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RESEARCH ARTICLE

Polymorphisms in miRNAs Gene (146a, 149, 196a) and Susceptibility to ARV-associated Hepatotoxicity

Hari Om Singh[1,*], Sushma Jadhav[1], Dharmesh Samani[1] and Tapan N. Dhole[1,2]

[1]*Department of Molecular Biology, National AIDS Research Institute, Pune, India;* [2]*Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, 226014-Lucknow, India*

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Abstract: ***Background*:** Micro RNAs act as a regulatory layer for pharmacogenomics-related gene expression. It could play a role in the efficacy and toxicity of the drug. The SNPs in miRNA genes are linked with different functional consequences.

***Methods*:** Hence, we examined the miR (146a G/C, 149C/T, and 196aC/T) polymorphisms in 34 patients with hepatotoxicity, 123 patients without hepatotoxicity, and 155 healthy controls using a PCR-RFLP method.

***Results*:** In patients with hepatotoxicity, miR196aCT genotype and combined genotype GCT showed a risk for hepatotoxicity severity with borderline significance (OR=2.08, P=0.07; OR=2.88, P=0.06). While comparing between patients with hepatotoxicity and healthy controls, the combined genotypes CCC and GCT have shown a susceptibility to hepatotoxicity severity (OR=2.89, P=0.05; OR=2.60, P=0.09). The miR196TT genotype was associated with the individuals of advanced HIV disease stage (OR=3.68, P=0.04). In HIV patients who consumed alcohol and did not have hepatotoxicity, the miR 196aCT genotype showed susceptibility to acquisition of hepatotoxicity with borderline significance (OR=2.36, P=0.06).

***Discussion*:** The miR149TT and 196aTT genotypes showed a risk of acquisition of hepatotoxicity to nevirapine usage among HIV patients without hepatotoxicity (OR=4.19, P=0.07; OR=1.97, P=0.84). In HIV patients with and without hepatotoxicity, the miR 196aCT genotype showed a risk of acquisition of hepatotoxicity and its severity to the combined use of alcohol and nevirapine, respectively (OR=14.18, P=0.08; OR=2.29, P=0.08). In multivariate logistic regression, taking nevirapine, 196aCT genotype had an independent risk factor for hepatotoxicity severity (OR=5.98, P=0.005; OR=2.38, P=0.05).

***Conclusion*:** In conclusion, miR196aC/T polymorphism and combined genotypes GCT and CCC may facilitate the risk for acquisition of hepatotoxicity and its severity.

Keywords: *miRNA* polymorphisms, NNRTI regimen, Genetic predisposition, HIV patients, ARV- associated hepatotoxicity.

1. INTRODUCTION

Undesirable effects have been reported with the use of all Antiretroviral (ARV) drugs [1]. The incidences of grade 3 or 4 hepatotoxicities reported are as follows: 10.8% in the efavirenz-treated group and 8.9% in the nevirapine-treated group, respectively [2]. While in another report, a higher incidence of hepatotoxicity was reported with nevirapine usage than efavirenz use [3, 4]. Nagpal *et al.*, 2010 reported an incidence of nevirapine-induced hepatotoxicity (3.19%) in HIV-infected individuals in India. MicroRNAs (miRNAs) play a central role as a novel regulatory layer affecting drug metabolism and drug targets. The miRNAs bind with 3'UTR of ABCB1 (ATP Binding Cassette Subfamily B member 1). Disruption of miRNAs binding sites could be a potential cause of reduced transport of efavirenz by ABCB1 resulting in lower plasma efavirenz level. The microRNAs regulate the expression of pharmacogenomic-related genes and can play a pivotal role in drug efficacy and toxicity [5]. MiRNAs modulate gene expression at the post-transcriptional level and polymorphisms in *miRNA* sequences related to the progression and development of liver diseases [6]. Cellular miRNA play a critical role in the pathogenesis of HIV, intervening in viral infection, latency and mediating cell intrinsic resistance [7, 8]. MicroRNAs regulate the expression of genes and polymorphisms in the gene found to be related either resistance to HIV-1 infection or progression to AIDS [9].

MicroRNAs are small non-coding RNAs about 17-22 nucleotide in length that regulate gene expression by translational repression or mRNA degradation in many cellular processes [10]. The miRNAs processing results in translational repression because of interaction between miRNAs and mRNA of target genes on 3'UTR region. MiRNA is found in the liver, pairs with the genomic RNA of a virus and positively regulate the replication of the virus. MicroRNAs have been found to be associated with many disorders including HIV susceptibility [11]. Changes in some of the miRNAs profiles have been associated with depletion of CD4 and CD8 cells during chronic infections [8]. HIV infec-

*Address correspondence to this author at the Department of Molecular Biology, NARI, Pune- 411026, India; Tel: 91-020-27331200 (1244-O); Fax: 91- 20-272121071; E-mails: hariomsgpgims@gmail.com; hsingh.nari@gov.in; hsingh@nariindia.org

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tion causes changes in the profile of miR-29a, miR-29b, miR-125b, miR-223, miR-382, miR-198 in infected $CD4^+$ T cell, PBMCs or serum [12-17]. MiRNA-196a2 (rs11614913) polymorphism was associated with the decreased Central Nervous System (CNS) AIDS-NHL [18].

MiR 146a has a significant role in negative regulation of acute responses during the activation of the innate immune system and plays a role in the regulation of most biological processes such as differentiation and surveillance of hematopoietic cells [19, 20]. The *miR*146aG/C (rs2910164) polymorphism causes a change from a G: U pair to a C: U mismatch in the stem structure of *miRNA*146a precursor that results in a reduced amount of mature *miR146a* [21, 22]. Up or down-regulation of *miRNA*146a is observed in human disorders, such as inflammatory diseases [22]. The *miR146a*G/C *(*rs2910164) polymorphism was associated with an increased risk of cancers such as breast [23, 24], hepatocellular [25], oral [26], colorectal [27, 28], lung [29], childhood acute lymphoblastic leukemia [30], head and neck [31], and gastric [32]. The *miR-27*a (rs895819 T/C) polymorphism may not be linked with preeclampsia susceptibility (PE), however, the *miR-27a* TC+CC genotype was linked with elevated BMI in PE [33].

Until now, *miR* (146a G/C, 149C/T, and 196aC/T) polymorphisms have not been studied in patients with ARV-associated hepatotoxicity. Hence, we examined the genetic variation *miR* (146aG/C, 149C/T, and 196aC/T) gene in patients with and without ARV-associated hepatotoxicity in Western Indian population.

2. MATERIAL AND METHODS

2.1. Participants

This is a case-control study. A total of 157 HIV patients were recruited from November 2014 to February 2017. Thirty-four HIV patients with hepatotoxicity (Grade III/IV) taking NNRTI-containing ART regimen and 123 patients without hepatotoxicity confirmed by liver function test (LFT) were enrolled in the study. They were age-matched with 155 healthy individuals who were consecutively recruited from outpatient clinics of National AIDS Research Institute, Pune. In hepatotoxicity cases, having hepatitis B, hepatitis C, tuberculosis and concurrent untreated opportunistic infections, immune reconstitution syndrome and under any other known hepatotoxic drugs were excluded from the case group. In the group of HIV patients without hepatotoxicity, individuals having evidence of hepatotoxicity, hepatitis B, hepatitis C, tuberculosis and receiving any other known hepatotoxic drugs were excluded. Similarly, 152 individuals (unrelated family), age-matched, serum negative from HIV-ELISA test, free of Hepatitis B, C and Tuberculosis were recruited. Clinical data were obtained by questionnaire and case records. LFT was done to evaluate the status of the liver enzyme. For male patients with hepatotoxicity, the total Bilirubin was >3.22mg/ml, SGOT>93.8 U/ml, SGPT>229.5 U/ml and Alkaline phosphatase >550.8 U/ml and for female patients with hepatotoxicity the total Bilirubin was >3.22mg/ml, SGOT>163.2 U/ml, SGPT>173.4 U/ml and Alkaline phosphatase >550.8 U/ml were considered as cases. Total Bilirubin <1.24mg/ml, SGOT<32 U/ml, SGPT<34 U/ml and Alkaline phosphatase <108 U/ml for male and female were considered as HIV-infected control. Estimation of the CD4 count was done by Fluorescence-activated cell sorting (FACS).CD4 status was used to classify patients into different sub-groups. CD4 range from < 200 *cells/mm*^{3} was defined as advanced HIV disease stage, 201-350*cells/mm*^{3} as intermediate HIV disease stage and >350*cells/mm*^{3}onward as early HIV disease stage. Ortho HCV ELISA test system and Murex HBsAg Confirmatory Version 3 ELISA were used to do ELISA for hepatitis C and HBsAg testing. A questionnaire was used to record the status of tobacco and alcohol usage. The study was approved by the institutional ethics committee board and all eligible participants gave informed written consent.

