Polymorphisms of HIV RT Gene Among the ART Naïve Native Drug Exposed Rural PLHA

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ABSTRACT

Background: The number of people living with human immunodeficiency virus (HIV) is increasing day by day in India. The disease has now spread from urban areas to rural areas. The proof reading of the reverse transcriptase enzyme is poor, which may lead to genetic diversity within the HIV strains, which in turn leads to problems like failure or resistance in antiretroviral treatment. This study is designed to find out the polymorphisms of the reverse transcriptase gene of HIV, after the native drug pressure among antiretroviral therapy (ART) naïve rural people living with HIV/AIDS (RPLHA). **Materials and Methods**: A total of 207 HIV-Reactive patients were allowed to take native drugs from the local area and were advised to attend the center for HIV after six months for a follow-up. At the time of the follow-up visit, a second blood sample was taken from 20 reactive native-drug exposed ART-naïve patients. The plasma was separated and transported at 20°C to the YRG Care Center for genotyping. **Results:** Among the 20 HIV-reactive samples processed for gene sequencing analysis to detect the genotypic variations, only one sample (5%) showed high-level mutational resistance variations and the predominant polymorphisms detected were V35T (100%), K122E (94.44%), and V60I (88.88%). **Conclusions:** The presence of drug-resistance mutations, although minimal, was important, as the drug-resistant strains could spread among the RPLHA and to their sexual partners. There was a definite need to generate a drug resistance database and the polymorphic pattern of Indian strains concern to the future clinical management of the disease, and a vaccine design to contain the disease.

Key words: Anti retroviral therapy, HIV, PLHA, Reverse transcriptase gene

INTRODUCTION

HIV-1, demonstrates high genetic diversity due to lack of proof reading ability of its enzyme; Reverse transcriptase and retroviral replication are highly error-prone processes. As a result of the high mutation rates, the human immunodeficiency virus (HIV)-1 virus strains show extreme genetic divergence.[1] The goal of the present study is to genotypically characterize the HIV-1 RT genes in the anti-retroviral drug-naïve, native, drug-exposed Rural People living with HIV/AIDS. As treatment programs are expanded, sequences obtained before Antiretroviral (ARV) therapy exposure provide the baseline rates of the polymorphism at positions thought to be related to or not related to drug resistance.[2] This sets the stage for evaluation of the transmission of resistance,[3,4] choice of initial treatment, and identification of the genetic changes resulting from treatment failure.[5] It is also decided to assess the impact of the native drugs on the reverse transcriptase gene, among the newly diagnosed PLHA.

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MATERIALS AND METHODS

Study design and study population

This study was conducted using stored plasma specimens from the ART-naïve, native, drug-exposed rural people living with HIV/AIDS. All the samples were collected between June 2004 and July 2010. The reactive patients were allowed to take native drugs from the local area and were advised to attend the center for HIV after six months for a follow-up. These specimens were collected during the follow-up visits of the infected population, in Meenakshi Medical College. These RPLHA were able to provide written informed consents. A total of 20 RPLHA were randomly selected from a group of 207 HIV-positive persons. Every fifth individual

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was selected to obtain 40 specimens for this sub-study, but only 20 were processed and analyzed in this current study. Moreover, care was taken to ensure equal distribution of factors such as age, marital status, and CD4 counts, to make this number representative of the entire cohort. This study was conducted at the Meenakshi Medical College and the samples were processed for Gene sequencing at the YRG Care Center, Chennai. The study was approved by the Institutional Review Board of the Meenakshi University, Chennai. Whole blood samples were collected in K3 EDTA evacuated tubes (Beckon Dickinson, NJ). All 40 samples were diagnosed with HIV infection by using the Triple serological tests using Rapid/ELISA (NACO Strategy III).

