for the group showing ARV therapy associated hepatotoxicity. 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. FACS analysis was utilized to estimate the CD4+ count. CD4+ cell counts of <200 cells/mm3, 201-350 cells/mm3, and >350cells/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^{0}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 [26]. PCR was performed in a volume of 20µl. 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 polymorphisms. The sequences and location of SNPs were employed for genotyping of *MDR1* C1236T and C3535T polymorphisms. Genotypes for *MDR1* C1236T were: 93kb 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. Additional staff of the laboratory was did the re-genotyping in 20% of the samples to check the disparities in genotyping. 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 ± 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 (25). Linkage disequilibrium (LD) was analyzed between both the loci by computing the relative LD value (D') as D' = Dij/ Dmax (28). The Dij value was compared among various groups by the comparison of confidence intervals.

3. 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 hepatotoxicity and constituted the first case group of HIV positive individuals showing ARV therapy associated hepatotoxicity. The remaining 131 individuals didn’t show hepatotoxicity (by LFT) and formed the second study group of HIV positive individuals on ARV therapy without hepatotoxicity. Control population consisted of 155 healthy people. The demographic profiles of the participants are outlined in table 1. The mean age ± SD of HIV infected individuals without hepatotoxicity, with HIV hepatotoxicity and control population was 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.

3.1 MDR1 polymorphisms in the cases with hepatotoxicity versus the cases without hepatotoxicity and control population

The incidence of polymorphisms of *MDR1* in the two study populations is shown in table 2. *MDR1* polymorphisms were not found to be distinct between HIV infected populations with and without hepatotoxicity. Although, among the cases with hepatotoxicity, the predominance of *MDR1* 1236TT genotype was more as compared to the non-hepatotoxic cases (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 cases with hepatotoxicity as compared to the non-hepatotoxic 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). Also, *MDR1* polymorphism were not significantly different between the individuals with ARV associated hepatotoxicity and the healthy controls.

3.2 MDR1 polymorphism in the HIV-infected people without hepatotoxicity versus control population

The occurrence of polymorphisms of *MDR1* (1236C/T, 3435C/T) in people with HIV infection verus 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 HIV-infected people (without hepatotoxicity) and healthy population. Although, 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.

3.3 Haplotypes distribution

We have likewise investigated the occurrence of different *MDR1* haplotypes among the two study groups and the 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 the HIV-infected individuals with hepatotoxicity than their non-hepatotoxic counterparts (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 individuals with hepatotoxicity than the non-hepatotoxic ones (0.30% versus 0.11%, OR=1.96, 95%CI: 0.98-3.94, P=0.06). This suggest that haplotype TC was associated with increased 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 the people with hepatotoxicity than healthy individuals (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 patients with hepatotoxicity compared to the control population (0.30% versus 0.13%, OR=1.94, 95%CI: 0. 87-4.37, P=0.11). The incidence of *MDR1* haplotypes among the HIV infected individuals without hepatotoxicity was not significantly different from the healthy population

3.4 MDR1 polymorphisms and stages of HIV-1

The incidence of *MDR1* polymorphism among people 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 control population (24.2% versus 41.94%, OR=0.43, P=0.09). *MDR1* 1236TT genotype was likely to be related with the decreased risk for progression of HIV disease. The incidence of *MDR1* 3435CT and 3435TT genotypes did not vary among the individuals in different stages of HIV infection and healthy population.

3.5 Gene-environment interaction

The distribution of *MDR1* polymorphisms among HIV infected individuals with and without hepatotoxicity and the 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 people consuming tobacco in both the study populations and the control group. *MDR1* 1236TT genotype was overrepresented among the tobacco consumers than the non-consumers in the hepatotoxic group (28.6% versus 14.8%) (Table-6). Also its occurrence was higher among the alcohol consumers than the non-consumers in the hepatotoxic 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 HIV-infected non-hepatotoxic people (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 non-hepatotoxic individuals 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 patients on nevirapine with hepatotoxicity than efavirenz users (21.7% versus 9. 1%, OR= 2.11, 95%CI: 0. 18 - 24.66, P=0.55). This suggest that *MDR1*1236TT genotype along with nevirapine usage was likely to be related with the increased risk for *severity of hepatotoxicity because of combined effect of gene polymorphism and environment.* The occurrence of *MDR1* 1236CT and 1236TT genotypes were also higher in the non-hepatotoxic HIV-infected people 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 HIV-infected individuals with hepatotoxicity, 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 *MDR1* 1236TT genotype with nevirapine and consumed alcohol was likely to be associated with increased *severity of hepatotoxicity because of combined effect of gene polymorphism and environment.* The incidence of 3435CT genotype of *MDR1* was greater among the alcohol consumers on navirapine than alcohol non-consumers on navirapine, in the non-hepatotoxic group (44.74% versus 33.33%, OR= 2.04, 95%CI: 0. 64 - 6.53, P=0. 23) (Table 9).

