Detection of Human Immunodeficiency Virus Type 1 (HIV-1) A/AE Circulating Recombinant Form (CRF) in India: Possible Implications

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Abstract

Background: Rapidly evolving viruses such as human immunodeficiency virus (HIV-1) develop marked sequence differences in their genome over the course of an epidemic and in individuals infected for longer duration. This is because of the error prone reverse transcriptase (RT), which rapidly incorporates mutations resulting in genomic diversity, altered cell tropism, immune escape, and variable resistance to antiretroviral drugs. As a result, radically different genomic combinations may be generated in individuals infected by genetically diverse viruses that have mosaic genomes.

Methods: Whole blood sample was collected from 25 HIV-1 infected patients. Chromosomal DNA was isolated from the patient’s peripheral blood mononuclear cells (PBMCs). Full-length gag gene (~1.5 kb) was amplified. PCR products were subjected to direct automated sequencing. For identification of recombinants Simplot version 2.5 was used.

Results: Out of 25 gag genes that were sequenced, the gene amplified from a 29 years old HIV-1 seropositive male revealed a putative recombinant sequence. This sequence showed maximum homology with HIV-1 subtype A. Simplot analysis revealed the sequence to be a likely recombinant with the following composition: Initial stretch of 1 to 200 nucleotides representing AE circulating recombinant form (CRF), 201 to 440 nucleotides representing HIV-1 subtype A, 441 to 660 nucleotides representing AE CRF again, 661 to 700 nucleotides representing HIV-1 subtype A and the remaining stretch of the nucleotides from 701 to 1076 representing AE CRF.

Conclusion: We document a putative HIV-1 subtype A/AE CRF. It is important to monitor various CRFs that are being generated and horizontally spread in the community. This has significant implications for development of candidate vaccine for India.

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Key Words: HIV-1; CRF (Circulating Recombinant Form)
describe a putative AE recombinant form of HIV-1 detected on sequencing of partial gag gene.

**Material and Methods**

Whole blood sample was collected from 25 HIV-1 infected patients, seropositive by ELISA and Western Blot. The patients were grouped as per revised CDC criteria [11]. Chromosomal DNA was isolated from patients peripheral blood mononuclear cells (PBMCs). No passage through cell culture or donor PBMCs was done. Full-length gag gene (~1.5 kb) was amplified using primers G00 (forward) (GACTAGCCGGAGGCTAGAAG, HXB2 764-782, GenBank accession # K03455) and G01 (reverse) (AGGGGTCTGTTGCCAAAGA, HXB2 2264-2281). Further, two polymerase chain reaction (PCR) products were generated using semi-nested and nested PCR with overlapping of ~100bps residues between each of these products. Semi-nested PCR was performed for amplification of first subgenomic region of the gag gene (~506bp) using primers G00(forward) (GACTAGCCGGAGGCTAGAAG, HXB2 764-782) and G75 (reverse) (CTTCTTATCTTTTACCATGC, HXB2 1249-1270). The second sub genomic region of gag gene (~716 bp) was amplified by nested PCR using primers G 60 (forward) (CAGCCAAAATTACCTTATATGGCAG, HXB2 1173-1197) and G25 (reverse) (ATTGCTTCAGCCAAAACCTTCTTG, HXB2 1867-1889). PCR products were subjected to direct sequencing with BigDye Terminator chemistry on an automated DNA sequencer model ABI PRISM @ 310 Genetic Analyser (Applied Biosystems, Foster City, California). Sequences derived from the sub-genomic fragments of the gag gene were overlapped using Genedoc multiple sequence alignment editor and sharing utility version 2.1.02 software [12]. The subtype was defined by NCBI BLAST (www.ncbi.nlm.nih.gov/blast). Multiple sequence alignment was done using Clustal X version 1.7 [13]. Genedoc multiple sequence alignment editor and sharing utility version 2.1.02 [12] and BioEdit sequence analysis editor 5.0.9 software [14]. For identification of recombinants Simplot version 2.5 [15] was used.

