

Molecular epidemiology of HIV

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The phylogenetic analysis of the nucleotide sequences of the *env* gene has enabled classification of HIV-1 into three groups. The group M of HIV-1 infection has been classified into 9 different genetic subtypes A-K, with E and I being classified as circulating recombinants forms (CRFs). The groups O and N are less frequently encountered in human infections. Presently group M of HIV-1 globally causes 99.6 per cent of all human infections. The epidemiological trends suggest that subtype C strains would dominate the HIV pandemic in the coming years. The geographic spread of subtype C strains is also very diverse with prevalence in Africa, Latin America and Asia. Data from India show a high prevalence of subtype C. In north and western India, 78.4 and 96 per cent of HIV-1 strains respectively were shown to be subtype C. Among female sex workers in Kolkata 95 per cent of the HIV-1 strains were subtype C. The south Indian subtype data are very similar to the data from the rest of India. The HIV-2 groups (subtypes) recognized are A-H. Unlike HIV-1, HIV-2 strains are predominantly found in Africa. The Indian HIV-2 strains identified till date are subtype A. This is also the predominant strain circulating in western African countries. This group (subtype) is estimated to cause 0.11 per cent of all HIV infections in humans.

Key words HIV-1 - HIV-2 - sequence diversity - subtypes

The acquired immunodeficiency syndrome (AIDS) continues to spread unchecked since its first documentation in 1981. Two decades later, nearly 50 million individuals are living with HIV/AIDS world wide, according to figures released by the Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO)¹. There are two types of human immunodeficiency virus (HIV) responsible for this infection/disease. A major proportion of the infection worldwide is caused by the HIV-1 virus, which was identified in 1983^{2,3}. The type 2 (HIV-2) virus was first detected in West Africa

and is significantly prevalent in those regions, Portugal and India with rare cases reported from western countries, Korea and Philippines⁴⁻¹⁰.

Since, the commencement of the AIDS epidemic two decades ago, HIV-1 has evolved, differing from one geographical location to another¹¹. This variability at the genomic level can be attributed to high mismatch error rate of the HIV reverse transcriptase (RT) enzyme coupled with the absence of proof reading capacity, diploid genome, the rapid turnover of the virus *in vivo*, viral fitness, immune response or

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therapeutic selection pressures and recombination events that are taking place during replication. It has been reported that HIV-1 RT has an average error rate of 1/1700 per detectable nucleotide incorporation¹¹⁻¹⁶. Further, certain regions of the genome are mutational hotspots with an error rate as high as 1 per 70 nucleotides¹². As a result when the nucleotide sequence of HIV-1 is analyzed, a great deal of heterogeneity is observed. The most notable manifestation of HIV-1 genetic diversity is the phylogenetic clustering of viral isolates worldwide referred to as clades or subtypes based on the 20-50 per cent differences in envelope (*env*) nucleotide sequences. The inherent diversity of HIV-1 is compounded by three separate introduction of the virus from chimpanzees to humans^{17,18}. Due to this reasons, there are now three groups of HIV-1: M (Major/Main), N (Non-M, Non-O/New) and O (Outlier). The *env* proteins of group M and O can show a variation as much as 30-50 per cent. The N subtype appears to be phylogenetically equidistant from M and O¹¹.

The M group is the most prevalent among the three groups. It now has nine subtypes (A-D, F-H, J, K), all of which have originated from Central Africa. The amino acid distances in the *env* gene between the subtypes in the major group have reached 25-35 per cent, while in the *gag* gene it is about 15 per cent^{18,19}. Within subtypes A and F, there are separate sub-clusters that are related closely to each other than to other subtypes. They are designated A1, A2 and F1, F2 respectively¹⁸. Within subtypes B, C and G there are geographically localized sub-clusters that share a common ancestry as suggested in phylogenetic trees: subtype B from Thailand²⁰, subtype C from India²¹ and Ethiopia²², and subtype G from Spain and Portugal (Table I)^{18,23}.

Majority of the HIV-1 strains analyzed till date are subtyped. However, there are a few HIV-1 strains with genomes having regions represented from different subtypes (recombinants) seen in geographic areas where more than one type is circulating. This recombinant status is reinforced by the finding that irrespective of the regions of their genome analyzed they fail the criteria of a single designated subtype. These 'hybrid strains' are the products of recombinant events taking place in the virus. If two different

subtypes of HIV-1 infect a single cell, following replication the result can be a mosaic genome comprising regions from each of the two subtypes. This is due to the "template switching" ability of the reverse transcriptase enzyme. Two types of recombinant forms have been identified - circulating recombinant forms (CRF) and the unique recombinant forms (URF)¹⁸.