4. 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 patient drugs are substrates of P-glycoproteins. The occurrence of *MDR1* polymorphism changes

from the population to population [17]. We analyzed the *MDR1* genotypes and haplotypes among the HIV infected individuals on ARV therapy and a subclass of those showing hepatotoxicity.

We found that the occurrence of *MDR1* 3435C/T polymorphism in our control population is identical to the investigations done in European, North Indian, Turkish, and Asian populations [29–34] and contrasted with the similar studies done in the Chinese, Iranian and Thailand populations [26, 32, 35]. Also, the genotypic dispersal of *MDR1* 1236C/T polymorphism in our healthy people was almost alike to the similar investigations in the North Indian population [34]. However, it contrasted from the Mexican, Chinese and South African populations [18, 30, 37, 38]. We have done a genotype-phenotype analysis and found that the *MDR1*1236TT is likely to be related with the increase the severity of hepatotoxicity (OR = 1.37, P = 0.57). However, due to the small sample size of the group showing hepatotoxicity, 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 [24].

We also studied the gene-gene interactions to understand the synergistic impact of *MDR1* polymorphism on ARV-related hepatotoxicity. The gene-gene interactions are known to have greater effects on gene expression than a single gene [39]. In our investigation, haplotype TC was associated with a greater risk for the severity of hepatotoxicity (OR = 1.96, P = 0.06); whereas, the haplotypes TT and CC were related with a reduced risk (OR = 0.16, P = 0.006; OR = 0.46, P = 0.06; OR = 0.09, P = 0.003; OR = 0.34, P = 0.03). Thus it is likely that the individuals possessing haplotype TC are more prone to a severe NNRTI-induced-hepatotoxicity and the ones with TT and CC haplotypes may have reduced risk for acquiring hepatotoxicity.

Likewise, we analyzed the relationship between the *MDR1* genotypes and the stage of HIV infection. In our investigation, the incidence of *MDR1* genotypes did not vary significantly among people in 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 [40]. Additionally, the patients with 3435 TT genotype had an increase in the CD4 + counts, following a treatment for half an year [22].

An analysis of the gene-environment interaction helps to know its impact on the disease etiology [41, 42]. We did a case-only analysis to study the gene-environment interaction. We did not rather go for a case-controlled analysis in light of the fact that a case-control investigation, requires a coordination of cases with the control population [43]. HIV patients who are naïve to ARV therapy and consume alcohol have been found to have a reduction in the CD4 + cell count [44]. Also, in the HIV infected women who consume tobacco, a diminished response to ARV therapy has been observed [45].

In our study, in the patients with hepatotoxicity, who consume alcohol, *MDR1*1236TT genotype exposed a risk for severe hepatotoxicity (OR = 1.50, P = 0.88). Among the patients who did not have NNRTI induced hepatotoxicity and used alcohol, the incidence of 3435CT genotype posed a higher risk of HIV disease progression (OR = 2.47, P = 0.12) because of combined effect of gene and environment. Among the

hepatotoxic patients on nevirapine, presence of *MDR1* 1236TT genotype was likely to be associated with the higher threat for the severe hepatotoxicity (OR = 2.11, P = 0.55). Whereas among the non-hepatotoxic individuals on nevirapine, *MDR1* 1236CT, 1236TT genotypes exposed a higher risk of HIV disease progression (OR = 1.66, P = 0.45; OR = 1.96, P = 0.55). In people with hepatotoxicity on nevirapine who consume, *MDR1* 1236TT genotype exposed a higher vulnerability to severe hepatotoxicity (OR = 2.21, P = 0.55) because of combined effect of gene and environment. In the HIV infected patients without nevirapine-linked-hepatotoxicity, who consume alcohol, *MDR1* 3435CT genotype showed a vulnerability for the progression of HIV infection (OR = 2.04, P = 0.23). This suggests that HIV infected individuals with *MDR1* 1236TT and 3435CT genotypes with or without ARV-related hepatotoxicity may have a combined vulnerability to hepatotoxicity severity and progression of HIV disease. Also, individuals on nevirapine, possessing 3435 T allele has a reduced risk of hepatotoxicity [25]. People 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 relationship between *ABCB1* variations and plasma EFV concentrations [19].