None of the study participants were exposed to ARV drugs in the past, by self-report. Drug Resistance Assay the HIV-1 genotyping assay was performed using the homebrew methodology. Reverse transcriptase (RT) regions of the polymerase (pol) gene of the HIV genome were amplified by complementary DNA (cDNA) conversion and nested polymerase chain reaction (PCR) as follows. The viral RNA was extracted from the plasma Siam viral RNA extraction kit (Qiagen Inc, Valencia, CA) and reverse transcribed to cDNA with a single temperature cycle, that is, 25°C for 10 minutes, 42°C for 1 hour, and 70°C for 15 minutes. RT from the polymerase gene was amplified from cDNA using the relevant primers. All PCR amplifications were performed on the Gene Amp PCR System 9700 (Perkin Elmer, Waltham, MA). The Amplified products from the first round of PCR were initially analyzed on an ethidium bromide (ETBR)-stained agarose (1%) gel. If the amplicon was visible on the gel, it was column purified using a PCR purification kit (iNtRON Biotechnology, Inc, Gyeonggi-Do, Korea). Those samples that were not detected after the first-round of PCR were subject to a second-round of nested PCR. The amplicon was again checked on an agarose gel and subsequently column purified. The purified PCR products were subjected to bidirectional sequencing on an automated ABI 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA). Both forward and reverse sequences were assembled and manually edited with the Seqscape Version 2.5 multiple sequence alignment software and exported as FASTA format consensus RT sequences. The consensus sequences of each specimen were assessed for drug resistance using the Stanford HIV drug resistance database available at http://hivdb.stanford.edu/.[6]

RESULTS

Among the 20 participants eleven were male and the remaining nine were female with a median age of 36 years (interquartile range (IQR) : 22–55). Almost all (100%) were of Tamil ethnicity and 100% were married. Most were daily wage workers (75%). All participants were ART-naive by self-report. No major significant differences were observed between participants with and without drug-resistance mutations. All samples were found to be of subtype C in the Stanford HIV drug-resistance database.

HIV-1 drug resistance pattern

Among all (n=20) the isolates only 18 were successfully amplified at the RT region of the pol gene. There were major mutations found that were directly associated with high-level resistance to ARV drugs and the overall prevalence of this was 5% (n=1) against the non-nucleoside reverse transcriptase inhibitor (NNRTI). The isolate was found to harbor two mutations (K103KN and G190AG) associated with reduced susceptibility to NNRTI drugs, namely, Delavirudine (DLV), Efavirenz (EFV), Nevirapine (NVP), and Etravirine (ETR) [Table 1].

Polymorphisms

In the RT region, the predominant polymorphisms detected were V35T (100%), K122E (94.44%), V60I (88.88%), S48T (83.33%), T200A (83.33%), Q207E (77.77%), and K173A (72.22%).

DISCUSSION

The HIV pandemic continues to be a big issue all over the world because of the route of transmission and lack of effective vaccine. India being a country with the third largest HIV-infected population, still needs a lot of effort to be put in, to contain the spread of the disease. From the first six cases detected in Chennai in 1986, the HIV epidemic has now grown to affect a lot more people in India today**.** It has also spread beyond the classic high-risk groups in urban areas to become a disease of the general population, predominantly in rural areas.[7] The current scenario of the transmitted drug resistance among ART-naive individuals can be ascertained by HIV-1 drug-resistance studies. Optimizing the initial treatment regimen for HIV-infected individuals is most important for producing a maximal and sturdy virological response. Therefore, testing for the presence of drug resistance strains prior to the initiation of therapy may be economical and beneficial to the individual.[6] In the current study, RT regions from the pol gene of HIV-1 viral strains from RPLHA were characterized. Major mutations, directly conferring resistance to ARV drugs has been described at positions K103KN and G190AG. These were found to confer resistance to NNRTI drugs, such as, DLV, EFV, ETR and NVP.