If the recombinant is identified in at least three epidemiologically unlinked individuals characterized by full-length genome sequencing, they are designated CRFs. Three near full-length genomic sequences are preferred, but two complete genomes in conjunction with partial sequences of a third strain are sufficient (for CRF, the partial sequences must also confirm the CRFs mosaic structure)²⁴. There are currently 16 recognized CRFs²⁵. Most of the CRFs are described from Africa, though 5 have originated outside of Africa¹⁸. The formerly designated subtypes E and I are now reclassified as CRFs²⁶. In addition to CRF, several HIV-1 strains with unique mosaic structures have been reported in epidemiologically linked persons. These forms known as unique recombinant forms (URF) have not show any evidence of epidemic spread and are thought to arise due to secondary recombination of a CRF¹⁸. Currently there are about 30 of them²⁷.

Though the sequence diversity within HIV-1 group O is nearly as great as within group M, clades are not clearly differentiated phylogenetically. Hence, subtypes within the HIV-1 O group are not yet defined. Since not many group N strains have been sequenced, no subtype has been determined till date.

Because of the high degree of divergence, the homologous recombination between group M and O viruses were not expected. Contrary to this, there are recent reports of the intergroup recombinants reported from Cameroon^{24,28}.

Like HIV-1, phylogenetic clusters have also been described for HIV-2. Phylogenetic analyses have shown high degree of closeness between HIV-2 and simian immunodeficiency virus isolated from sooty mangabeys (SIVsm)²⁹⁻³¹. All the different subtypes described are considered to have arisen by

independent cross-species transmission from sooty mangabey to human transmission events and belong to different lineages¹⁷. Hence these subtypes are analogous to the groups described for HIV-1¹⁷. Currently there are 8 subtypes (groups) identified and designated as A to H³²⁻³⁵. Among these 8 groups, A and B are circulating in the human population and C-H represent only few unique infections. Hence, there is a proposal to call A and B as groups and C-H as putative-groups³⁵.

The present drive in the area of HIV prevention includes the attempts at vaccine development. The most cost effective control measures would be social intervention to prevent transmission and a prophylactic vaccine. The development of a therapeutic vaccine will have a modest impact if it achieves increased longevity of infected individuals with reduced viral loads in infected individuals reducing the risk of such individuals as transmitters. There are certain issues confronting HIV vaccine development drive. These include: agreement on measure of vaccine elicited protective responses, impact of subtype variations and emergence of recombinants. More evidence for cross-clade cytotoxic lymphocytic responses is to be obtained as this has implications for attempts at custom designed region specific vaccines.

Significance of genotype determination

Impact on biological properties: The possibility that HIV subtype may influence viral transmissibility and pathogenicity has been suggested. However, results of various studies have shown it extremely difficult to find consistent associations between HIV-1 subtypes and correlates of transmission and pathogenesis. Also, it seems unlikely that a single characteristic such as subtype can account for significant differences in transmission and disease progression.

Data available from a study done in South Africa³⁶ shows subtype B to be associated with male homosexual transmission and subtype C with heterosexual transmission.

In the context of disease progression subtypes A and G have been associated with longer AIDS-free

survival period³⁷. There are some striking differences in the pathogenesis of HIV-1 between areas where B subtype and non-B subtypes are prevalent. In contrast to areas where subtype B is prevalent, rapid progression to AIDS is seen in areas where non-B subtypes are predominant^{15,38,39}. However, Kaleebu *et al*⁴⁰ has shown the lack of association of subtypes with disease progression in Uganda. In Uganda, an area where HIV-1 subtypes A and D circulate, no increase has been shown in the prevalence of one subtype over the other for over a decade⁴¹.

Several reports have shown that viruses with subtypes A, C or inter subtype recombinant long terminal repeats (LTRs) are more likely to be transmitted from mother to infant compared to viruses with subtype D LTRs. The transmission rates was 3.2 times more for subtype A, 4.8 times more for inter subtypes and 6.1 times more likely to be for subtype C⁴³.