The fact that this work can just assess 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 may have a synergistic impact on the severity of ARV therapy linked hepatotoxicity in the HIV infected people. In the patients on nevirapine who consume alcohol, *MDR1* 1236TT and 3435CT genotypes, had a combined effect on vulnerability to hepatotoxicity severity 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

Acknowledgment

We greatly appreciate and acknowledge the clinic in-charges and other supporting staff of NARI for the recruitment of study participants. I would like to thank Kamini Jakhar for helping in editing the manuscript.

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

Consent for publication: Yes

Source of funding: study was not supported by any extramural funds.

Competing interests: None

Conflict of interests: Nil

Ethical approval: NARI/EC/ICF version 1.0, dated 28 August 2013

Consent to Participate: Taken

Author's contribution

HS: Overall supervision

DS: Experimental work

VC: Manuscript writing

TN D: Clinical input and Critical Review of manuscript

All authors read and approved the final manuscript: Yes

References

1. O'Brien ME, Clark RA, Besch CL, Myers L, Kissinger P. (2003). Patterns and correlates of discontinuation of the initial HAART regimen in an urban outpatient cohort. *J Acquir Immune Defic Syndr,* 34:407-14.

2. Van Dyke RB, Wang L, Williams PL, for the Pediatric ACTGCT. (2008). Toxicities Associated with Dual Nucleoside Reverse-Transcriptase Inhibitor Regimens in HIV-Infected Children. The *Journal of Infectious Diseases*, 198:1599-608.
3. Minzi OM, Irunde H, Moshiro C. (2009) .HIV patients presenting common adverse drug events caused by highly active antiretroviral therapy in Tanzania. *Tanzan J Health Res,* 11:5-10.
4. Nagpal M, Tayal V, Kumar S, Gupta U. (2010). Adverse drug reactions to antiretroviral therapy in AIDS patients at a tertiary care hospital in India: A prospective observational study. *Indian J Med Sci.* 64:245-52.

5. Mascolini M. (2001). HIV news from Buenos Aires: Part 1-the Zahir and the band-aid. 1st IAS conference on HIV pathogenesis and treatment; July 8-11; Buenos Aires: *APAC Mon*, 274-89.
6. Croop JM. (1993) P-glycoprotein structure and evolutionary homologies. *Cytotechnology,* 12:1-32.
7. Jones PM, George AM. (2004). The ABC transporter structure and mechanism: perspectives on recent research. *Cell Mol Life Sci.*61:682-99.

