ABSTRACT

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Title: Studies on Molecular Diversity of Human Immunodeficiency Virus -1

India having the third major HIV epidemic in the earth with an estimation of 2.1 million people is living with HIV and more than 65 thousand deaths in past years. Transmission of HIV with anti-retroviral drug resistance mutations has a negative impact on prevention of HIV through ART. Studies on genetic diversity of HIV are hardly reported in India. There are limited data available on drug resistance mutation in treatment naïve patients, treatment failure on first line ART in resource limited settings, DRM in second line ART failure patients as well as on virological failure. Evidence on subtype diversity and drug resistance testing of infants (Age 6 weeks to 18 months) born to HIV positive mothers are also very rare.

This study aims to find out circulating subtypes, drug resistance mutations and phylogeny diversity of HIV from different individuals including babies born to HIV infected mothers, individuals both on ART and not on ART, and patients with virological failure.

HIV-1 subtype was detected and HIV drug resistance testing was performed with the samples of ART naïve individual, patients with treatment failure of first line anti-retroviral therapy, virologic failure patients and infant born to HIV positive mother. The Viroseq (Abbott diagnostics, Wiesbaden, Germany) and Qiamp (Qiagen, GMBH, Hilden Germany) both the platform was used. Obtained sequence were analysed in the HIV drug resistance database of Stanford University. Clade typing and phylogenetic reconstructions was performed using the REGA sub typing tool of the HIV drug resistance database, and nucleotide sequences were aligned using the Clustal W multiple sequence alignment program. Phylogenetic analysis was conducted using MEGA v3.0 software. The neighbor-joining method and Kimura parameter model were used for tree construction with reliability estimated from 1000 boot strap replicates.

All the obtained sequences were found subtype C with some recombinants. In pre-treatment HIV patients two recombinants of subtype C were submitted to GenBank and accession no (LC570898 & LC529744) was obtained.

The PI major drug resistance mutations detected in the protease gene were M46L and V32I. Whereas accessory PI drug resistance mutations were L23I, L24I and V82Deletion, for the RT gene L74V, M184V D67N, K70N, K70A, K65Q, D67S, L74I,

M184R, M184 Deletion, T215 Deletion, K65Deletion D67DN, L74Q, K65L, M184R, L74Q L74Y were detected as NRTI and E138EA E138R K101KE, K103N, G190GA V179D, Y188L Y181C Y181Deletion, Y188Deletion, G190Deletion Y181L, Y188R, G190G_REQRN Y188C, K238T were detected as NNRT. High variation of major and accessary PI mutations, NRTI- and NNRTI-resistant mutation was observed. NRTI and NNRTI DRMs were met at a frequency of 34 (77.27%) and 15(34.09%) amongst 44 first line ART failure patients, with M184V (34.09%), T215F (25.0%) and K219E (20.45%) being the most frequent among NRTI associated mutations, and Y188L (18.18%), K103N (6.81%) and A98G (6.81%) among NNRTI associated ones. PI DRMs were observed in 5/44 (11.3%) patients, with V82L, V82S and I84V being the commonest.

Virologic failure was detected among 15 samples out of 365 HIV/AIDS patients on second line ART. DR mutations were detected in 5 out of 9 samples and among the rest 4 samples no HIV DR mutation was detected. Among NRTI based drugs, M184V and M41L were predominant mutations (80%). For NNRTI based drugs, A98G and Y181C were predominant (80%) conferring resistance to DLV and NVP. Again for Ps, 154V, A71V, V82A and M46L were seen in 40% of the cases conferring resistance to IDV, SQV, LPV, NFV and ATV. With the predominant subtype C, few recombinant of C, D and J were found in infants born to HIV positive mothers whereas no drug resistance mutations were found in infants.

This study has been conducted with four different kinds of subjects with subtype diversity and implications of ART on their treatment. The pattern of DRM in pre-treatment patients may help to select appropriate ARV drugs at right time. For early identification of virologic failure, mandatory viral load testing is highly recommended by this study. Rapid access of ART in India warrants urgent need of HIV drug resistance surveillance for proper monitoring on adherence to ART that minimize the development of DRMs. HIV diversity may have consequences for diagnosis, pathogenesis, transmission, clinical management, epidemic dynamics and vaccine development.

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