2.2. DNA Extraction

Two ml blood sample was collected and stored at -70°C until DNA extraction. Extraction of DNA was done using AxyPrep Blood Genomic DNA Miniprep Kit as per the protocol given by the manufacturer.

2.3. Genotyping

2.3.1. miRNAs(146a G/C, 149C/T, and 196a C/T) Polymorphism

Genotyping of *miRNAs*(146a G/C, 149C/T, and 196a C/T) polymorphisms were done using PCR-restriction fragment length polymorphism (PCR-RFLP). Primers for amplification of *miR* (146a G/C, 149C/T, and 196a C/T) polymorphisms were taken as described [21, 34]. The reaction mixture for PCR was prepared in a total volume of 25 µl with 10 pmol primers, 10X standard Taq buffer, genomic DNA (100-150 ng), 2.5mM deoxynucleotide triphosphates (dNTPs), and 1 unit of Taq DNA polymerase (New England BioLabs, USA). The reaction conditions for *miR* 146aG/C were: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 10 min. The amplification conditions for *miR*149C/T, and 196aC/T polymorphisms were: 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 35 sec, annealing at 61°C for 35 sec and extension at 72°C for 35 sec and a final extension at 72°C for 10 min. The sizes of amplified products of *miR* (146a G/C, 149C/T, and 196a C/T) polymorphisms were 147 bp, 194 and 149 bp, respectively. The amplified products were digested using restriction enzymes *SacI, PvuII*and *MspI* (MBI FermentasInc, Hanover, MD, USA) at 37°C for 16 hours, respectively. Genotyping of *miR* (146aG/C, 149C/T, and 196a C/T) polymorphisms was done on 10% polyacrylamide gel using molecular weight markers and visualized after staining with ethidium bromide. Based on sequences and location of SNP, genotypes of *miR* (146a G/C,149C/T, and 196a C/T) polymorphisms were assigned as follows: for *miR* 146aG/C,GG: 147bp, GC: 147, 122 and 25 bp, CC: 122 and 25 bp, for 149 C/T: CC: 254bp , CT: 254, 194 and 60bp, TT:194 and 60bp, for 196a C/T: CC: 149bp, CT: 149, 125 and 24bp, TT: 125 and 24bp. All reactions were carried out in Veriti 96 well thermal cycler (Applied Biosystems, CA, and USA). Re-genotyping of 20% samples was done by other laboratory personnel to confirm the discrepancy in genotyping, similar genotypes were noticed. Ten % of samples were sequenced to assess the genotyping error.

2.3.2. Data Analysis

The mean age variable was considered with an appropriate standard deviation. Deviations from Hardy-Weinberg equilibrium in control were assessed by Chi-square ($\chi 2$) goodness -of -fit test. Chi-squared test was also used to compare genotype frequencies among HIV patients with, without hepatotoxicity and healthy controls. SHEsisPlus online analysis tool was used to compare haplotype frequency among HIV patients with, without hepatotoxicity and healthy controls. Odds Ratios (ORs) and 95% confidence interval (CI) were determined by unconditional binary logistic regression using SPSS software version 17.0. Data were analyzed for the two-sided statistical significance and *P*-value less than 0.05 was considered for the statistically significant difference. Linkage disequilibrium (LD) was assessed between both the loci by calculating the relative LD value (D') as D' = Dij/Dmax. The Dij values were compared between HIV patients with *vs*. without hepatotoxicity, hepatotoxicity *vs*. healthy controls, HIV patients *vs*. healthy controls by comparison of confidence intervals.

2.3.3. Results

A total of 157 HIV patients, 34 with hepatotoxicity, 123 without hepatotoxicity and 155 healthy controls were enrolled in the study. The mean age difference between study groups were 35.14 ± 8.96, 39.29 ± 1.34 and 36.75±4.56 years, respectively. The demographic profiles of HIV patients with, without hepatotoxicity and healthy controls are presented in Table **1**.

2.3.4. miR Polymorphisms and HIV Patients with Hepatotoxicity

In single and double locus model, none of the genotypes/alleles of *miR* (146a G/C, 149C/T, and 196a C/T) polymorphism were different significantly between patients with and without hepatotoxicity. However, *miR*196aCT genotype was overrepresented in patients with hepatotoxicity as compared to without hepatotoxicity (47.1% *vs*. 30.1%, OR=2.08, 95%CI: 0.92 – 4.70, P=0.07). The frequency of *miR* 196a CT+TT combined genotype was much prevalent in patients with hepatotoxicity in comparison to without hepatotoxicity (50.0% *vs*. 39.0%, OR=1.69, 95%CI: 0.77 – 3.71, P=0.19).

Similarly, *miR (*146a G/C, 149C/T and 196a C/T) polymorphisms did not significantly differ between patients with hepatotoxicity and healthy controls. The distribution of *miR*146TT, 149TT genotypes occurred higher in HIV patients with hepatotoxicity as compared to healthy controls (8.8% *vs*. 7.1%, OR=2.31, 95%CI: 0.43– 12.52, P=0.33; 32.4% *vs*. 30.3%, OR=2.42, 95%CI: 0.73 – 8.09, P=0.15, respectively). Frequency distributions of *miR* 146aGT, 146aGT+TT, 149CT, 149CT+TT, 196aCT, 196aCT+TT genotypes and 196aT allele were almost similar in patients with hepatotoxicity and healthy controls (50.0% *vs*. 49.0%, 58.8% *vs*. 56.1%, 41.2% *vs*. 45.8%, 73.5% *vs*. 76.1%,47.1% *vs*. 44.5%,50.0% *vs*. 48.4%,26.47% *vs*. 26.13%, respectively). The *miR*149T allele was significantly reduced in patients with hepatotoxicity as compared to healthy controls (52.9% *vs*. 53.23%, OR=0.52, 95%CI: 0.27– 1.00, P=0.05) (Table **2**).

2.3.5. miR Polymorphisms and HIV Patients without Hepatotoxcity

The genotype distribution of *miR* (146a G/C, 149C/T, and 196a C/T) polymorphisms followed the Hardy-Weinberg equilibrium in the healthy control population (*P*=0.61, 0.31 and 0.06). In single and both locus model, *miR* polymorphisms did not differ significantly between HIV patients without hepatotoxcity and healthy control. The prevalence of *miR* 196aTT genotype was prominent in HIV patients as compared to healthy controls (8.9% *vs*. 3.9%, OR=2.34, 95%CI: 0.70 – 7.78, P=0.17). Frequency of *miR*196a CT genotype was underrepresented in HIV patients without hepatotoxcity as compared to healthy controls (30.1% *vs*. 44.5%, OR=0.56, 95%CI: 0.33– 1.074, P=0.08) (Table **3**).

2.3.6. Gene-gene Interaction

The occurrence of combined genotype GCT (*miR* 146a*G/149*C/196a*T) appeared to be higher in patients with hepatotoxicity as compared to without hepatotoxicity (0.18% *vs*. 0.09%, OR=2.88, 95%CI: 0.96-8.68, P=0.06). The prevalence of combined genotype CCC (*miR*146a*C/149*C/196a*C) was found to be higher in patients with hepatotoxicity as compared to without hepatotoxicity (0.20% *vs* 0.09%, OR=2.89, 95%CI: 0.98 – 8.6, P=0.05). The frequency of combined genotype CTC (*miR*146a*C/149*T/196a*C) was predominantly higher in patients with hepatotoxicity as compared to without hepatotoxicity (0.20 % *vs*. 0.14%, OR=1.81, 95%CI: 0.72 - 4.57, P=0.21). While comparing the combined genotype GCT (*miRNAs* 146a*G/149*C/196a*T) between patients with hepatotoxicity and healthy controls, the prevalence of GCT genotype was substantially higher in patients with hepatotoxicity (0.18% *vs*. 0.09%, OR=2.60, 95%CI: 0.87- 7.92, P=0.09). Frequency of combined genotype CTT (*miR*146a*C/149*T/196a*C) occurred higher in HIV patients without hepatotoxicity as compared to healthy controls (0.03% *vs*. 0.02%, OR=1.31, 95%CI: 0.23-7.62, P=0.76) (Table **4**).