8. Rosenberg MF, Callaghan R, Ford RC, Higgins CF. (1997). Structure of the multidrug resistance P-glycoprotein to 2.5 nm resolution determined by electron microscopy and image analysis. *J Biol Chem,* 272:10685-94.
9. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR. (1989) Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A*, 86:695-8.
10. Pileri SA, Sabattini E, Falini B, Tazzari PL, Gherlinzoni F, Michieli MG, Damiani D, Zucchini L, Gobbi M, Tsuruo T, et al.(1991). Immunohistochemical detection of the multidrug transport protein P170 in human normal tissues and malignant lymphomas. *Histopathology,* 19:131-40.
11. Sakaeda T, Nakamura T, Okumura K. (2003). Pharmacogenetics of MDR1 and its impact on the pharmacokinetics and pharmacodynamics of drugs. *Pharmacogenomics.*4:397-410.
12. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu Rev Pharmacol Toxicol,* 39:361-98.
13. Sugawara I, Kataoka I, Morishita Y, Hamada H, Tsuruo T, Itoyama S, Mori S. (1988) Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK 16. *Cancer Res*, 48:1926-9.
14. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 84:7735-8.
15. Stormer E, von Moltke LL, Perloff MD, Greenblatt DJ.(2002). Differential modulation of Pglycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. *Pharm Res,* 19:1038-45.
16. Tozzi V. (2010). Pharmacogenetics of antiretrovirals. *Antiviral Res,* 85(1):190-200.
17. Chelule PK, Gordon M, Palanee T, Page T, Mosam A, Coovadia HM, Cassol S. (2003). MDR1 and CYP3A4 polymorphisms among African, Indian, and white populations in KwaZulu-Natal, South Africa. *Clin Pharmacol Ther,* 74:195-6.
18. Dong Q, Xu B, Tan Y, Liu Z, Tian L, Zhang B, Lin CK, Kung HF, Sung JJ, He ML. (2009) The genetic variability of MDR1 C3435T polymorphisms in four Southern Chinese populations. *Biomed Pharmacother,* 63:658-62.
19. Haas DW, Smeaton LM, Shafer RW, Robbins GK, Morse GD, Labbe L, Wilkinson GR, Clifford DB, D'Aquila RT, De Gruttola V, Pollard RB, Merigan TC, Hirsch MS, George AL Jr, Donahue JP, Kim RB. (2005). Pharmacogenetics of long-term responses to antiretroviral regimens containing Efavirenz and/or Nelfinavir: an Adult Aids Clinical Trials Group Study. *J Infect Dis,* 192:1931-42.j
20. Li YH, Wang YH, Li Y, Yang L. (2006) MDR1 gene polymorphisms and clinical relevance. *Acta Genetica Sinica,* 33:93-104.

21. Salem AH, Fletcher CV, Brundage RC. (2014). Pharmacometric characterization of efavirenz developmental pharmacokinetics and pharmacogenetics in HIV-infected children. *Antimicrob Agents Chemother,* 58:136-43.

22. Fellay J, Marzolini C, Meaden ER, Back DJ, Buclin T, Chave JP, Decosterd LA, Furrer H, Opravil M, Pantaleo G, Retelska D, Ruiz L, Schinkel AH, Vernazza P, Eap CB, Telenti A;Swiss HIV Cohort Study. (2002). Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet,* 359:30-6.

23. Leschziner GD, Andrew T, Pirmohamed M, Johnson MR. (2007). ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.*7:154-79.

24. Ritchie MD, Haas DW, Motsinger AA, Donahue JP, Erdem H, Raffanti S, Rebeiro P, George AL, Kim RB, Haines JL, Sterling TR. (2006) Drug transporter and metabolizing enzyme gene variants and nonnucleoside reverse-transcriptase inhibitor hepatotoxicity. *Clin Infect Dis,* 43:779-82.

25. Haas DW, Bartlett JA, Andersen JW, Sanne I, Wilkinson GR, Hinkle J, Rousseau F, Ingram CD, Shaw A, Lederman MM, Kim RB; Adult AIDS Clinical Trials Group. (2006) Pharmacogenetics of nevirapine-associated hepatotoxicity: an Adult AIDS Clinical Trials Group collaboration. *Clin Infect Dis,* 43:783-6.

26. Pongstaporn W, Pakakasama S, Chaksangchaichote P, Pongtheerat T, Hongeng S, Permitr S. (2015). MDR1 C3435T and C1236T polymorphisms: association with high-risk childhood acute lymphoblastic leukemia. *Asian Pac J Cancer Prev*, 16:2839-43.

27. Sole X, Guino E, Valls J, Iniesta R, Moreno V. (2006). SNPStats: a web tool for the analysis of association studies. *Bioinformatics,* 22:1928-9.

28. Cox A, Camp NJ, Nicklin MJ, di Giovine FS, Duff GW. (1998). An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers. *Am J Hum Genet, 62:1180-*8

29. Ghodke Y, Chopra A, Shintre P, Puranik A, Joshi K, Patwardhan B. (2011). Profiling single nucleotide polymorphisms (SNPs) across intracellular folate metabolic pathway in healthy Indians. *Indian J Med Res,* 133:274-9.

30. Lakhan R, Misra UK, Kalita J, Pradhan S, Gogtay NJ, Singh MK, Mittal B. (2009). No association of ABCB1 polymorphisms with drug-refractory epilepsy in a north Indian population. *Epilepsy Behav,* 14:78-82.

31. Rubis B, Holysz H, Barczak W, Gryczka R, Lacinski M, Jagielski P, et al. (2012). Study of ABCB1 polymorphism frequency in breast cancer patients from Poland. *Pharmacol Rep,* 64(6):1560-6.