Table 1: Polymorphisms of the HIV RT Gene

Age / Sex	NRTI	NNRTI	Other mutations																		
M / 40	None	None	V35T	E36A	T39D	S48T	V60I	D121Y	K122E	S162A	K173A	Q174K	D177E	T200A	Q207E						
F / 33	None	None	V35T	E36AE	T39D	S48T	V60I	D121Y	K122E	S162A	K173A	D177E	T200A	Q207E	R211K						
M / 40										Could Not Amplified											
F / 22	None	None	V35T	T39N	S48T	V60I	D121HY	K122E	I135T	T139AT	S162A	K173A	D177E	G196E	T200A	Q207E					
		K103KN,																			
M / 25	None	G190AG	V35T	T39N	S48T	V60I	D121Y	K122E	I135T	S162A	K173A	Q174K	D177E	T200E	Q207E						
M / 45	None	None	V35T	T39DN	S48ST	V60I	K102Q	D121Y	K122E	I135V	S162A	K173A	Q174KQ	D177E	I178IM	T200A	Q207DN	R211K			
F / 38	None	None	V35T	E36A	T39D	S48T	V60I	K122E	I135T	S162Y	K173T	D177E	T200A	Q207E							
M / 35	None	None	V21I	V35T	T39E	S48T	V60I	K122E	I135T	D177E	K173A	T200E	E204K	Q207E							
F / 30										Could Not Amplified											
M / 55	None	None	V35T	T39E	S48T	V60I	K122E	D123DN	I135R	K166R	K173A	D177E	T200A	I202V	Q207E	R211KR					
M / 29	None	None	V35T	T39N	S48T	V60I	D121Y	K122E	I135R	S162A	K173A	D177E	Q197K	T200A	Q207E	R211K					
F / 23	None	None	V35T	E36A	T39D	V60I	D121Y	K122E	I135T	S162H	K166R	K173A	D177E	Q197L	T200E	E204K	Q207E	F214L			
M / 51	None	None	T27S	V35T	E36A	T39E	S48T	V60I	D121Y	K122E	K166R	K173A	D177E	T200A	Q207E						
M / 37	None	None	V35M	E36A	T39E	S48T	V60I	D121Y	K122E	I135R	K173AV	D177E	T200A	Q207E							
F / 46	None	None	V35T	T39E	S48T	K122E	I135K	S162C	K173A	D177E	G196K	T200A	Q207E	R211K							
F / 38	None	None	V35T	E36A	T39D	S48T	V60I	K122E	D123S	I135T	S162Y	K173A	Q174KQ	D177E	T200A	Q207E	R211K				
F / 33	None	None	K20R	V35T	T39E	S48T	V60I	D121H	K122E	I135T	S162A	T165I	D177E	T200A	Q207EK	R211K					
F / 40	None	None	V35T	E36AE	T39E	S48T	V60I	E53DE	D121Y	K122E	D123DE	S162N	K166R	K173T	D177E	Q197H	T200A	Q207G			
M / 53	None	None	V35T	E36A	T39E	S48T	D121H	K122E	I135T	A158S	S162A	K173A	D177E	T200A	Q207EGQR	R211K					
M / 33	None	None	V35T	T39D	S48T	E53DE	V60I	D121DH	K122EK	I135IT	K166R	K173A	D177E	T200A	Q207E	R211K					

HIV: Human immunodeficiency virus, RT: Reverse transcriptase

A study by Deshpande *et al,* from Mumbai indicated that two isolates out of 128 (1.6%) had the M184V mutation, indicating primary drug resistance to 3TC.[8] A phenotypic study by Hira *et al*, conducted in Mumbai has shown a higher prevalence (6.7%) of primary drug resistance to reverse transcriptase inhibitors.[9] Another genotypic study by Balakrishnan *et al,* from Chennai involving drug-naïve patients attending the HIV clinic has also shown the absence of primary drug resistance.[2] Our study shows 5% prevalence for Primary NNRTI drug-resistance and none for NRTI. There is not much variation found in drug resistance compared to the alert cutoff level, because of the native drugs used by the ART-naïve reactive patients.

Enormous polymorphic substitutions were observed in the current study. V35T, K122E, S48T, V60I, and T200A were observed in more than 80% of the sequences. In a study by Iqbal *et al*., at the RT region, the predominant polymorphisms detected were V60I (98.2%), S48T (94.5%), K122E (90.9%), T200A (85.5%), and D177E (80%).[6] Phenotypic studies were required to observe the actual association of these mutations in relation to HIV-1 drug resistance. These observations revealed that HIV-1 polymorphisms differed in different geographical locations and population groups within the same subtype. These polymorphic variations were important in terms of vaccine design.

CONCLUSION

Although HIV-1 drug resistance is usually acquired during failure of ARV therapy, drug-resistant strains are also transmitted between individuals. HIV-1 drug resistance has been observed among ART-naive RPLHA, although minimal, (alert cut off 5% — WHO) continuous surveillance is very much needed, because in the current scenario, the scale-up of ARV medications is high in India. The presence of drug-resistant mutations, although minimal, is significant, as drug-resistant strains can spread among RPLHA and to their sexual partners. In addition, future prospective studies with large sample size are needed to optimize HIV treatment strategies and to acquire a wider knowledge of drug resistance and polymorphic mutations among RPLHA. Generating a HIV drug-resistance database, using Indian strains, will be very important for the future clinical management of the HIV disease.

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