2.3.7. miR Polymorphisms and HIV Disease Stages

Frequency of *miR* 146aCC genotype was much prevalent in intermediate, and early HIV disease stages as compared to healthy controls (9.68% *vs*. 7.1%, OR=2.19, 95%CI**:** 0.25-6.17, P=0.31; 10.52% *vs*. 7.1%, OR=1.91, 95%CI: 0.18-8.40, P=0.51, respectively). The occurrence of *miR*149CT genotype was higher in advanced HIV disease stage as compared to healthy controls (52.05% *vs*. 45.8%, OR=1.78**,** 95%CI**:** 0.64-3.14, P=0.18). The frequency of *miR* 149TT genotype was distributed higher in early HIV disease stage as compared to healthy controls (36.84% *vs*. 30.3%, OR=2.11**,** 95%CI: 0.28-4.42, P= 0.29). The prevalence of *miR* 196aTT genotype was increased in the intermediate HIV disease stage as compared to healthy controls (6.45% *vs*. 3.9%, OR=2.13, 95%CI: 0.18-8.71, P=0.42). The distribution of *miR* 196aTT genotype occurred at a higher frequency in advanced HIV disease stage as compared to healthy controls (12.33% *vs*. 3.9%, OR=3.68, 95%CI: 0.87-10.06, P=0.043) (Table **5**).

Table 1. The demographic profile of HIV patients with, without hepatotoxicity and healthy controls.

Subjects	HIV Patients with Hepatotoxicity (Grade III and IV)	HIV Patients without Hepatotoxicity	Healthy Controls
Number	N=34	N= 123	N= 155
Mean Age (Range)	35.14 ± 8.96	39.29 ± 1.34	36.75±4.56
Females	16(47.05%)	45 (36.6%)	38 (24.5%)
Males	18(52.94%)	78 (63.4%)	117 (75.5%)
NNRTI Regimen			
Efavirenz N=21	11 (32.4%)	10 (8.1%)	NA
Nevirapine N=136	23 (67.6%)	113 (91.9%)	NA
Alcohol Habit			
User N=51	7 (20.6%)	41 (33.3%)	0
Non user N=114	27 (79.4%)	82 (66.7%)	0
Tobacco Habit			
User N=50	7(20.6%)	41 (33.3%)	0
Non user N=115	27 (79.4%)	82 (66.7%)	0
CD4+ Status			
<200(N=89)	16 (47.1%)	73 (59.4%)	NA
201-350 (N=48)	17 (50.0%)	31 (25.2%)	NA
>350(N=20)	1 (2.9%)	19 (15.4%)	NA

0= data was not recorded in questionnaire form. NA= Not applicable.

Table 2. Frequency distribution of *miR (*146a G/C, 149C/T, and 196a C/T) genotypes/alleles in total HIV patients, HIVpatients with hepatotoxicity and without hepatotoxicity.

***miR*146a G/C Genotype**	**Total HIV Patients N= 157(%)**	**Healthy Controls N= 155(%)**	***P*-Value**	**OR(95%CI)**
GG	64(40.76%)	68 (43.9%)	-	1 (Reference)
GC	81(51.59%)	76 (49.0%)	0.86	1.02 (0.39 – 2.67)
CC	12(7.64%)	11 (7.1%)	0.91	1.16 (0.44 – 3.07)
GC + CC	93(59.23%)	87 (56.1%)	0.65	1.14 (0.71 – 1.83)
***miR*146a Allele**	**Total HIV Patients N= 314(%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95% CI)**
G	209(66.56%)	212 (68.39%)	-	1 (Reference)
C	105(33.43%)	98 (31.61%)	0.68	1.09 (0.77 – 1.54)
***miR*149C/T Genotype**	**Total HIV Patients 157(%)**	**Healthy Controls N= 155**	***P*-Value**	**OR(95%CI)**
CC	36(22.92%)	37 (23.9%)	-	1 (Reference)
CT	74(47.13%)	71 (45.8%)	0.92	1.07 (0.59– 1.59)
TT	47(29.93%)	47 (30.3%)	0.94	1.03 (0.53 – 1.98)
CT + TT	121(77.07%)	118 (76.1%)	0.95	1.05 (0.60 – 1.84)

(Table 2) contd….

***miR*149C/T Allele**	**Total HIV Patients N= 314(%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
C	146(46.49%)	145 (46.77%)	-	1 (Reference)
T	164(52.22%)	165 (53.23%)	1.00	0.99 (0.71 – 1.37)
***miR* 196a C/T Genotype**	**Total HIV Patients N= 157(%)**	**Healthy Controls N= 155**	***P*-Value**	**OR(95%CI)**
CC	92(58.59%)	80 (51.6%)	-	1 (Reference)
CT	53(33.75%)	69 (44.5%)	0.11	0.67 (0.41 – 1.09)
TT	12(7.64%)	6 (3.9%)	0.41	1.74 (0.57– 5.48)
CT + TT	65(41.40%)	75 (48.4%)	0.25	0.75 (0.47 – 1.21)
***miR*196a C/T Allele**	**Total HIV Patients N= 314**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
C	192(61.14%)	229 (73.87%)	-	1 (Reference)
T	77(24.52%)	81 (26.13%)	0.56	1.13 (0.77 – 1.66)
***miR*146a G/C Genotype**	**HIV Patients with Hepato-toxicity N= 34 (%)**	**HIV Patients without Hepato-toxicity N= 123 (%)**	***P*-Value**	**OR(95%CI)**
GG	14 (41.2%)	50 (40.7%)	-	1 (Reference)
GC	17 (50.0)	64 (52.0%)	0.95	0.98 (0.44 – 2.19)
CC	3 (8.8%)	9 (7.3%)	0.87	1.13 (0.26 – 4.87)
GC + CC	20 (58.8%)	73 (59.35%)	0.99	0.99 (0.46 – 2.18)
***miR*146a Allele**	**HIV Patients with Hepato-toxicity N= 68 (%)**	**HIV Patients without Hepato-toxicity N= 246 (%)**	***P*-Value**	**OR(95% CI)**
G	45 (66.18%)	164 (66.67)	-	1 (Reference)
C	23 (33.82%)	82 (33.33%)	0.94	1.02 (0.58 – 1.81)
***miR*149C/T Genotype**	**HIV Patients with Hepato-toxicity N= 34 (%)**	**HIV Patients without Hepato-toxicity N= 123 (%)**	***P*-Value**	**OR(95%CI)**
CC	9 (26.5%)	27 (22.0%)	-	1 (Reference)
CT	14 (41.2%)	60 (48.8%)	0.45	0.69 (0.26 – 1.81)
TT	11 (32.4%)	36 (29.3%)	0.88	0.92 (0.33 – 2.56)
CT + TT	25 (73.53%)	96 (78.0%)	0.57	0.78 (0.32 – 1.87)
***miR149*C/T Allele**	**HIV Patients with Hepato-toxicity N= 68 (%)**	**HIV Patients without Hepato-toxicity N= 246 (%)**	***P*-Value**	**OR(95%CI)**
C	32 (47.1%)	114 (46.34%)	-	1 (Reference)
T	32 (52.9%)	132 (53.66%)	0.86	0.95 (0.55 – 1.64)
***miR* 196a C/T Genotype**	**HIV Patients with Hepato-toxicity N= 34 (%)**	**HIV Patients without Hepato-toxicity N= 123 (%)**	***P*-Value**	**OR(95%CI)**
CC	17 (50.0%)	75 (61.0%)	-	1 (Reference)
CT	16 (47.1%)	37 (30.1%)	0.07	2.08 (0.92 – 4.70)
TT	1 (2.9%)	11 (8.9%)	0.43	0.42 (0.050– 3.54)
CT + TT	17 (50.0%)	48 (39.0%)	0.19	1.69 (0.77 – 3.71)

(Table 2) contd….