32. Shi NJ, Zhang WX, Zhang N, Zhong LN, Wang LP. (2017). Correlation of MDR1 gene polymorphisms with anesthetic effect of sevoflurane-remifentanil following pediatric tonsillectomy. *Medicine (Baltimore),* 96:e7002.

33. Tan EK, Chan DK, Ng PW, Woo J, Teo YY, Tang K, et al. (2005). Effect of MDR1 haplotype on risk of Parkinson disease. Arch Neurol, 62(3):460-4.

34. Rustemoglu A, Gumus-Akay G, Yigit S, Tasliyurt T. (2011). Analysis of common MDR1 (ABCB1) gene C1236T and C3435T polymorphisms in Turkish patients with familial Mediterranean fever. *Genet Mol Res.*10:3411-20.
35. Saidijam M, Mahjub H, Shabab N, Yadegarazari R. (2015).Simultaneous analysis of multidrug resistance 1(MDR1) C3435T, G2677T/A, and C1236T genotypes in Hamadan City population, West of Iran. *Iran Biomed J,* 19:57-62.
36. Jafar T, Prasad N, Agarwal V, Mahdi A, Gupta A, Sharma RK, et al. (2011). MDR-1 gene polymorphisms in steroid-responsive versus steroid-resistant nephrotic syndrome in children. *Nephrol Dial Transplant*, 26:3968-74.
37. Gutierrez-Rubio SA, Quintero-Ramos A, Duran-Cardenas A, Franco-Topete RA, Castro-Cervantes JM, Oceguera-Villanueva A, et al. (2015). 1236 C/T and 3435 C/T polymorphisms of the ABCB1 gene in Mexican breast cancer patients. *Genet Mol Res*, 14:1250-9.
38. Masebe TM, Bessong PO, Nwobegahay J, Ndip RN, Meyer D. (2012). Prevalence of MDR1 C3435T and CYP2B6 G516T polymorphisms among HIV-1 infected South African patients. *Dis Markers,* 32:43-50.
39. Palmer LJ, Cardon LR. (2005). Shaking the tree: mapping complex disease genes with linkage disequilibrium. *Lancet,* 366:1223-34.
40. Bellusci CP, Rocco CA, Aulicino PC, Mecikovsky D, Bologna R, Sen L, et al. (2010). MDR1 3435T and 1236T alleles delay disease progression to pediatric AIDS but have no effect on HIV-1 vertical transmission. *AIDS,* 24:833-40.
41. Deng Y, Newman B, Dunne MP, Silburn PA, Mellick GD.(2004). Case-only study of interactions between genetic polymorphisms of GSTM1, P1, T1 and Z1 and smoking in Parkinson's disease. *Neurosci Lett.* 366:326-31.
42. Hunter DJ, Hankinson SE, Hough H, Gertig DM, Garcia-Closas M, Spiegelman D, et al. (1997). A prospective study of NAT2 acetylation genotype, cigarette smoking, and risk of breast cancer. *Carcinogenesis,* 18:2127-32.
43. Greenland S. (1980) The effect of misclassification in the presence of covariates. *Am J Epidemiol,* 112:564-9.
44. Samet JH, Cheng DM, Libman H, Nunes DP, Alperen JK, Saitz R. (2007). Alcohol consumption and HIV disease progression. J Acquir Immune Defic Syndr.46:194-9.
45. Feldman JG, Minkoff H, Schneider MF, Gange SJ, Cohen M, Watts DH, et al. (2006). Association of cigarette smoking with HIV prognosis among women in the HAART era: a report from the women's interagency HIV study. *Am J Public Health*. 96(6):1060-5.

Tables

Table 1: Characteristics of the study populations

Subjects	HIV infected individuals with ARV associated hepatotoxicity	HIV infected individuals without hepatotoxicity	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

Table 2: Incidence of *MDR1* (1236 C/T and 3435 C/T) genotypes/alleles among the HIV infected individuals with ~~of~~ ARV associated hepatotoxicity and HIV infected individuals without hepatotoxicity

Genotype *MDR1* (1236 C/T)	**HIV infected individuals with ARV associated hepatotoxicity N= 34 (%)**	**HIV infected 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**	**HIV infected individuals with ~~of~~ ARV ~~related~~ associated 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 associated 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 associated 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)
Genotype *MDR1* (1236 C/T)	**Individuals with ARV associated 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)
MDR1 **(1236 C/T) Allele**	**Individuals with ARV associated hepatotoxicity**	**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 *MDR1* (3435 C/T)	**I Individuals with ARV associated 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)
MDR1 **(3435 C/T) Allele**	**Individuals with ARV associated 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 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.

