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Genotypic HIV type-1 drug resistance among patients with immunological failure to first-line antiretroviral therapy in south India

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Abstract

Background—HIV type-1 (HIV-1) monitoring in resource limited settings relies on clinical and immunological assessment. The objective of this study was to study the frequency and pattern of reverse transcriptase (RT) drug resistance among patients with immunological failure (IF) to first-line therapy.

Methods—A cross-sectional study of 228 patients with IF was done, of which 126 were drug-naive (group A) when starting highly active antiretroviral therapy (HAART) and 102 were exposed to mono/dual therapy prior to HAART initiation (group B). A validated in-house genotyping method and Stanford interpretaion was used. Means, sd, median and frequencies (as percentages) were used to indicate the patient characteristics in each group. The χ^2 test and Fisher's exact test were used to compare categorical variables as appropriate. All analyses were performed using SPSS software, version 13.0. *P*-values <0.05 were considered to be statistically significant.

Results—RT drug resistance mutations were found in 92% and 96% of patients in groups A and B, respectively. Median (interquartile range) CD4+ T–cell count at failure was 181cells/ml (18–999) and time to failure was 40 months (2–100). M184V (80% versus 75%), thymidine analogue mutations (63% versus 74%), Y181C (39% versus 39%) and K103N (29% versus 39%) were predominant RT mutations in both groups. Extensive nucleoside reverse transcriptase inhibitor cross-resistance mutations were observed in 51% and 26%of patients in group B and A, respectively.

Conclusions—Alternative strategies for initial therapy and affordable viral load monitoring could reduce resistance accumulations and preserve available drugs for future options in resource-limited settings.

Introduction

The introduction of potent and cost-effective generic antiretroviral therapies (ARTs) has revolutionized the quality of life and longevity in HIV patients in India. As per the World Health Organization (WHO), the first-line regimen consists of two nucleoside reverse

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transcriptase inhibitors (NRTIs) and a non-NRTIs (NNRTIs), including combinations of zidovudine (AZT)/stavudine (d4T) plus lamivudine (3TC) plus nevirapine (NVP)/efavirenz (EFV), which is widely used in India. Currently tenofovir disoproxil fumarate (TDF)/abacavir (ABC) have been recommended for use in first-line therapy because of their efficacy and tolerance in clinical studies and for preserving second-line NRTI options [1], but these drugs are less accessible in India. Despite these treatment resources, selection of drug resistance as a consequence of incomplete suppression of HIV type-1 (HIV-1) replication poses a challenge to sustained ART. Patterns of resistance mutations after first-line treatment might differ depending on prior drug exposure and ART combinations, duration, adherence and HIV-1 subtype, and can determine the response to subsequent regimens. Studies among treated patients in subtype B and non-B viruses worldwide have reported evidence of drug resistance in up to 78% of viraemic patients receiving ART [2–4] and in 6–16% of untreated individuals [5,6]

The majority of the scientific literature regarding the emergence and management of resistance to triple-drug therapy, particularly to first-line highly active antiretroviral therapy (HAART), relates to the detection of resistance near the onset of virological failure [2,4,6,7,]. However, in resource-limited settings, treatment monitoring with HIV viral load testing is often not available because of cost. Thus, in these settings, monitoring of ART is guided by CD4+ T-cell count and clinical parameters to identify treatment failure [1]. The result is that patients are often maintained on a virologically failing regimen until immunological or clinical failure occurs, thereby accumulating multidrug resistance and hence falling short of available NRTI options for second-line in resource-limited settings.

In India, studies of drug resistance have identified a maximum frequency of 81% among treated patients [8,9] and 56% among the paediatric population [10], although these studies had small sample sizes and identified resistance at virological failure that does not reflect the current scenario. Here, we present the frequency and pattern of resistance mutations to reverse transcriptase (RT) inhibitors among patients receiving first-line treatment who developed confirmed immunological failure (IF). The results are analysed to identify the specific RT mutations associated with IF, comparing those with and without mono/dual exposure prior to HAART.

Methods

A cross-sectional study was conducted among 228 HIV-1-infected patients with evidence of IF on a first-line regimen[1] during the period March 2004 to June 2007, and were consecutively enrolled during their routine clinic visit to YRG CARE medical centre (Chennai, India), a tertiary HIV referral centre for south India. These patients were divided into two groups: 126 patients who were not exposed to mono/dual therapy (group A) and 102 patients who had been exposed to mono/dual therapy (group B) prior to initiation of first-line HAART. Those patients who had experienced changes in treatment because of toxicities (for example, switch from NVP to EFV/d4T to AZT) were considered to have received first-line HAART and were included in group A.

Inclusion criteria required that patients were aged >18 years, had been on first-line HAART for >6 months with self-reported good adherence (>95%), and were experiencing IF as per the WHO criteria [1].

Patients were excluded if they had been on drug holidays for >4 weeks before genotypic resistance testing. The study protocol was reviewed and approved by the Institutional Review Board of YRG CARE. Written informed consent was obtained from all study

participants before enrolment. Plasma was separated and stored aseptically within 6 h of collection at −75°C ±5°C until testing.

HIV-1 drug resistance assay was done using an in-house method that was previously validated with an assay analytical sensitivity of >1,000 copies/ml [11,12]. Interpretation of the genotype in terms of drug resistance was based on an algorithm established by the Stanford HIV-1 Sequence Database and all major clinically relevant RT mutations were analysed [13]. Plasma viral load was not done for these patients. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 [14].

The prevalence of drug resistance mutations was reported with 95% confidence intervals calculated on the basis of normal approximation of binomial distribution. Means, sd, median and requencies (as percentages) were used to indicate the patient characteristics in each group. The χ^2 test and Fisher's exact test were used to compare categorical variables, as appropriate. All analyses were performed using SPSS version 13.0 (SPSS Inc, Chicago, IL, USA). All tests of statistical significance were two-sided and associations with $P<0.05$ were considered to be statistically significant.