***miR*196a C/T Allele**	**HIV Patients with Hepato-toxicity N= 68 (%)**	**HIV Patients without Hepato-toxicity N= 246 (%)**	***P*-Value**	**OR(95%CI)**
C	50 (73.53%)	187 (76.02%)	-	1 (Reference)
T	18 (26.47%)	59 (23.98%)	0.58	1.19 (0.64 – 2.23)
***miR* 146a G/C Genotype**	**HIV Patients with Hepato-toxicity N= 34 (%)**	**Healthy Controls N= 155(%)**	***P*-Value**	**OR(95%CI)**
GG	14 (41.2%)	68 (43.9%)	-	1 (Reference)
GT	17 (50.0%)	76 (49.0%)	0.59	1.29 (0.52 – 3.18)
TT	3 (8.8%)	11 (7.1%)	0.33	2.31 (0.43– 12.52)
GT + TT	20 (58.8%)	87 (56.1%)	0.47	1.38 (0.57 – 3.31)
***miR*146aG/C Allele**	**HIV Patients with Hepato-toxicity N= 68 (%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
G	45 (66.18%)	212 (68.39%)	-	1 (Reference)
T	23 (33.82%)	98 (31.61%)	0.39	1.33 (0.70 – 2.52)
***miR*149C/T Genotype**	**HIV Patients with Hepato-toxicity N= 34 (%)**	**Healthy Controls N= 155**	***P*-Value**	**OR(95%CI)**
CC	9 (26.5%)	37 (23.9%)	-	1 (Reference)
CT	14 (41.2%)	71 (45.8%)	0.63	1.32 (0.43 – 4.03)
TT	11 (32.4%)	47 (30.3%)	0.15	2.42 (0.73 – 8.09)
CT + TT	25 (73.5%)	118 (76.1%)	0.33	1.66 (0.59 – 4.68)
***miR*149C/TAllele**	**HIV Patients with Hepato-toxicity N= 68(%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
C	32 (47.1%)	145 (46.77%)	-	1 (Reference)
T	36 (52.9%)	165 (53.23%)	0.05	0.52 (0.27– 1.00)
***miR*196a C/T Genotype**	**HIV Patients with Hepato-toxicity N= 34 (%)**	**Healthy Controls N= 155**	***P*-Value**	**OR(95%CI)**
CC	17 (50.0%)	80 (51.6%)	-	1 (Reference)
CT	16 (47.1%)	69 (44.5%)	0.37	1.50 (0.62 – 3.62)
TT	1 (2.9%)	6 (3.9%)	0.76	0.78 (0.14– 17.30)
CT + TT	17 (50.0%)	75 (48.4%)	0.36	1.50 (0.63 – 3.58)
***miR*196a C/T Allele**	**HIV Patients with Hepato-toxicity N= 68 (%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
C	50 (73.53%)	229 (73.87%)	-	1 (Reference)
T	18 (26.47%)	81 (26.13%)	0.22	1.54 (0.77 – 3.06)

N, the total number of HIV-patients with hepatotoxicity (34), without hepatotoxicity (123) and healthy controls (155). Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele with other genotypes/alleles. Significant P values and related OR (95% CI) are shown in bold.

Table 3. Frequency distribution of *miR* (146a G/C, 149C/T, and 196a C/T) genotypes/alleles in HIVpatients and healthy controls.

***miR* 146a G/C Genotype**	**HIV Patients N= 123 (%)**	**Healthy Controls N= 155(%)**	***P*-Value**	**OR(95%CI)**
GG	50 (40.7%)	68 (43.9%)	-	1 (Reference)
GC	64 (52.0%)	76 (49.0%)	0.78	1.09 (0.61 – 1.93)
CC	9 (7.3%)	11 (7.1%)	0.49	1.48 (0.48 – 4.64)
GC + CC	73 (59.3%)	88 (56.1%)	0.67	1.13 (0.65 – 1.97)
***miR* 146a G/C Allele**	**HIV Patients N= 246 (%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
G	164 (66.67)	212 (68.39%)	-	1 (Reference)
C	82 (33.33%)	98 (31.61%)	0.58	1.13 (0.74 – 1.72)
***miR*149C/T Genotype**	**HIV Patients N= 123 (%)**	**Healthy Controls N= 155**	***P*-Value**	**OR(95%CI)**
CC	27 (22.0%)	37 (23.9%)	-	1 (Reference)
CT	60 (48.8%)	71 (45.8%)	0.36	1.39 (0.68 – 2.83)
TT	36 (29.3%)	47 (30.3%)	0.98	1.05 (0.52 – 2.14)
CT + TT	96 (78.1%)	118 (76.1%)	0.34	1.38 (0.71 – 2.70)
***miR*149C/T Allele**	**HIV Patients N= 246 (%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
C	114 (46.34%)	145 (46.77%)	-	1 (Reference)
T	132 (53.66%)	165 (53.23%)	0.66	1.09 (0.74 – 1.62)
***miR* 196a C/T Genotype**	**HIV Patients N= 123 (%)**	**Healthy Controls N= 155**	***P*-Value**	**OR(95%CI)**
CC	75 (61.0%)	80 (51.6%)	-	1 (Reference)
CT	37 (30.1%)	69 (44.5%)	0.08	0.56 (0.33– 1.074)
TT	11 (8.9%)	6 (3.9%)	0.17	2.34 (0.70 – 7.78)
CT + TT	48 (39.0)	75 (48.4)	0.26	0.73 (0.42 – 1.27)
***miR* 196a C/T Allele**	**HIV Patients N= 246 (%)**	**Healthy Controls N= 310**	***P*-Value**	**OR(95%CI)**
C	187 (76.02%)	229 (73.87%)	-	1 (Reference)
T	59 (23.98%)	81 (26.13%)	0.20	0.77 (0.52 – 1.15)

N, the total number of HIV-patients (123) and healthy controls (155). Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele with other genotypes/alleles.

2.3.8. Gene-environment Interaction

Occurrence of *miR* 149TT, 196aCT genotypes was higher in tobacco consuming patients with hepatotoxicity as compared to non-users (42.86% *vs*. 29.63%, OR=1.56, 95%CI: 0.20 – 12.54, P=0.67; 71.43% *vs*. 40.74%, OR=5.17, 95%CI: 0.58 – 46.45, P=0.14, respectively). The prevalence of *miR* 196a CT and 196aTT genotypes was higher in tobacco using HIV patients as compared to non-users (34.15% *vs*. 28.05%, OR=1.58, 95%CI: 0.66-3.78, P=0.30, 9.76% *vs*. 8.54%, OR=1.41, 95%CI: 0.35 – 5.61, P=0.63, respectively) (Table **6**).

A higher prevalence of *miR*196aCT genotype was observed in alcohol consuming patients with hepatotoxicity as compared to non-users (71.23% *vs*. 40.74%, OR=5.17, 95%CI: 0.58 – 46.45, P=0.14). Frequency of *miR*149TT genotype was increased in alcohol consuming HIV patients as compared to non-consumers (34.14% *vs*. 26.83%, OR=2.29, 95%CI: 0.69 – 7.50, P=0.17). The distribution of *miR* 196aCT genotype was observed to be higher in alcohol consuming HIV patients without hepatotoxicity as compared to non-users (39.02% *vs*. 25.61%, OR=2.36, 95%CI: 0.95 – 5.89, P=0.06) (Table **7**).

The *miR* 149TT and 196aTT genotypes were distributed higher in patients with hepatotoxicity taking nevirapine as compared to non-users (32.35% *vs*. 14.28%, OR=4.19, P=0.07; 8.08% *vs*. 4.76%, OR=1.97, P=0.84, respectively).

Table 4. Frequency distribution of combined genotype of *miR (*146a G/C, 149C/T, and 196a C/T) polymorphisms in HIV patients with, without hepatotoxicity and healthy controls.