Impact on HIV testing: It is necessary for commercially available assays to be able to diagnose infections of all HIV-positive individuals including those infected with less prevalent, more diverse subtypes. Kits for diagnosis and monitoring HIV infections were first developed for HIV-1 clade B and initially not optimized for use with others. Current assays used for detection of HIV-1 antibodies can detect all subtypes of groups M and O as also HIV-2⁴⁴.

The initial HIV amplification assays have encountered problems during estimation of HIV-1 RNA for some non- B subtypes⁴⁵. The newer versions of these assays, which are in use today, have been shown to reliably quantify HIV-1 RNA from subtype A-G infections. However, variations in results were seen during quantitation of group O. Primer mismatch has been linked to the failure to quantify viral load from group O infections by the AMPLICOR HIV-1 Monitor version 1.5, which were however detected by LCx HIV RNA Quantitative⁴⁶.

Impact on antiviral resistance: A major obstacle to the long-term efficacy of antiretroviral therapy is the emergence of HIV-1 variants with reduced susceptibility to antiretroviral agents⁴⁷. Genotypic analyses of viruses of different clades have shown

nucleotide change (silent mutations), polymorphisms and secondary mutations within reverse transcriptase and protease regions implicated in the emergence of resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) used in HIV-1 treatment. In treatment-naïve patients, many of these changes are not predicted to confer resistance to drugs among the different clades. They may however facilitate the development of resistance¹¹. It is believed that some of these pre-existing genetic polymorphisms may accelerate the emergence of a few NNRTI resistance mutations in certain HIV-1 non-B subtypes¹¹.

In the absence of any drug exposure, protease sequences from B and non-B HIV-1 are polymorphic among 30 per cent of the protease gene coded amino acids⁴⁸. Some of these amino acid substitutions may occur at high rates in non-subtype B viruses at positions associated with drug resistance in subtype B.

Unlike HIV-1 group M, HIV-1 group O and HIV-2 are inherently resistant to NNRTI because of a base substitution in the RT gene^{49,50}.

Methodologies for genotype determination

There are a number of methods, to understand the genetic heterogeneity of HIV-1 subtypes, the reference method being sequencing and phylogenetic analysis. This method not only determines the subtype but also examines the relationship between a set of sequences¹⁸. However, there are other methods that are economically feasible and more accessible in the developing world.

Heteroduplex mobility assay (HMA) makes use of the difference in electrophoretic mobility of a heteroduplex formed between the amplified PCR product of a sample and a reference strain to identify genotypes⁵¹. HMA can employ different target sequences with the *gag* and the *env* region (gp120) being the preferred region. The *env* gp41 based HMA is considered to be an useful tool to monitor subtypes in countries with divergent strains of HIV-1. It circumvents problems arising due to variation in sequences as it targets a relatively conserved region⁵².

Hyendrickx *et al*⁵³ have used a combination of *gag/env* primers to detect recombinant strains of HIV-1 group M isolates.

Another well described and simple laboratory method for the differentiation of HIV-1 subtypes is the V3 serological sub-typing⁵⁴⁻⁵⁶. This method is based on the binding of antibody to peptides from the V3 loop of the envelope from different subtypes⁵⁷. Genotyping and serotyping have shown a good correlation in areas where a single subtype circulates⁵⁸. Serotyping with the V3 peptide depends on an immune response to an antigen coded by a very small antigenic domain and thus a single amino acid substitution can affect serotyping. By contrast, genotypic methods are based on the analysis of a much larger domain and are hence more specific. Though simple, it is however not very practical in areas where multiple subtypes co-circulate. Further, it is not reliable in differentiating subtype C and A⁵⁹. The V3 serological sub-typing results with HIV-2 are of limited value, as these are inconsistent with the genotyping result, especially since V3-A and V3-D peptides, display poor discrimination. This also reaffirmed the high level similarity between the V3 sequences of different HIV-2 subtypes⁶⁰. The V3 loop of the HIV-2 appears to be highly conserved whereas the V2 region appears to be a highly variable region⁵.

Both heteroduplex mobility assay and serotyping are useful techniques in areas where a single subtype predominates. In areas where multiple subtypes co-circulate, more than one genomic region will need to be analyzed to rule out recombination. In such circumstances the best technique is the sequencing of whole genome. This technique is however labour-intensive, time consuming and expensive.

