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

Mohana Krishnan K, Amsavathani SK

Department of Microbiology, Sri Muthukumaran Medical College and Research Institute, Mangadu, Chennai, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, India

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. The goal of the present study is to genotypically characterize the HIV-1 RT genes in the anti-retroviral drug-naive, 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. This sets the stage for evaluation of the transmission of resistance, choice of initial treatment, and identification of the genetic changes resulting from treatment failure. It is also decided to assess the impact of the native drugs on the reverse transcriptase gene, among the newly diagnosed PLHA.

MATERIALS AND METHODS

Study design and study population

This study was conducted using stored plasma specimens from the ART-naive, 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
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/.

**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, Delavirdine (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.[8] 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  | None            |
| F / 33   | None | None  | None            |
| M / 40   | None | None  | None            |
| F / 22   | None | None  | None            |
| M / 25   | None | None  | None            |
| M / 45   | None | None  | None            |
| F / 38   | None | None  | None            |
| M / 35   | None | None  | None            |
| F / 30   | None | None  | None            |
| M / 55   | None | None  | None            |
| M / 29   | None | None  | None            |
| M / 53   | None | None  | None            |
| M / 37   | None | None  | None            |
| F / 66   | None | None  | None            |
| F / 38   | None | None  | None            |
| M / 33   | None | None  | None            |
| M / 53   | None | None  | None            |

HIV: Human immunodeficiency virus, RT: Reverse transcriptase

CONCLUSION

Although HIV-1 drug resistance is usually acquired during failure of ARV therapy, drug-resistant strains are also transmitted between individuals. HIV drug resistance has been observed among ART-naive RPLHA. Generating a HIV drug-resistance database, using Indian strains, will be very important for future clinical management of the HIV disease.

The authors would like to thank Dr. Sunithi Solomon, Dr. Balakrishnan, Dr. Vidya, and Dr. Saravanan for their immense help in carrying out the molecular study at YRG Care.

ACKNOWLEDGMENT

The authors would like to thank Dr. Sunithi Solomon, Dr. Balakrishnan, Dr. Vidya, and Dr. Saravanan for their immense help in carrying out the molecular study at YRG Care.
REFERENCES

1. Lakhashe S, Thakar M, Godbole S, Tripathy S, Paranjape R. HIV infection in India: Epidemiology, molecular epidemiology and pathogenesis. J Biosci 2008;33:515-25.

2. Balakrishnan P, Kumarasamy N, Kantor R, Solomon S, Visya S, Mayer KH, et al. HIV type 1 genotypic variation in an antiretroviral treatment-naïve population in southern India. AIDS Res Hum Retroviruses 2005;21:301-5.

3. Boden D, Hurley A, Zhang I, Cao Y, Guo Y, Jones E, et al. HIV-1 drug resistance in newly infected individuals. JAMA 1999;282:1135-41.

4. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, et al. Antiretroviral drug resistance among patients recently infected with HIV. N Engl J Med 2002;347:385-94.

5. Kantor R, Katzenstein D, Gonzales MJ, Carvalho A, Wynhoven B, Soares M, et al. Genotypic analyses of RT and protease sequences from persons infected with non-subtype B HIV-1. 10th Conference on Retroviruses and Opportunistic Infections, Feb 10-14, 2003; Boston, Massachusetts, USA.

6. Iqbal HS, Solomon SS, Madhavan V, Solomon S, Balakrishnan P. Primary HIV-1 drug resistance and polymorphic patterns among injecting drug users (IDUs) in Chennai, Southern India. J Int Assoc Physicians AIDS Care (Chic) 2009;8:323-7.

7. Profile of the HIV epidemic in India. Dr. Sunith Solomon. VII Sir Dorabji Tata Symposium March 10-11, 2006 HIV/AIDS Research Issues.

8. Deshpande A, Recordon-Pinson P, Deshmukh R, Faure M, Jauvin V, Garrigue I, et al. Molecular characterization of HIV type 1 isolates from untreated patients of Mumbai (Bombay), India, and detection of rare resistance mutations. AIDS Res Hum Retroviruses. 2004;20:1032-5.

9. Hira SK, Panchal K, Parmar PA, Bhatia VP. High resistance to antiretroviral drugs: The Indian experience. Int J STD AIDS 2004;15:173-7.

How to cite this article: Krishnan KM, Amsavathani SK. Polymorphisms of HIV RT gene among the ART naïve native drug exposed rural PLHA. J Global Infect Dis 2012;4:110-3.

Source of Support: Nil. Conflict of Interest: None declared.