***miR*146aG/C, *miR*149 C/T and *miR*196a C/T Combined Genotype**	**HIV Patients with Hepatotoxicity (N = 68)**	**HIV Patients without Hepatotoxicity (N = 262)**	***P*-Value**	**OR(95%CI)**
GTC	0.2077	0.2651	-	1(Reference)
GCC	0.1894	0.206	0.76	1.18 (0.41 - 3.40)
CTC	0.2037	0.1412	0.21	1.81 (0.72 - 4.57)
CCC	0.1345	0.1479	0.83	0.86 (0.23 - 3.19)
GCT	0.1872	0.0907	0.06	2.88 (0.96 - 8.68)
GTT	0.0775	0.1049	0.92	0.94 (0.28 - 3.20)
CTT	0	0.033	1	0.00 (-Inf - Inf)
CCT	0	0.0113	1	0.00 (-Inf - Inf)
Combined *miR*146aG/C, *miR*149 C/T and *miR*196aC/T Genotype	**HIV Patients with Hepatotoxicity (N = 68)**	**Healthy Controls (N=310)**	***P*-Value**	**OR(95%CI)**
GTC	0.2077	0.2687	-	1(Reference)
GCC	0.1894	0.2249	0.65	0.76 (0.24 - 2.45)
CTC	0.1345	0.1457	0.79	1.27 (0.23 - 7.14)
CCC	0.2037	0.0994	**0.05**	**2.89 (0.98 – 8.69)**
GCT	0.1872	0.0936	0.09	2.60 (0.87- 7.92)
GTT	0.0775	0.0966	0.11	0.13 (0.01 - 1.51)
CTT	0	0.0468	0.14	5.31 (0.58 - 48.96)
CCT	**0**	0.0242	0.46	2.96 (0.17 - 51.71)
Combined *miR*146aG/C, *miR*149C/T and *miR*196a C/T Genotype	**HIV Patients (N= 262)**	**Healthy Controls (N=310)**	***P*-Value**	**OR(95%CI)**
GTC	0.2651	0.2687	-	1(Reference)
GCC	0.206	0.2249	0.49	0.77 (0.37 - 1.60)
CTC	0.1479	0.1457	0.75	1.17 (0.46 - 2.96)
CCC	0.1412	0.0994	0.66	1.20 (0.54 - 2.65)
GCT	0.1049	0.0966	0.84	1.09 (0.48 - 2.49)
GTT	0.0907	0.0936	0.9	0.94 (0.35 - 2.51)
CTT	0.033	0.0242	0.76	1.31 (0.23 - 7.62)
CCT	0.0113	0.0468	0.31	0.27 (0.02 - 3.37)

N, the total number of chromosomes in HIV patients with hepatotoxicity 68, without hepatotoxicity 262 and healthy controls 310. Odds ratios and 95% CIs were derived from logistic regression models comparing the combined genotype GTC with other genotypes. Significant P values and related OR (95% CI) are shown in bold.

Table 5. Frequency distribution of *miR* (146a G/C,149C/T, and 196a C/T) polymorphisms in different disease stages of HIV patients and healthy controls.

***miR* 146a G/C Genotype**	**Healthy Controls N=155 (%)**	**Early HIV Disease Stage**		**Intermediate HIV Disease Stage**		**Advanced HIV Disease Stage**	
		N=19(%)	OR (P)	N= 31 (%)	OR (P)	N= 73 (%)	OR (P)
GG	68 (43.9%)	9 (47.36%)	1 (Reference)	14 (45.16%)	1 (Reference)	27 (36.99%)	1 (Reference)
GC	76 (49.0%)	8 (42.10%)	0.75 (0.60)	14 (45.16%)	0.87 (0.76)	42 (57.53%)	1.28 (0.47)
CC	11 (7.1%)	2(10.52%)	1.91 (0.51)	3 (9.68%)	2.19 (0.31)	4 (5.48%)	0.92 (0.86)
***miR*149C/T Genotype**	**Healthy Controls N= 155(%)**	**Early HIV Disease Stage**		**Intermediate HIV Disease Stage**		**Advanced HIV Disease Stage**	
		N=19(%)	OR (P)	N= 31 (%)	OR (P)	N= 73 (%)	OR (P)
CC	37 (23.9%)	5 (26.31)	1 (Reference)	8 (25.80%)	1 (Reference)	14 (19.17%)	1 (Reference)
CT	71 (45.8%)	7 (36.84%)	1.067 (0.92)	15 (48.38%)	1.43 (0.51)	38 (52.05)	1.78 (0.18)
TT	47 (30.3%)	7(36.84%)	2.11 (0.29)	8 (25.80%)	1.28 (0.69)	21 (28.76%)	0.83 (0.70)
***miR* 196a C/T Genotype**	**Healthy Controls N= 155(%)**	**Early HIV Disease Stage**		**Intermediate HIV Disease Stage**		**Advanced HIV Disease Stage**	
		N=19(%)	OR (P)	N= 31 (%)	OR (P)	N= 73 (%)	OR (P)
CC	80 (51.6%)	15 (78.95%)	1 (Reference)	19 (61.29%)	1 (Reference)	41 (56.16%)	1 (Reference)
CT	69 (44.5%)	4 (21.05%)	0.33(0.068)	10 (32.26%)	0.67 (0.39)	23 (31.51%)	0.69 (0.30)
TT	6 (3.9%)	0 (0.0%)	NA	2 (6.45%)	2.13 (0.42)	9 (12.33%)	**3.68 (0.043)**

N= number of subjects, (%) = frequency of subjects, Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele with other genotypes. Significant P values and related OR (95% CI) are shown in bold.

Table 6. Frequency distribution of *miR (*146a G/C, 149C/T, and 196a C/T) genotypes in tobacco using HIV patients with and without hepatotoxicity.

***miR* 146a G/C Genotype**	**Tobacco User N= 7 (%)**	**Tobacco Non User N= 27 (%)**	***P*-Value**	**OR(95%CI)**
HIV Patients with Hepatotoxicity				
GG	4 (57.14%)	10 (37.03%)	-	1 (Reference)
GC	3 (42.86%)	14 (51.85%)	0.35	0.41 (0.060 – 2.72)
CC	0 (0.0%)	3 (11.11%)	NS	-
***miR*149C/T Genotype**	**Tobacco User N= 7 (%)**	**Tobacco Non User N= 27 (%)**	***P*-Value**	**OR(95%CI)**
CC	0 (0.0%)	9 (33.33%)	NS	NA
CT	4 (57.14%)	10 (37.03%)	-	1 (Reference)
TT	3 (42.86%)	8 (29.63%)	0.67	1.56 (0.20 – 12.54)
***miR* 196a C/T Genotype**	**Tobacco User N= 7 (%)**	**Tobacco Non User N= 27 (%)**	***P*-Value**	**OR(95%CI)**
CC	2 (28.57%)	15 (55.56%)	-	1 (Reference)
CT	5 (71.43%)	11 (40.74%)	0.14	5.17 (0.58 – 46.45)
TT	0 (0.0%)	1 (3.70)	NS	-

(Table 6) contd….

HIV Patients				
***miR* 146a G/C Genotype**	**Tobacco User N= 41 (%)**	**Tobacco Non User N= 82 (%)**	***P*-Value**	**OR(95%CI)**
GG	18 (43.90%)	32 (39.02%)	-	1 (Reference)
GC	21 (51.22%)	43 (52.43%)	0.55	0.78 (0.34 – 1.77)
CC	2 (4.88%)	7 (8.53%)	0.20	0.31 (0.051 – 1.85)
***miR*149C/T Genotype**	**Tobacco User N= 41 (%)**	**Tobacco Non User N= 82 (%)**	***P*-Value**	**OR(95%CI)**
CC	9 (21.95%)	18 (21.95%)	-	1 (Reference)
CT	21 (51.22%)	39 (47.56%)	0.94	0.96 (0.35 – 2.62)
TT	11 (26.83%)	25 (30.49%)	0.87	0.91 (0.30 – 2.77)
***miR* 196a C/T Genotype**	**Tobacco User N= 41 (%)**	**Tobacco Non User N= 82 (%)**	***P*-Value**	**OR(95%CI)**
CC	23 (56.10%)	52 (63.41%)	-	1 (Reference)
CT	14 (34.15%)	23 (28.05%)	0.30	1.58 (0.66 – 3.78)
TT	4 (9.76%)	7 (8.54%)	0.63	1.41 (0.35 – 5.61)

N= number of subjects, **(%) =** frequency of subjects, Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele with other genotypes.

