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)

Introduction

One of the major characteristics of the human immunodeficiency viruses (HIV) is their extremely high genetic variability. This extensive heterogeneity is the result of high error rate of reverse transcriptase [1] and fast turnover of virions in HIV infected individuals [2]. In an infected antiretroviral therapy (ART) naïve individual, 1×10^{10} virus particles are produced each day of which 10^4 - 10^5 have at least one mutation per genome. A rate of 3.4×10^{-5} misincorporations per replication cycle *in vivo* has been suggested [2,3]. The reverse transcriptase enzyme is known to be highly recombinogenic [4], as a result radically different genomic combinations may be generated in individuals infected by genetically diverse viruses. Recombination, though is most obvious among members of different subtypes (intersubtype or interclade), it is likely to occur among members of the same subtype (intrasubtype or

intraclade). The prevalence of intersubtype recombinant strains is increasing and is likely to create even more HIV-1 antigenic diversity [5,6]. Several recombinant viruses have now spread epidemically to establish distinct lineages [7].

Currently, nine different subtypes and two sub-subtypes have been identified (A through K) within group M of HIV-1 [8]. Prototype viruses representing the genetic subtypes E and I have not yet been found. 14 circulating recombinant forms (CRFs) have so far been identified [9]. The viruses originally identified as subtype E and I are intersubtype recombinants and have been termed circulating recombinant forms (CRFs), CRF01 AE and CRF04 cpx, respectively.

With the spread of several subtypes of HIV-1 in India, it is likely that recombinant viruses may exist. A recombinant virus with evidence of A and C intersubtype recombination has been reported [10]. In this paper we

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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) (GACTAGCGGAGGCTAGAAG, HXB2 764-782, GenBank accession # K03455) and G01 (reverse) (AGGGGTCGTTGCCAAGA, 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) (GACTAGCGGAGGCTAGAAG, HXB2 764-782) and G75 (reverse) (CTTCTATTACTTTTACCCATGC, HXB2 1249-1270). The second sub genomic region of *gag* gene (~716 bp) was amplified by nested PCR using primers G 60 (forward) (CAGCCAAAATTACCCTATAGTGCAG, HXB2 1173-1197) and G25 (reverse) (ATTGCTTCAGCCAAAACCTCTTGC, 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 ($T/t=1.5$) 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

