HIV-1 drug resistance among untreated patients in India: Current status

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ABSTRACT

HAART has dramatically improved survival and quality of life among people living with HIV and AIDS globally. However, drug resistant mutations of HIV are a great challenge to the benefits of HAART. Antiviral resistance can be mediated either by changes in the molecular target of therapy (the primary mechanism observed in HIV-1) or in other viral proteins that indirectly interfere with a drug's activity. Drug resistant mutations easily evolve in the presence of sub-optimal adherence. With the introduction of generic HAART, there has been a steep increase in the number of patients put on HAART in India. It should also be noted that since most patients pay for medications out of their own pockets, interruptions in therapy due to monetary constraints are not uncommon. There is little information on HIV drug resistance in resource constrained settings like India where the predominant circulating HIV-1 sub-type is C. The transmissibility of drug-resistant forms of the virus is also a major concern especially when formulating treatment guidelines. This article reviews published data available on the patterns of HIV-1 drug resistance among treatment naïve in India.

KEY WORDS: Antiretroviral drugs, HIV drug resistance, non-B subtypes, primary drug resistance

$\mathcal{I}$ndia with an estimated 5.134 million HIV-infected individuals at the end of 2004, has the second largest number of HIV infections in the world.[1] Since the introduction of generic antiretrovirals (ARVs) in 2000, there has been a steep increase in the number of individuals initiating antiretroviral therapy (ART) primarily due to the reduction in cost of ART from INR 35000 a month to approximately INR 1000 a month.[2,3]

Highly active antiretroviral therapy (HAART) has dramatically improved survival and quality of life in people living with HIV and AIDS.[4] However, these benefits can be greatly compromised by the drug-resistant forms of the virus. As the first therapeutic regimen is probably the most important for virologic suppression, drug-resistant variants of HIV greatly challenge the efficacy of HAART in producing adequate viral suppression.[5] In settings of incomplete viral suppression, drug-resistant mutations can easily evolve resulting in widespread drug resistance.[5,6]

There are numerous factors that result in the development of drug-resistant strains of the virus. The high replication capacity of HIV and its error-prone transcription is a major factor contributing to the development of resistance. It has been shown that retroviral replication is a highly error-prone process

with varying estimates of roughly 7×10^{-6} to 1.4×10^{-4} base-pair substitutions occurring per nucleotide per replication cycle.[6-8] Another significant source of genetic variation is recombination. Recombination between HIV-1 genomes has been demonstrated and probably occurs *in vivo* as a result of simultaneous infection of an individual by two distinct HIV-1 strains.[9-11] However, the observed degree of HIV-1 genetic diversity may also be influenced by selective pressure such as the host's immune response, cell tropism of the virus and the genetic makeup of the host.[12]

Irregularity in adherence to ART is probably the most important factor contributing to the development of resistance. Patients in India commonly interrupt ART as most patients pay for ART out of their own pockets. Patients also tend to combine the HAART with drugs from alternate systems of medicine. The interactions between alternate medicines and ARV agents are unknown. As many patients access care in the private sector, they are often prescribed mono and dual therapy, as the level of knowledge about HIV and HAART among the average clinician is quite low. It is also common that patients purchase their medications over-the-counter at pharmacies and could hence purchase sub-optimal regimens.

Since ART has been available in the western world since 1987, there have been numerous reports of the transmission of HIV strains with resistance to single or multiple antiretroviral drugs.[13-15] Moreover, some subtypes of HIV-1 can be less susceptible to protease inhibitors or non-nucleoside reverse-

transcriptase inhibitors (NNRTI) than the subtype B strains that are prevalent in the United States and Europe.[16-18] Although the transmission of drug-resistant strains of HIV has been well-documented, a concern with testing chronically infected patients is that, drug-resistant mutations will disappear in the absence of drug selection pressures and would hence be undetectable by resistance assays. Drug-resistant mutations also become undetectable if the infecting strains revert to a wild type or become overgrown by fitter wild-type viruses, persisting as archived viruses or as minority species and that may not be detectable by current assays.[13-15,19]

Despite widespread use of ARV agents, little information is available on the prevalence of HIV-1 drug resistance in India.[20-24] As treatment programs are expanded, the prevalence of HIV-1 drug resistance among ART naïve patients is of paramount importance in selecting treatment regimens and planning national policies. This review discusses the current situation of HIV-1 drug resistance among ART naïve patients in India.

HIV-1 primary drug resistance
In India, the number of individuals seeking treatment for infection with HIV has increased as the cost of highly active antiretroviral therapy (HAART) has decreased 20- fold after the introduction of generic HAART in India in the year 2000.[25] The most common regimens are three drug combinations of zidovudine (ZDV) or stavudine (d4T) plus lamivudine (3TC) plus nevirapine (NVP) or efavirenz (EFV).[2,3,26,27] Generic ART has been shown to be safe and tolerable and has also been shown to suppress viral replication in clinical trials in India.[28] However, one of the biggest issues with the management of HIV disease in India is the high rate of sub-optimal adherence. This sub-optimal adherence is brought about by a combination of various factors like financial constraints, forgetfulness and stigma. Hence, the monitoring of prevalence of drug-resistant strains is a major public health concern especially since India is home to the second largest HIV infected population in the world.