Table 7. Frequency distribution of *miR* (146a G/C, 149C/T, and 196a C/T) genotypes in alcohol using HIV patients with and without hepatotoxicity.

***miR* 146a G/C Genotype**	**Alcohol User N= 7 (%)**	**Alcohol Non User N= 27 (%)**	***P*-Value**	**OR(95%CI)**
HIV Patients with Hepatotoxicity				
GG	5 (71.43%)	9 (33.33%)	-	1 (Reference)
GC	2 (28.57%)	15 (55.55%)	0.35	0.41 (0.060 – 2.72)
CC	0 (0.0%)	3 (11.11%)	NS	NA
***miR* 149C/T Genotype**	**Alcohol User N= 7 (%)**	**Alcohol Non User N= 27 (%)**	***P*-Value**	**OR(95%CI)**
CC	0 (0.0%)	9 (33.33%)	NS	-
CT	4 (57.14%)	10 (37.04%)	-	1 (Reference)
TT	3 (42.86%)	8 (29.63%)	0.51	0.50 (0.061 – 4.04)
***miR* 196a C/T Genotype**	**Alcohol User N= 7 (%)**	**Alcohol Non User N= 27 (%)**	***P*-Value**	**OR(95%CI)**
CC	1 (14.29%)	16 (59.26%)	-	1 (Reference)
CT	5 (71.23%)	11 (40.74%)	0.14	5.17 (0.58 – 46.45)
TT	1(14.29%)	0 (0.0%)	NS	-
HIV Patients without Hepatotoxicity				
***miR* 146a G/C Genotype**	**Alcohol User N= 41 (%)**	**Alcohol Non User N= 82 (%)**	***P*-Value**	**OR(95%CI)**
GG	17 (41.46%)	33 (40.24%)	-	1 (Reference)
GC	22 (53.65%)	42 (51.22%)	0.85	0.92 (0.39 – 2.15)
CC	2 (4.87%)	7 (8.54%)	0.23	0.33 (0.055 – 2.01)

(Table 7) contd….

***miR*149C/T Genotype**	**Alcohol User N= 41 (%)**	**Alcohol Non User N= 82 (%)**	***P*-Value**	**OR(95%CI)**
CC	7 (17.07%)	20 (24.39%)	-	1 (Reference)
CT	20 (48.78%)	40 (48.78%)	0.61	1.32 (0.45 – 3.90)
TT	14 (34.14%)	22 (26.83%)	0.17	2.29 (0.69 – 7.50)
***miR* 196a C/T Genotype**	**Alcohol User N= 41 (%)**	**Alcohol Non User N= 82 (%)**	***P*-Value**	**OR(95%CI)**
GC	22 (53.66%)	53 (64.63%)	-	1 (Reference)
CT	16 (39.02%)	21 (25.61%)	0.06	2.36 (0.95 – 5.89)
TT	3 (7.32%)	8 (9.76%)	0.93	0.94 (0.21 – 4.26)

N= number of subjects, (%) = frequency of subjects, Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele with other genotypes.

The occurrence of *miRNA* 146aGC, 149CT, 196aCT genotypes was found to be higher in HIV patients taking nevirapine as compared to non-users (53.98% *vs*. 30%, OR=3.03, 95%CI: 0.70 – 13.09, P=0.14; 49.56% *vs*. 40.0%, OR=1.93, 95%CI: 0.39 -9.55, P=0.42; 31.86% *vs*. 10%, OR=4.68, 95%CI: 0.56 – 38.69, P=0.15) (Table **8**).

In alcohol and nevirapine consuming patients without and with hepatotoxicity, frequency of *miR* 196aCT genotype was found to behigher as compared to non-users **(**40.54% *vs*. 27.63%, OR=2.29, 95%CI: 0.89 – 5.88, P=0.09; 80% *vs*. 38.88%, OR=14.18, 95%CI: 0.70– 287.88, P=0.08, respectively). In HIV patients consuming alcohol and nevirapine, the occurrence of *miR* 149TT genotype was found to be higher in comparison to non-users (32.43% *vs*. 27.63%, OR=2.40, 95%CI: 0.68 – 8.55, P=0.18). In HIV patients without hepatotoxicity taking both alcohol and Efavirenz, the prevalence of *miR* 149TT genotype was higher in comparison to non-users (50.0% *vs*. 16.67%, OR=4.00, 95%CI: 0.13 - 119.23, P=0.42) (Table **9**).

2.3.9. Risk Factors of ARV Associated Hepatotoxicity: Multivariate Logistic Regression Analysis

Association of age, sex, tobacco, alcohol, and *miR* (146a G/C, 149C/T and 196a C/T) polymorphisms with ARV-associated hepatotoxicity was evaluated by multivariate logistic regression analysis. Polymorphisms of *miR* gene, age, sex, tobacco, alcohol usage were not independent risk factors for ARV-associated hepatotoxicity. While comparing between HIV patients with and without hepatotoxicity, *miR* 196aCT genotype, taking nevirapine with hepatotoxicity showed a risk for ARV-associated hepatotoxicity (OR=5.98, P=0.005; OR=2.38, P=0.05, respectively) (Table **10**).

3. DISCUSSION

Globally, this is the first report that described the genetic polymorphisms (*miR*-146aG/C-rs2910164, *miR*-149C/T-rs2292832, and *miR*-196aC/T -rs11614913) of *miR* gene with respect to patients with and without hepatotoxicity. MicroRNAs are short noncoding RNAs that bind genes and silence their expression. miRNAs play a regulatory role in controlling the metabolism of the drug. Polymorphisms of *miR* gene influence the expression, maturation. It could be an important risk determinant for disease susceptibility. The aberrant expression of *miRNAs* was associated with etiology, diagnosis, prognosis of a disease. Susceptibility to developing drug-induced hepatotoxicity is dependent on ethnic variations and also linked with the genetic background of the host. Individual single nucleotide genotypic analysis of *miRNAs* gene in patients with ARV –associated hepatotoxicity might not be sufficient to predict its functional consequences, but combined genotype of *miRNAs* gene may provide the functional significance of *miRNA* polymorphisms on the disease susceptibility and control of drug metabolism.

In the present study, *miR*146aG/C and *miR*196aC/T polymorphisms were comparable with the study described by Bansal *et al.*, 2014 [35], while the *miR*149C/T polymorphism was not comparable with the study carried out by Sushma *et al.*, 2015 [36]. In our study, *miR* (146a G/C, 149C/T, and 196aC/T) polymorphisms did not differ significantly among patients with, without hepatotoxicity and healthy controls. However, *miR* 196aCT genotype was associated with hepatotoxicity severity with borderline significance (OR=2.08, P=0.07). In patients with hepatotoxicity, the *miR* 146aTT and *miR* 149TT genotypes showed a higher risk of hepatotoxicity severity (OR=2.31, P=0.33; OR=2.42, P=0.15). Whereas *miR*149T allele was associated with the reduced susceptibility to hepatotoxicity severity (OR=0.52, P=0.05). In HIV patients, *miR* 196aTT genotype showed a risk for acquisition of hepatotoxicity (OR=2.34, P=0.17). Studies reported that *miR*146aG/C polymorphism was not associated with cancers [26-29, 37, 38], other studies have reported an association between *miR146a* G/C polymorphism and increased risk of cancers [23-25, 32]. Polymorphism 146aG/C in *miR* gene was associated with reduced genetic susceptibility to breast cancer [35]. Pornpitra *et al.*, (2015) revealed no significant association between *miR*-149 C/T polymorphism and HCC risk. The *miR*-149C/T polymorphism was involved with decreased cancer risk [39]. The *miR*-149 CC genotype was associated with OSCC risk [36]. Several studies have been reported that 196aC/T polymorphism was associated with various kind of cancers [29, 40]. The *miR*-196a2CC genotype was significantly associated with OSCC (P=0.001).