**Results**

Of the 25 HIV-1-infected patients, the gene amplified from a 29 years old HIV-1 seropositive male revealed a putative recombinant sequence. This individual hailed from Rajasthan and had received multiple transfusions in 1994 following a road traffic accident. 1076 bases of the gag gene from this strain, labelled as #80495 were sequenced. The sequence along with alignment with the reference strains is shown in Fig 1. This sequence showed maximum homology with HIV-1 subtype A. Simplot analysis revealed the sequence to be a putative recombinant gene with the following composition: initial stretch of 1 to 200 nucleotides representing AE CRF, 201 to 440 nucleotides representing HIV-1 subtype A, 441 to 660 nucleotides representing AE CRF again, 661 to 700 nucleotides representing HIV-1 subtype A and the remaining stretch of the nucleotides from 701 to 1076 representing AE CRF. The study strain was a putative mosaic sequence. The Simplot homology graph against the reference strains is shown in Fig 2. This depicts the plot of similarity (generated by SimPlot) of a set of reference sequences to the #80495 gag partial sequence (1076 nucleotides). Each curve is a comparison between gag partial sequence (1076 nucleotides) being analysed and a reference gag region. Each point plotted is the percent identity within a sliding window 200 bp wide centred on the position plotted, with a step size between points of 20 bp. Positions containing gaps were excluded from the comparison. The colours are consistent with those used for the similarity curves. The Simplot bootscan graph is shown in Fig 3. Each curve is a comparison between gag partial sequence (1076 nucleotides) being analysed and a reference gag region. Each point plotted is the percent identity within a sliding window 200 bp wide centred on the position plotted, with a step size between points of 20 bp. Positions containing gaps were excluded from the comparison. 100 Bootstraps were considered. DNA distance was calculated by Kimura-2-parameter (T=1.5) and Neighbour-joining method was used to calculate branch length. The colours are consistent with those used for the similarity curves.

**Discussion**

HIV-1 is a uniformly persistent, pathogenic and transmissible agent. The considerable plasticity of the HIV-1 genome plausibly contributes to its success in humans [16]. Continuing discoveries of divergent strains of HIV-1 [17] and circulating recombinant forms (CRFs) [7] around the globe have immense significance. The ability of this virus to mutate rapidly and establish successful epidemics in different geographic locations underscores the significance of global monitoring of HIV-1 strains. The HIV-1 sequence database is growing exponentially, but the distribution of submitted sequences is not representative of the worldwide picture. Subtype C predominates in India and causes 75-96% infections in Sub-Saharan Africa. It accounts for more than 50% of HIV-1 isolations around the world [18].

Sequencing data of small portions of subgenomic fragments of the viral genome, though relevant, may not be ideal for characterisation of the so-called mosaic genomes due to intersubtype recombination. In addition, in the context of subtype vaccine development, identification of mosaic genomes is important. Molecular analyses of various HIV-1 isolates reveal sequence variations over many parts of the viral genome. The virus variants isolated from HIV infected individuals worldwide share remarkable diversity.

Partial gag sequence (1076 bases) of the strain 80495 of the present study was identified as a probable mosaic sequence. This sequence showed maximum homology with HIV-1 subtype A. Simplot analysis revealed the sequence to be a putative AE CRF. That mosaic HIV-1 is present in India is important but not surprising since several subtypes of HIV-1 have been reported from India [10,19,20,21]. Using HMA to detect the presence...
Fig 1: Shows alignment of partial (1076 nucleotides) sequence of gag gene from isolate #80495. The reference sequences used for alignment are A1.UG85.U455 (ACC No M62320), C.IN.95.95IN21068 (ACC No AF067155), B.FR.83.HXB2 (ACC No K03455) and 01_AE.CF.90.90CF11697 (ACC No AF197340).
of HIV-1 subtypes in India, Sahni et al [20] have reported six putative homotypic recombinants between subtype C2 & C3, two homotypic recombinant between subtype C3 & C4 and one heterotypic recombinant between subtype B2 & C4. However no sequence data was available on these putative recombinants. Lole et al [10] have earlier reported an A/C recombinant from Pune. In Thailand epidemic, an early balance between a subtype B strain and an A-E recombinant was quickly shifted to dominance of the recombinant variant [22]. A similar A-E mosaic variant, apparently a descendant of the same recombination event, has been found in an infected individual in the Central African Republic, but till date, a purely subtype E genome has not been found [22]. It is possible that the recombinant variants of subtype E may have some significant advantage over the parental E strain, and that parent E subtype virus got eliminated by selection. Such advantages may include alterations in tropism, replication efficiency, or immune recognition. In addition, recombination may allow more efficient transmission in a susceptible population.

Therefore, a comprehensive molecular epidemiology database for the country is necessary and would be of immense value in developing future strategies to combat the scourge of the HIV epidemic in India and globally.

Conflicts of Interest
None identified

References


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