HIV-1 primary resistance to nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs
A study by Deshpande *et al* from Mumbai indicated that two isolates out of 128 (1.6%) had the M184V mutation, indicating primary drug resistance to 3TC.[20] This primary resistance was detected among drug-naïve patients in the early stages of HIV disease (CD4 + T- cell count more than 400/uL), attending the out patient clinic of the Sir JJ Hospital, Mumbai and they were recruited to the study in 2003. A phenotypic study by Hira *et al* conducted in Mumbai has shown a higher prevalence (6.7%) of primary drug resistance to reverse transcriptase inhibitors.[22] The patients (14/208) for this study were recruited in the years 1997-2003 and they were referred from different clinics in and around Mumbai. The resistance profiles were as follows: 2 (4%) isolates were resistant to AZT, 8 (3%) isolates were resistant to 3TC, 3 (11%) isolates were resistant to NVP and one isolate was resistant to d 4T.

A genotypic study by Eshleman *et al* from Pune with 12 acute

seroconvertors has shown that none of them had primary resistance.[24] These patients were recruited in the years 1999–2001, based on the presence of p24 antigenemia and the absence of confirmed HIV-1 antibody and subsequent seroconversion within 4 months. Similarly, another genotypic study by Balakrishnan *et al* from Chennai involving drug naïve patients attending HIV clinic also has shown the absence of primary drug resistance.[21] These patients were recruited in 2001-2003 and all the 50 cases were residents of Tamil Nadu and Andhra Pradesh.

Interestingly, a recent study by Sachdeva *et al* from Chandigarh has demonstrated the presence of coexisting strains of wild type and drug-resistant mutants among drug naïve patients, attending voluntary counseling and testing center (VCTC) from 2000 to 2002. A highly sensitive nested amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for the detection of the minority population of zidovudine and lamivudine-resistant variants of HIV-1. For the detection of zidovudine resistance they used the two most common resistance-associated codons 70 and 215 primer, while for lamivudine they used codon 184 specific primers.[29-31] Of 60 patients studied, they found a very high prevalence (32%) of M184V/I point mutations. Of these patients, 16 (84%) showed the presence of both Val and Ile variants (26.67% of the total patients) and 3 (16%) showed Val (5.0% of the total patients) coexisting with the wild type. Primary mutations K 70R was observed in 78.33% patients, whereas very low (2%) frequency AZT specific T215F primary mutation was seen in those drug naïve patients.

HIV-1 primary resistance to protease inhibitor [PI] drugs
Indian literature available has failed to identify the presence of primary drug resistance to protease inhibitors except for the study by Hira *et al*.[22] This study has shown 2.5% (2/79) prevalence of SQV primary drug resistance.

Primary and secondary mutations/polymorphisms
Extensive genetic variations (minor mutations) have been observed with Indian strains. However most of them are polymorphisms (mutations that were significantly more common in subtype C isolates than in subtype B isolates, but were present at similar frequencies in treated and untreated persons). There are some strains with amino acid substitutions at the drug-resistance positions, which may be associated with drug resistance (subtype C polymorphisms that occurred at significantly higher frequencies in treated persons with subtype B virus than in untreated persons with subtype B virus).[32]

Mutations at PI drug position
The most frequently observed positions of amino acid substitutions (primary mutations) are 12, 13, 15,19, 22, 35, 36, 43, 55, 60, 62, 63, 69, 74, 79, 89 and 93. Mutations at 36, 63 and 93 were observed in more than 90%, although these mutations are associated with drug resistance in subtype B strains.[33] Substitutions at positions of secondary mutations to PIs are less frequent (10, 20, 24, 46, 71 82 and 82). However, amino acid substitution at the position 82 was 18% in the Chennai study.[21] The Pune study has shown the limited evolution in the pol region

among acute seroconverters.[24] The sequence identity baseline and one year samples ranged from 99.7 to 99.9% and between baseline and two year samples ranged from 99.4 to 100%.

Mutations at NRTI and NNRTI drug position

As in the PI drugs positions, a higher level of polymorphisms are seen in RTI drug positions also. They are 35, 39, 48, 60, 13, 177, 200, 207, 214 and 245. Other minor mutations are at the positions 20, 22, 67, 118 and 210 in NRTI region and 103, 230 and 238 in NNRTI region.

Subtypes

India is the first Asian country outside Africa where HIV-2 and HIV-1/HIV-2 mixed infections have been reported.[34,35] However, there was very little evidence of epidemic spread of HIV-2 (unpublished data from YRG CARE, Chennai) in the Indian sub-continent, while the treatment options are very limited with currently available drugs for HIV-2 and mixed infections. Although the predominance of subtype C in India has already been reported by different investigators, the recent studies with pol region also indicate the prevalence of subtype C.[20-24] However, the study from Mumbai has shown the presence of A-C intersubtype recombinants in 2% of cases whereas subtype A and CRF01_AE were observed rarely.[20]

Future perspectives

It is clear from the currently available reports that among the untreated HIV-1 patients, the prevalence of known drug-resistance mutations is very low, when compared to the alert cut-off (5%), which has been defined by the ad hoc working group of the world health organisation (WHO).[36] In view of expanded treatment access to ARV amongst the Indian HIV-infected population, there is a need to assess the utility and feasibility of integrating HIV drug-resistance surveillance into the national ARV treatment programmes. Accelerated roll-out of ART could also lead to the emergence and transmission of ARV-resistant viruses. Although HIV drug resistance can not be prevented completely, there might be a possibility of combating its spread and impact. Moreover, in India there are very few reference laboratories that currently have the facility to perform genotyping / phenotyping techniques. Hence larger studies and continuous surveillance are needed among drug naïve HIV infected individuals, particularly with recent infections. Also generating a HIV drug-resistance database using Indian strains will be very important for the future clinical management of HIV disease.

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