Global scenario

HIV-1: All groups of HIV-1 are found in Africa. While group M is prevalent all over the continent, groups N and O are geographically restricted to Central Africa^{15,61-63}. Subtypes A and D are prevalent in East Africa, subtype A in West Africa, and subtype C in south Africa. In West and Central Africa, the most prevalent genetic form is however a recombinant virus CRF02_AG¹⁸. Subtype B is the

Table I. Geographic distribution of HIV-1 groups and non-recombinant subtypes

Groups	Non- recombinant subtypes	Geographic/country distribution
M	A	East Africa, West Africa and Central Africa Eastern Europe and Central Asia
	B	North America, South America, East Africa, Central Africa, North Africa/ Middle East, Europe, Australia, New Zealand, Japan, China, Korea, Philippines and Malay Peninsula
	C	India, Brazil, South Africa, East Africa, Nepal and China
	D	East Africa, Central Africa, West Africa, Eastern Europe and Central Asia
	F	Central Africa, West Africa, Latin America, Caribbean and North America
	G	West Africa, Central Africa, North Africa, Middle East, Eastern Europe, Taiwan and Korea
	H	Central Africa, Eastern Europe and Central Asia
	J	Central Africa, West Africa
	K	Cameroon, DR Congo
O	Nil	West Africa (Cameroon, Gabon, Niger, Nigeria, Senegal, and Togo), France and Norway
N	Nil	Cameroon

Source: References 19,27

most prevalent form in western and central Europe, the Americas and Australia. Subtype C is most prevalent in the Indian subcontinent and recombinants CRF01_AE and subtype B in South East Asia¹⁸. Globally subtype C is the most predominant subtype causing 47.2 per cent of infections²⁶. Even in areas that were traditionally non-C in nature, it is becoming more predominant. Subtype A and CRF02_AG was estimated to be the second leading cause of the pandemic (27%), followed by subtype B strains (12.3%)²⁶. To date, 16 CRFs of HIV subtypes have been identified from different parts of the globe²⁵ (Table II) and are spreading sub-epidemics in areas where these strains have emerged.

Apart from these CRFs, unique recombinants have also been described. Many AC and AD recombinants are reported from Eastern African countries like Tanzania, Zambia, Uganda and Kenya, where subtype

A, C and D circulate^{27,64}. The AC recombinant was also reported from India, where subtype C and A co-circulate, though C is predominant⁶⁵. The other URFs reported are the B'01, a recombination of subtype B and CRF01_AE from Thailand, USA and Myanmar and recombinant B'C from Myanmar.

HIV-2: Unlike HIV-1, HIV-2 does not show any difference in geographical distribution as all subtypes/groups are found in West Africa. The two most prevalent HIV-2 subtypes are A and B. Majority of the HIV-2 characterized till date appears to be group A reported from West African countries⁶⁶⁻⁶⁸. Group B viruses seem to be more geographically limited (Fig. 1). It has been reported from Abidjan, Ivory Coast, France and Portugal^{5,7,69,70}. Groups C, D, E and F are designated based on the analysis of partial sequences of viral genomes. Groups C and D were identified from Liberia and groups E and F

Table II. The overview on currently existing circulating recombinant forms (CRF) of HIV-1

Name	Reference strain	Subtypes	Country / Region
CRF01_AE	CM240	A, E	Mekong region and the Malay peninsula, Central Africa, USA
CRF02_AG	IbNG	A, G	West and Central Africa
CRF03_AB	Kal153	A, B	Russia, Ukraine
CRF04_cpx	94CY032	A, G, H, K, Unclassified	Cyprus and Greece
CRF05_DF	VI1310	D, F	Belgian, DR Congo
CRF06_cpx	BFP90	A, G, J, K	Burkina Faso, Mali
CRF07_BC	CN54	B', C	China
CRF08_BC	GX-6F	B', C	Southern China
CRF09_cpx	96GH2911	Not published yet	West Africa, USA
CRF10_CD	TZBF061	C, D	Tanzania
CRF11_cpx	GR17	A, CRF01_AE, G, J	Cameroon, Central African Republic, Gabon, DR Congo
CRF12_BF	ARMA159	B, F	Argentina, Uruguay
CRF13_cpx	96CM-1849	A, CRF01_AE, G, J, Unclassified	Cameroon
CRF14_BG	X397	B, G	Western Europe
CRF15_01B	99TH.MU2079	CRF01_AE, B	Thailand
CRF16_A2D	KISII5009	A2, D	Kenya, South Korea and Argentina

Adapted and modified from HIV Sequence Database (<http://www.hiv-web.lanl.gov/content/hiv-db/CRFs/CRFs.html>)²⁵

were reported from Sierra Leone^{30,32}. Group G is represented by the phylogenetic analysis of full-strength genomic sequences of a strain collected from an asymptomatic blood donor from Ivory Coast³⁴.