	790	800	810	820	830	840	850	860	870	880
B.FR.83.HXB2_	ATGGGTGCGA	GAGCGTCAGT	ATTAAGCGGG	GGAGAATTAG	ATCGATGGGA	AAAAATTTCG	TTAAGGCCAG	GSGGAAGAA	AAAATATAAA	TTAAAACATA
C.IN.95.95IN21068_	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-
A1.UG.85.U455_	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-
01_AE.CF.90.90CF11697_	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-
80495_	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-	-----A-
	890	900	910	920	930	940	950	960	970	980
B.FR.83.HXB2_	TAGTATGGGC	AAGCAGGGAG	CTAGAACGAT	TCGCAGTTAA	TCCTGGCCCTG	TTAGAAACAT	CAGAAGGCTG	TAGACAAATA	CTGGGACAGC	TACAACCATC
C.IN.95.95IN21068_	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-
A1.UG.85.U455_	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-
01_AE.CF.90.90CF11697_	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-
80495_	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-	-----G-CA-
	990	1000	1010	1020	1030	1040	1050	1060	1070	1080
B.FR.83.HXB2_	CCTTCAGACA	GGATCAGAAG	AACCTTAGATC	ATTATATATAT	ACAGTAGCAA	CCCTCTATTC	TGTGCATCAA	AGGATAGAGA	TAAAGACAC	CAAGGAAGCT
C.IN.95.95IN21068_	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-
A1.UG.85.U455_	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-
01_AE.CF.90.90CF11697_	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-
80495_	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-	-----A--G-
	1090	1100	1110	1120	1130	1140	1150	1160	1170	1180
B.FR.83.HXB2_	TTAGACAAGA	TAGAGGAAGA	GCAAAACAAA	AGTAAGAAAA	AAGCACACCA	AGCAGCAGCT	GACACAGGAC	ACAGCAATCA	GGTCAGCCAA	AATTACCCCTA
C.IN.95.95IN21068_	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-
A1.UG.85.U455_	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-
01_AE.CF.90.90CF11697_	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-
80495_	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-	-----A--T--A-
	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280
B.FR.83.HXB2_	TAGTGCAGAA	CATCCAGGGG	CAATGCTGAC	ATCAGGCCAT	ATCAGCTAGA	ACTTTAAATG	CATGGGTAAA	AGTAGTAGAA	GAGAGGCTTT	TCAGCCCGAGA
C.IN.95.95IN21068_	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--
A1.UG.85.U455_	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--
01_AE.CF.90.90CF11697_	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--
80495_	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--	-----TC--A--
	1290	1300	1310	1320	1330	1340	1350	1360	1370	1380
B.FR.83.HXB2_	AGTGATACCC	ATGTTTTCAG	CATTATCAGA	AGGAGCCACC	CCACAGATTT	TAAACACCA	GCTAAACACA	GTGGGGGGAC	ATCAAGCCAG	CATGCAGATG
C.IN.95.95IN21068_	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--
A1.UG.85.U455_	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--
01_AE.CF.90.90CF11697_	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--
80495_	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--	-----G--A--
	1390	1400	1410	1420	1430	1440	1450	1460	1470	1480
B.FR.83.HXB2_	TTAAAAGAGA	CCATCAATGA	GGAAGCTGCA	GAATGGGATA	GAGTGCATCC	AGTGCATGCA	GGGCTATTG	CACCAAGGCA	GATGAGAGAA	CCAGGGGAA
C.IN.95.95IN21068_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
A1.UG.85.U455_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
01_AE.CF.90.90CF11697_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
80495_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
	1490	1500	1510	1520	1530	1540	1550	1560	1570	1580
B.FR.83.HXB2_	GTGACATAGC	AGGAACCTACT	AGTACCCCTTC	AGGAACCAAT	AGGATGGATG	ACAATAATC	CACCTATCCC	AGTAGGAGAA	ATTATATAAA	GATGGATAAT
C.IN.95.95IN21068_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
A1.UG.85.U455_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
01_AE.CF.90.90CF11697_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
80495_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680
B.FR.83.HXB2_	CCTGGGATTA	AATAAATAG	TAAAGATGTA	TAGCCCTACC	AGCATCTCTG	ACATAAGACA	AGGACCAAG	GAACCCCTTA	GAGACTATGT	AGACCGGTTT
C.IN.95.95IN21068_	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--
A1.UG.85.U455_	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--
01_AE.CF.90.90CF11697_	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--
80495_	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--	-----T--G--
	1690	1700	1710	1720	1730	1740	1750	1760	1770	1780
B.FR.83.HXB2_	TATAAAACTC	TAAAGGCCGA	GCAAGCTTCA	CAGGAGGTAA	AAATTTGGAT	GACAGAAAC	TTGTTGGTCC	AAAATGCCAA	CCCAGATTGT	AAGACTATTT
C.IN.95.95IN21068_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
A1.UG.85.U455_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
01_AE.CF.90.90CF11697_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
80495_	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--	-----T--
	1790	1800	1810	1820	1830	1840	1850	1860		
B.FR.83.HXB2_	TAAAGGCAIT	GGGACACGC	GCTACACTAG	AAGAAATGAT	GACAGCATGT	CAGGAGATAG	GAGGACCCGG	CCATAG		
C.IN.95.95IN21068_	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--		
A1.UG.85.U455_	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--		
01_AE.CF.90.90CF11697_	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--		
80495_	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--	-----G--		

Fig 1 : Shows alignment of (1076 nucleotides) sequence of *gag* gene from isolate #80495. The reference sequences used for alignment are A1.UG.85.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).

Fig. 2 : Plot of similarity (generated by SimPlot) of a set of reference sequences to the #80495 *gag* partial sequence (1076 nucleotides). The reference sequences used for generating the similarity plot are A1.UG.85.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). D.CD.83.NDK (ACC No M27323), O.CM.91.MVP5180 (ACC No L20571) and N.CM.95.YBF30 (ACC No AJ006022).

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

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Fig. 3 : Plot of bootscan analysis (generated by SimPlot) of a set of reference sequences to the #80495 *gag* partial sequence (1076 nucleotides). The reference sequences used for generating the similarity plot are A1.UG.85.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). D.CD.83.NDK (ACC No M27323), O.CM.91.MVP5180 (ACC No L20571) and N.CM.95.YBF30 (ACC No AJ006022).

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