We have also looked for combined effect of different polymorphisms on the same gene by gene-gene interaction analysis. The combined genotypes had a potential impact on

Table 8. Frequency distribution of *miR (*146a G/C, 149C/T, and 196a C/T) genotypes in NNRTIs using HIV patients with hepatotoxicity and without hepatotoxicity.

***miR* 146a G/C Genotype**	**HIV Patients with Nevirapine users N= 136 (%)**	**HIV Patients efavirenz users N= 21 (%)**	***P*-Value**	**OR(95%CI)**
Total HIV Patients (With and Without Hepatotoxicity)				
GG	54 (39.70%)	10 (47.61%)	-	1 (Reference)
GC	72 (52.94%)	9 (42.85%)	0.58	1.48 (0.51 – 4.31)
CC	10(7.35%)	2 (9.52%)	0.73	0.93(0.15–7.16)
***miR*149C/T Genotype**	**HIV Patients with Nevirapine users N= 136 (%)**	**HIV Patients efavirenz users N= 21 (%)**	***P*-Value**	**OR(95%CI)**
CC	28(20.58%)	8 (38.09%)	-	1 (Reference)
CT	64(47.05%)	10 (47.61%)	0.37	1.83 (0.58 – 5.73)
TT	44 (32.35%)	3(14.28%)	0.07	4.19(0.90-22.03)
***miR* 196a C/T Genotype**	**HIV Patients with nevirapine users N= 136 (%)**	**HIV Patients efavirenz users N= 21 (%)**	***P*-Value**	**OR(95%CI)**
CC	78 (57.35%)	14(66.00%)	-	1 (Reference)
CT	47 (34.55%)	6(28.57%)	0.68	1.41 (0.46 – 4.44)
TT	11 (8.08%)	1 (4.76%)	0.84	1.97(0.23-44.08)
***miR* 146a G/C Genotype**	**Nevirapine User N= 23 (%)**	**Efavirenz User N= 11 (%)**	***P*-Value**	**OR(95%CI)**
HIV Patients with Hepatotoxicity				
GG	10 (43.48%)	4 (36.36%)	-	1 (Reference)
GC	11 (47.83%)	6 (54.54%)	0.54	0.60 (0.12 – 3.08)
CC	2 (8.69%)	1 (9.09%)	0.95	0.92(0.061–13.69)
***miR* 149C/T Genotype**	**Nevirapine User N= 23 (%)**	**Efavirenz User N= 11 (%)**	***P*-Value**	**OR(95%CI)**
CC	4 (17.39%)	5 (45.45%)	-	1 (Reference)
CT	8 (34.78%)	6 (54.54%)	0.86	1.67 (0.23 – 12.71*)
TT	11 (47.83%)	0 (0.0%)	NS	-
***miR* 196a C/T Genotype**	**Nevirapine user N= 23 (%)**	**Efavirenz Present N= 11 (%)**	***P*-Value**	**OR(95%CI)**
CC	12 (52.17%)	5 (45.45%)	-	1 (Reference)
CT	11 (47.83%)	5 (45.45%)	0.68	0.70 (0.14 – 3.64)
TT	0 (0.0%)	1 (9.09%)	NS	-
HIV Patients				
***miR* 146a G/C Genotype**	**Nevirapine User N= 113 (%)**	**Efavirenz User N= 10 (%)**	***P*-Value**	**OR(95%CI)**
GG	44 (38.94%)	6 (60%)	-	1 (Reference)
GC	61 (53.98%)	3 (30%)	0.14	3.03 (0.70 – 13.09)
CC	8 (7.08%)	1 (10%)	0.62	1.09(0.10 – 27.32)

(Table 8) contd….

***miR*149C/T Genotype**	**Nevirapine User N= 113 (%)**	**Efavirenz User N= 10 (%)**	***P*-Value**	**OR(95%CI)**
CC	24 (21.24%)	3 (30.0%)	-	1 (Reference)
CT	56 (49.56%)	4 (40.0%)	0.42	1.93 (0.39 – 9.55)
TT	33 (29.20%)	3 (30.0%)	0.95	1.38 (0.20 – 9.59)
***miR* 196a C/T Genotype**	**Nevirapine User N= 113 (%)**	**Efavirenz User N= 10 (%)**	***P*-Value**	**OR(95%CI)**
CC	66 (58.41%)	9 (90%)	-	1 (Reference)
CT	36 (31.86%)	1 (10%)	0.15	4.68 (0.56 – 38.69)
TT	11 (9.73%)	0 (0.0%)	NS	-

NS, not significant. N= number of subjects, **(%) =** frequency of subjects, Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele with other genotypes.

Table 9. Frequency distribution of *miR* (146a G/C, 149C/T and 196a C/T) genotypes in combined alcohol and NNRTI regimen using HIV patients with and without hepatotoxicity.

***miR* 146a G/C Genotype**	**Alcohol + Nevirapine User N= 5 (%)**	**Alcohol Non User + Nevirapine User N= 18 (%)**	***P*-Value**	**OR(95%CI)**
HIV Patients with Hepatotoxicity				
GG	4 (80%)	6 (33.33%)	-	1 (Reference)
GC	1 (20%)	10 (55.55%)	0.09	0.074 (0.004 – 1.48)
CC	0 (0.0%)	2 (11.11%)	NS	-
***miRNA* 149C/T Genotype**	**Alcohol User + Nevirapine N= 5 (%)**	**Alcohol Non- user + Nevirapine user N= 18 (%)**	***P*-Value**	**OR(95%CI)**
CC	0 (0.0%)	4 (22.22%)	NS	-
CT	2 (40%)	6 (33.33%)	-	1 (Reference)
TT	3 (60%)	8 (44.44%)	0.79	0.72 (0.058 – 8.86)
***miRNA* 196a C/T Genotype**	**Alcohol User + Nevirapine N= 5 (%)**	**Alcohol Non-user + Nevirapine User N= 18 (%)**	***P*-Value**	**OR(95%CI)**
CC	1 (20%)	11 (61.11%)	-	1 (Reference)
CT	4 (80%)	7 (38.88%)	0.08	14.18(0.70– 287.88)
TT	0 (0.0%)	0 (0.0%)	NS	-
***miRNA* 146a G/C Genotype**	**Alcohol User + Efavirenz N= 2(%)**	**Alcohol Non -user + Efavirenz User N= 9(%)**	***P*-Value**	**OR(95%CI)**
GG	1 (50%)	3 (33.33%)	-	1 (Reference)
GC	1 (50%)	5 (55.55%)	0.39	0.034 (0.0 – 79.36)
CC	0 (0.0%)	1 (11.11%)	NS	-
***miRNA* 149C/T Genotype**	**Alcohol User + Efavirenz N= 2(%)**	**Alcohol Non- user + Efavirenz User N= 9(%)**	***P*-Value**	**OR(95%CI)**
CC	0 (0.0%)	5 (55.55%)	NS	-
CT	2 (100%)	4 (44.44%)	-	1 (Reference)
TT	0 (0.0%)	0 (0.0%)	NS	-

(Table 9) contd….