The latest group, H was identified from a male patient originating from Ivory Coast³⁵.

Indian scenario

India has about 4.6 million people living with HIV next only to South Africa. The national HIV prevalence is between 0.4 and 1.3 per cent⁷¹.

HIV-1: The data from three centres in India show a high prevalence of subtype C. In north India 78.4 per cent of the strains were subtype C. The strain analysis also found 68 per cent of the circulating subtype to

be C3⁷². Another HIV-1 subtype study carried out in western India demonstrated 96 per cent of the tested samples to be subtype C with majority (66%) of them being C3⁷³. A study undertaken among female sex workers in Kolkata showed 95 per cent of strains to be subtype C⁷⁴. Among these subtype C strains, 68 per cent showed maximum homology to the C3-Indian reference strain (NIH, HMA panel). A study from south India also showed very similar findings to that observed in the north, west and eastern parts of India⁷⁵. Ninety five percent of the 83 strains analyzed showed homology with subtype C. Among the subtype C strains that were further characterized, 90.38 per cent had maximum homology with subtype C3. Subtype A was detected in 3.7 per cent of the individuals tested (Fig. 2)⁷⁵. The other subtypes reported in small proportion are subtypes A, B and a report of unique recombinant variant AC^{65,75,76}.

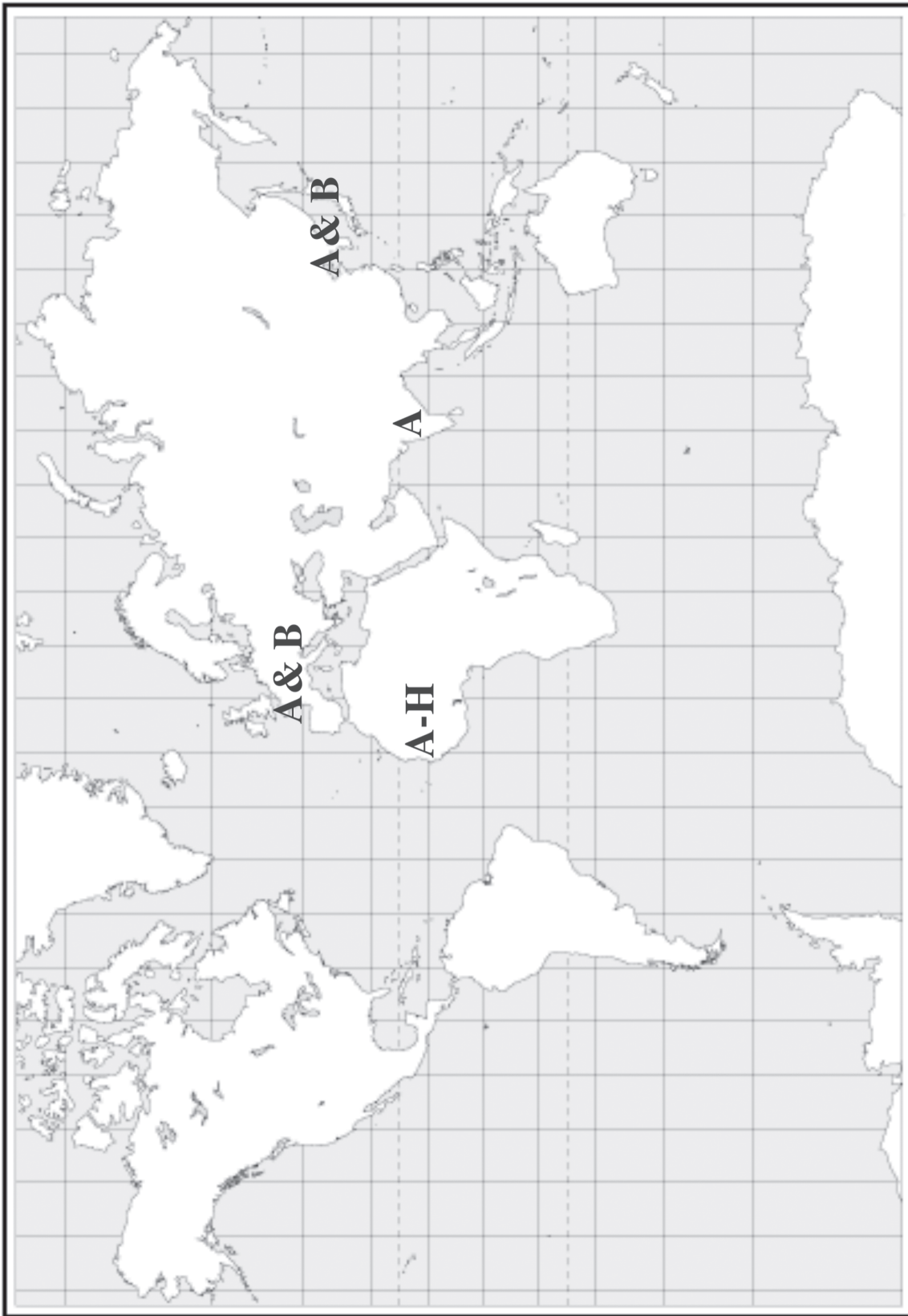
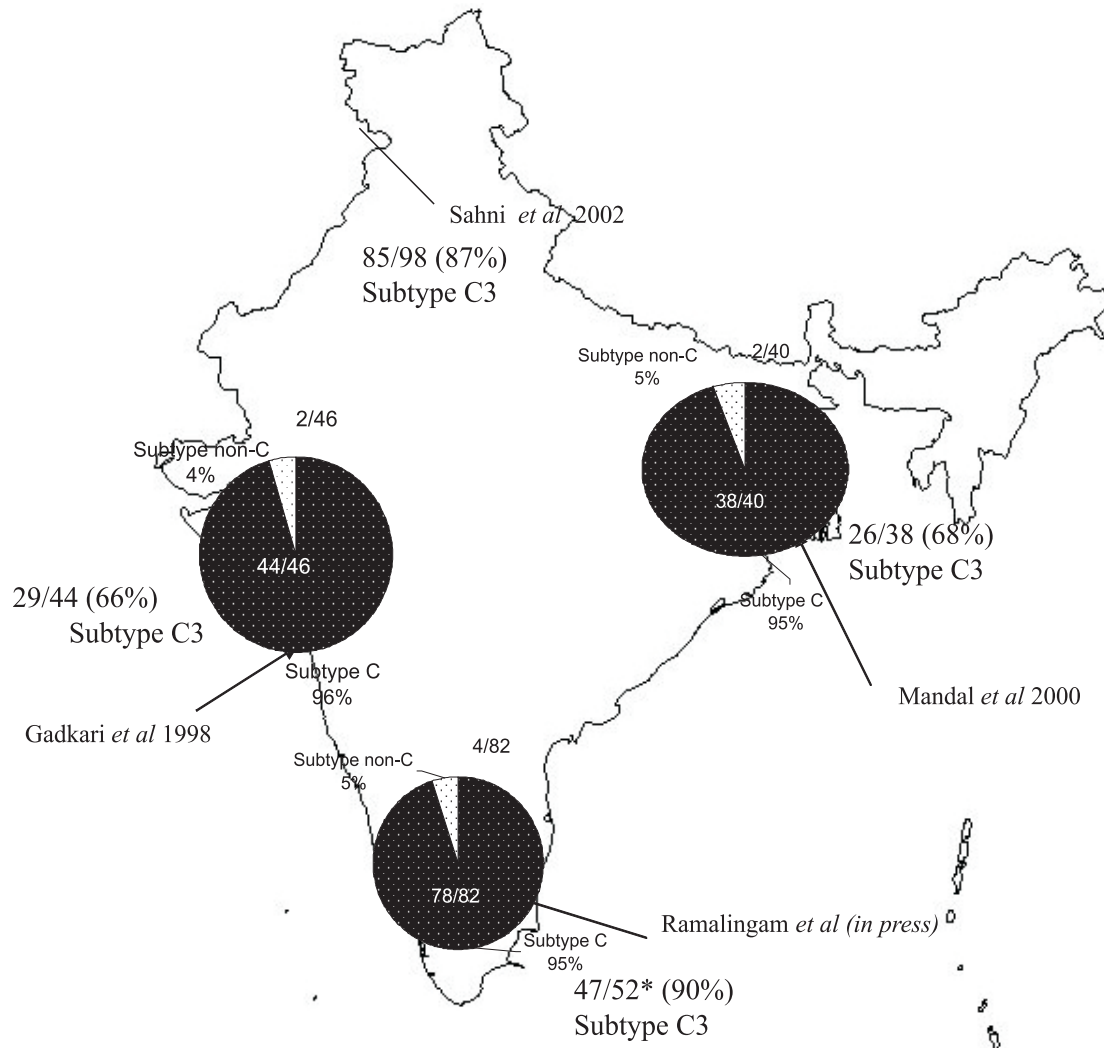


Fig.1. Global geographic distribution of HIV-2 subtypes/groups.