Table 3: Incidence of *MDR1* (1236 C/T and 3435 C/T) genotypes/alleles in HIV infected individuals without hepatotoxicity versus healthy controls.

Genotype *MDR1* (1236 C/T)	HIV infected individuals 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)
MDR1 (1236 C/T) Allele	HIV infected individuals 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 *MDR1* (3435 C/T)	HIV infected individuals 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)
MDR1 (3435 C/T) Allele	HIV infected individuals without hepatotoxicity N= 262 (%)	Healthy controls N= 310	*P*- 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 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.

Table 4: Incidence of *MDR1* haplotypes (1236C/T and 3435C/T) in HIV infected individuals with and without ARV associated hepatotoxicity and healthy controls

Haplotype *MDR1* (1236 C/T and 3435 C/T)	**Individuals with ARV associated hepatotoxicity y (N = 68)**	**Individuals without hepatotoxicity (N = 262)**	***P*- 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 associated hepatotoxicity (N = 68)**	**Healthy controls (N=310)**	***P*- 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 without hepatotoxicity(N= 262)**	**Healthy controls (N=310)**	***P*- 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 *P* values are shown in bold ($P<0.05$)

Table 5. Occurance of *MDR1* (1236 C/T and 3435 C/T) genotypes among individuals in different stages of HIV infection versus healhty controls

Genotype *MDR1* (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 *MDR1* (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 *MDR1* 1236 C/T and 3435 C/T) with other genotypes.

Table 6. Incidence of *MDR1* (1236 C/T and 3435 C/T) genotypes among HIV infected individuals with and without ARV induced hepatotoxicity (subclassified as tobacco users and non-users)

Genotype *MDR1* (1236 C/T)	Tobacco users N= 7 (%)		Tobacco non-users N= 27 (%)	*P*-Value	OR(95%CI)
HIV infected individuals with ARV 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)
HIV infected individuals without ARV induced 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 43 (%)**	**N=**	**Tobacco non-user N= 88 (%)**	***P*-Value**	**OR(95%CI)**
CC	7 (16.3%)		17 (19.3%)	-	1 (Reference)
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 *MDR1* 1236 C/T and 3435 C/T) with other genotypes.

Table 7. Frequency distribution of *MDR1* (1236 C/T and 3435 C/T) genotypes among HIV infected individuals with and without ARV induced hepatotoxicity (subclassified as alcohol users and non-users)

Genotype *MDR1* (1236 C/T)	Alcohol users N= 7 (%)	Alcohol non-users (%) N= 27	*P*-Value	OR(95%CI)
HIV infected individuals with ARV 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)
HIV infected individuals 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. Incidence of *MDR1* (1236 C/T and 3435 C/T) genotypes among HIV infected individuals with and without ARV induced hepatotoxicity (subclassified as NNRTIs users and non-users)

Genotype *MDR1* (1236 C/T)	**Individuals without hepatotoxicity on nevirapine N= 142 (%)**	**Individuals without hepatotoxicity on 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 without hepatotoxicity on nevirapine N= 142 (%)**	**Individuals without hepatotoxicity on 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 induced 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)
Genotype *MDR1* (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)

HIV infected individuals without hepatotoxicity				
Genotype *MDR1* (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 *MDR1* (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 *MDR1* 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 *MDR1* (1236 C/T)	**Alcohol+ Nevirapine users N= 5 (%)**	**Nevirapine users+ Alcohol non-user N= 18 (%)**	***P*- Value**	**OR(95%CI)**
HIV infected individuals with ARV 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 *MDR1* (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 *MDR1* (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 *MDR1* (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)
TT	0 (0.0%)	4 (44.44%)	NS	-
HIV infected individuals without hepatotoxicity				
Genotype *MDR1* (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 *MDR1* (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 *MDR1* (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 *MDR1* (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	-
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 *MDR1* 1236 C/T and 3435 C/T) with other genotypes.