***miRNA* 196a C/T Genotype**	**Alcohol User + Efavirenz N= 2(%)**	**Alcohol Non User + Efavirenz User N= 9(%)**	***P*-Value**	**OR(95%CI)**
CC	0 (0.0%)	5 (55.55%)	NS	-
CT	1 (50%)	4 (44.44%)	-	1 (Reference)
TT	1 (50%)	0 (0.0%)	NS	-
HIV Patients				
***miRNA* 146a G/C Genotype**	**Alcohol User + Nevirapine N= 37 (%)**	**Alcohol Non User + Nevirapine User N= 76 (%)**	***P*-Value**	**OR(95%CI)**
GG	14 (37.84%)	30 (39.47%)	-	1 (Reference)
GC	21 (56.76%)	40 (52.63%)	0.99	1.00 (0.41 – 2.45)
CC	2 (5.40%)	6 (7.90%)	0.40	0.43 (0.072 – 2.86)
***miRNA* 149C/T Genotype**	**Alcohol User + Nevirapine N= 37 (%)**	**Alcohol Non User + Nevirapine User N= 76 (%)**	***P*-Value**	**OR(95%CI)**
CC	6 (16.22%)	18 (23.68%)	-	1 (Reference)
CT	19 (51.35%)	37 (48.69%)	0.47	1.52 (0.48 – 4.76)
TT	12 (32.43%)	21 (27.63%)	0.18	2.40 (0.68 – 8.55)
***miRNA* 196a C/T Genotype**	**Alcohol User + Nevirapine N= 37 (%)**	**Alcohol Non User + Nevirapine User N= 76 (%)**	***P*-Value**	**OR(95%CI)**
CC	19 (51.35%)	47 (61.84%)	-	1 (Reference)
CT	15 (40.54%)	21 (27.63%)	0.08	2.29 (0.89 – 5.88)
TT	3 (8.11%)	8 (10.53%)	0.98	0.98 (0.21 – 4.51)
***miRNA* 146a G/C Genotype**	**Alcohol User + Efavirenz N= 4(%)**	**Alcohol Non User + Efavirenz User N= 6(%)**	***P*-Value**	**OR(95%CI)**
GG	3 (75.0%)	3 (50.0%)	-	1 (Reference)
GC	1 (25.0%)	2 (33.33%)	0.83	0.58 (0.004 – 83.17)
CC	0 (0.0%)	1 (16.67%)	NS	-
***miRNA* 149C/T Genotype**	**Alcohol User + Efavirenz N= 4(%)**	**Alcohol Non User + Efavirenz User N= 6(%)**	***P*-Value**	**OR(95%CI)**
CC	1 (25.0%)	2 (33.33%)	-	1 (Reference)
CT	1 (25.0%)	3 (50.0%)	0.81	0.67 (0.025 – 18.06)
TT	2 (50.0%)	1 (16.67%)	0.42	4.0 (0.13 – 119.23)
***miRNA* 196a C/T Genotype**	**Alcohol User + Efavirenz N= 4(%)**	**Alcohol Non -User + Efavirenz User N= 6(%)**	***P*-Value**	**OR(95%CI)**
CC	3 (75.0%)	6 (100.0%)	-	1 (Reference)
CT	1 (25.0%)	0 (0.0%)	NS	-
TT	0 (0.0%)	0 (0.0%)	NS	-

NS, not significant. N= number of subjects, **(%) =** frequency of subjects, Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele with other genotypes.

Table 10. Multivariate analysis between HIV patients with and without hepatotoxicity.

Variables	B	S.E.	df	*P*-Value	OR(95%CI)
146a GG			2	0.88	
146a GC	-0.19	0.433	1	0.65	0.82 (0.35-1.92)
146a CC	0.075	0.797	1	0.92	1.07(0.22-5.14)
149 CC			2	0.86	
149CT	-0.198	0.51	1	0.69	0.82(0.35-2.239)
149TT	0.043	0.56	1	0.93	1.04 (0.34-3.13)
196aCC			2	0.08	
196aCT	0.86	0.44	1	**0.05**	**2.38** (1.00-5.67)
196aTT	-0.78	1.10	1	0.47	0.45 (0.05-3.98)
Age	0.049	0.031	1	0.10	1.05 (0.98-1.11)
Sex	-0.319	0.447	1	0.47	0.72 (0.30-1.74)
Tobacco user	-0.469	0.569	1	0.40	0.62 (0.20-3.98)
Alcohol user	0.527	0.602	1	0.38	1.69(0.52-5.51)
NNRTI drug user	1.789	0.529	1	**0.005**	**5.98** (2.12-16.88)

miR (146a G/C, 149C/T and 196a C/T) polymorphisms, Age 18-50 year, sex, tobacco user, alcohol user, NNRTI drug user. Significant values (<0.05) represented in bold.

the gene expression as compared to the presence of single variation [41]. While comparing between patients with *vs* without hepatotoxicity and patients with hepatotoxicity *vs*. healthy controls, we found that the combined genotype GCT was likely to be associated with susceptibility to hepatotoxicity severity (OR=2.88, P=0.06; OR=2.60, P=0.09). On comparing between patients with hepatotoxicity and healthy controls, the combined genotype CCC was associated with susceptibility to hepatotoxicity severity (OR=2.89, P=0.05). The combined risk of the genotype of two SNPs on *miR*-149 and *miR*-196a2 was associated with cancer [36]. We hypothesize that the combined genotype may influence the processing of the mature *miRNA* for translational repression.

Also, we analyzed the distribution of *miR* polymorphisms among HIV disease stages and healthy controls. The *miR* 196aTT genotype was associated with the advanced HIV disease stage (OR=3.68, P=0.04). This suggests that HIV patients with *miR* 196aTT genotype may facilitate the risk for the advancement of HIV disease. The frequency of *miR*196aTT genotype was higher in advanced stages of oral squamous cell carcinoma when compared to early stage [36]. Hsi-Feng, (2012) reported that HNSCC patients with *miR*196a TT genotype seemed to have a poorer prognosis.

We also analyzed the gene-environment interaction using the case method. We analyzed the interaction of *miR* polymorphisms with tobacco, alcohol and drug usage. A study suggested that heavy consumption of alcohol had a negative impact on the CD4 cell count in HIV patients lacking antiretroviral treatment [42]. A decreased response to the ART was observed in HIV infected women using tobacco [43]. In subgroup analysis, the *miR*196aCT genotype showed susceptibility to acquisition of hepatotoxicity and its severity among alcohol consuming HIV patients without and with hepatotoxicity (OR=5.17, P=0.14; OR=2.36, P=0.06). The *miR* 149TT genotype showed increased susceptibility to acquisition of hepatotoxicity among alcohol consuming HIV patients (OR=2.29, P=0.17). We hypothesized that HIV patients consuming alcohol with *miR*196a CT and *miR*149TT genotypes may be more prone to alcohol-induced hepatotoxicity. However, these findings need to be confirmed in a large sample size.

The *miR*149TT genotype was likely to be associated with susceptibility to hepatotoxicity severity in nevirapine using patients with hepatotoxicity (OR=4.19, P=0.07; OR=1.97, P=0.84). In both alcohol + nevirapine consuming patients with and without hepatotoxicity, the *miR*196a CT genotype was likely to be associated with susceptibility to acquisition of hepatotoxicity and its severity (OR=2.29, P=0.08; OR=14.18, P=0.08). In multivariate logistic regression, on comparing between patients with and without hepatotoxicity, nevirapine usage, 196aCT genotype emerged as an independent risk factor for hepatotoxicity severity (OR=5.98, P=0.005; OR=2.38, P=0.05). These findings need to be validated in larger samples.

The present study has certain limitations, it could only evaluate the association and not causality. 1) We allocated a ratio of 1:4 for case-controls. However, we did not manage matched enrollment in the controls. Notwithstanding this, the case-control ratio was approximately 1:3. 2) Also, we did not determine the plasma drug levels.

CONCLUSION

The *miR*196a CT genotype and combined genotypes GCT and CCC may have a role in the acquisition of hepatotoxicity and its severity and advancement of the disease.

Polymorphisms (rs2910164, rs2292832) of *miR* gene were not significantly associated with the acquisition of hepatotoxicity and its severity. Additionally, *miR*196aC/T and 149C/T polymorphisms in the presence of alcohol and nevirapine could facilitate the acquisition of hepatotoxicity and its severity. However, further study should be done in a larger sample size with other populations.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the institutional ethics committee board of Nari Ethics Committee, Pune, India.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study, Reported experiments on humans were in accordance with the ethical standards of the committee responsible for human experimentation (institutional national), and with the *Helsinki Declaration* of 1975, as revised in 2008 (http://www.wma.net/en/ 20activities/ 10ethics/10helsinki/).

CONSENT FOR PUBLICATION

Informed written consent was obtained from all participants involved in the study.

AUTHORS' CONTRIBUTIONS

Hari Om Singh: Overall supervision and manuscript preparation.

Sushama Jadhav: Experimental work.

Dharmesh Samani: Experimental work.

T.N Dhole: Clinical management.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available at www.nari-icmr.res.in

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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