In all the studies the method used for subtyping was HMA and other subtypes reported were A,B and E (currently designated as CRF 01_AE). * Only 52 subtype C strains were further typed

Fig.2. Genomic diversity of HIV-1 in four different regions of India.

As the disease progresses to AIDS, compared to the HIV-1 subtype B, lower frequency of shift from non-syncytium inducing strains (NSI) to syncytium inducing strains (SI) is observed in subtype C infected individuals³⁸. One of the reasons for this lack of shift to SI from NSI may be the increased level of expression of CCR5 in CD4+ T cells or the presence of increased number of CD4 T cells expressing CCR5. Compared to the West, a higher proportion of CD4+ T cells express CCR5 among the Indian healthy controls and HIV infected individuals⁴². This possibly gives the R5 HIV strain a replication advantage over X4 HIV strains.

HIV-2: Grez *et al*⁷⁷ documented a decade earlier that the HIV-2 strains circulating in India were subtype A strains. This was based on characterizing five strains on sequencing, V1-V4 region of the envelope. The percentage divergence of sequences in HIV-2 strains from south India is minimal when compared with the sequence from west India. It indicates that during the period of 10 yr, HIV-2 strains circulating in India have not undergone any significant divergence in the envelope V3 region. The HIV-2 subtype A predominant in western African countries is the one mainly found to be circulating in the Indian subcontinent as well. Subtype A of HIV-2 is seen

both in monotypic and dual infections with HIV-1⁷⁸. In this study⁷⁸, the mean pair-wise genetic distances among the south Indian strains were 7.8 per cent (range 4.3-13.1%). This was similar to the distance among the five strains isolated from western India in 1991 (mean 7.7%, range 5.6-10.5%). The mean distances of the south Indian strains from the western Indian subtype A strains were only 8.8 per cent (range 4.5-15.5%) for the V3 region. However, this was 12.9% (mean 5.9-19.4%) with reference to non-Indian subtype A strains. The polygenetic tree analysis also showed subtype A and non A strains clustered as two separate groups. Among the subtype A strains all the Indian strains were clustered together in the tree.

Bhanja *et al*⁷⁹ reported a HIV-2 strain, which exhibited a mean genetic variation of 13.5 per cent from the other four reference strains of HIV-2 from India. Phylogenetic analysis of this strain has revealed a close relatedness to the HIV-2 Rod sequence isolated in offshore Senegal.

Summary

Currently based on the phylogenetic analysis of the nucleotide sequences from the *env* gene, strains belonging to group M of HIV-1 infection have been classified into nine different genetic subtypes A-K, with E and I being classified as recombinants. The epidemiological trends suggest that *env* based subtype C strains would dominate the HIV pandemic in coming years. The geographic spread of subtype C strains is also very diverse. It is prevalent in different regions in Africa, Latin America and Asia. Genotype C3 is the predominant HIV-1 subtype circulating in the Indian subcontinent. Data from different parts of India show a high prevalence of subtype C. In north India, 78.4 per cent of HIV-1 strain were subtype C and 68 per cent of the strain circulating were C3. From western India, 96 per cent of samples subtyped were subtype C with 66 per cent of them being C3. Among female sex workers in Kolkata 95 per cent of the HIV-1 strains were subtype C and 68 per cent were C3. The south Indian subtype data are very similar to that from the rest of India.

The HIV-2 genotypes recognized are A-H. Unlike HIV-1, HIV-2 strains are predominantly found in

Africa. The Indian HIV-2 strains are identified as subtype A. This is also the predominant strain circulating in western African countries.

The issues confronting HIV vaccine development are the still uncertain availability of measures of protective immunity in volunteers, genetic variation among the HIV strains along with diversity in geographical distribution. Much progress has been made addressing some of these issues offering avenues of therapeutic or prophylactic vaccine development and testing⁸⁰⁻⁸¹.

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