Results

Out of the 228 patients with first-line failure according to WHO guidelines, the majority (67%) had a 50% decrease in their CD4+ T-cell count from the peak value, followed by 23% who had a persistent CD4+T-cell count of <100 cells/ml after 6 months of therapy, and 7% who had a decrease in CD4+ T-cell count to baseline levels (or less than baseline levels) after 6 months of NNRTI-based first-line therapy. Out of the 228 patients, genotypic information was available for 200 patients (100 in each group), as plasma from 28 patients (26 in group A and 2 in group B) could not be amplified. The demographic details and treatment history of individual groups are given in Table 1. Overall, RT drug resistance-associated mutations (RAMs)were observed in 94% (188/200) of patients, of whom NRTI resistance mutations was identified in 95% and NNRTI resistance mutations in 96%.

There was no difference between group A (92%) and group B (96%) in terms of selecting any one of the those RT RAMs. In total, 63% in group A and 74% in group B had thymidine analogue mutations (TAMs), with M41L and T215Y significantly ($P<0.05$) higher in group B (Table 2). The frequency of K65R mutation was higher in group B ($P=0.10$), whereas other nucleoside analogue mutations (NAMs) was evenly distributed. K65R was only found among patients failing d4T-containing regimens in both the groups. Single codon deletion at RT position 69 was found in 2 (1%) patients, one in each group, although no insertions were observed.

Among NNRTI mutations, Y181C was the predominant, followed by K103N and G190A with similar distribution between groups. Any one of the etravirine (ETR)-associated mutations were observed in 95%, of which only 1% had >3 ETR mutations (Table 2). The duration on HAART before IF was 37.5 and 43 months in groups A and B, respectively.

Overall, 26% of patients in group A and 51%in group B had developed high-level NRTI cross-resistance in terms of coselecting TAMs, K65R and Q151M (Table 2). All the RT sequences were monophyletically clustered with Indian subtype C using 1000 bootstrap.

Discussion

This study demonstrates high frequency of RT inihibitor resistance mutations among south Indian patients on treatment who were selected for sequencing and resistance analysis based on IF after a median of almost 4 years on first-line HAART.

The frequency of resistance among these patients was higher (94%) than those previously reported in India [8,9] and from other subtype B and non-B cohorts [2–4,15]. The high frequency of resistance is likely because most of the patients were genotyped at the time of IF, whereas most of the literature on drug resistance is based on virological monitoring and resistance testing at virological failure. In the current study, there was no significant difference with respect to time to IF among groups A and B (38 versus 43 months).

NAMs were observed at a similar frequency between the groups; not surprisingly, M184V was the predominant mutation and its frequency is in agreement with other patients failing HAART [8,9] and other studies in subtype C globally that included 3TC [4,16]. TAMs were the next frequent NRTI mutation observed (69%) at a higher frequency compared to the earlier reports from India [8,9] as the majority of the patients (93%) were on thymidine analogues, with d4T being predominant. T215Y, L210W and M41L, which constitute the TAM-1 pathway [17] were significantly higher in group B, and this might be associated with their prior AZT/3TC dual therapy prior to HAART.

Although subtype C isolates have been reported to have a higher tendency to develop K65R [18,19], by contrast, we observed a lower rate of K65R when compared with global reports, but a higher rate than that reported in India so far [8,9]. Prior studies have also found K65R to be associated with patients failing a d4T-containing regimen [20], which is in line with the findings our study. Unlike the previous reports, Q151M and its accessory mutations were observed among patients exposed to d4T rather than AZT/didanosine (ddI) [21], which could be a result of bias in the number of patients on failing AZT/ddI-containing regimen.

Both thymidine-analogue- and non-thymidine-analogue-based (that is, non-TDF/ABC-based) drugs have been recommended for use in first-line regimens in resource-limited settings [1], with the latter being a preferred option in terms of preserving NRTI options for second-line therapy. In the present setting, patients have been started on thymidine-analogue-based drugs and have been exposed for a longer period because of the lack of virological monitoring, thereby accumulating a higher rate of resistance mutations. Furthermore, prior exposure to mono/dual therapy in group B left only 26% of them with fully active second-line NRTI options, according to the WHO guidelines for resource-limited settings [1].

Among the NNRTI mutations, Y181C mainly selected among patients failing a regimen containing NVP, and K103N among those failing EFV, as reported earlier [17,20]. V106M was predominant over V106A, as reported previously, for subtype C viruses because the natural GTG polymorphism at codon 106 in clade C variants is distinct from clade B viruses [22].

In the present study, the number of patients with multiple ETR-associated mutations (>3) was negligible, showing that ETR could still be useful in these settings. Similar to other antiretrovirals, however, ETR is vulnerable to a rapid loss of response in the absence of other active drugs in the regimen [23]. With a high rate of cross-resistance in these settings, the introduction of newer drugs such as darunavir and integrase inhibitors might be useful for those with extensive resistance.

In total, 6% of the patients did not develop any of the RT resistance mutations despite good self-reported adherence. These patients might have mutations in the RNAse H and connection domains of the RT enzyme that have been recently reported [24], and which could have been overlooked because the assay covers only the RT polymerase region.

Some patient specimens (12.3%) could not be amplified, suggesting that these patients might have plasma viral load below the assay sensitivity limit (<1,000 copies/ml) or might

even have a suppressed viral load despite failing immunologically (immunological nonresponders). It would be interesting to study the rate of such patients and characteristics underlying them [25].

The study limitations include its cross-sectional design, which means it cannot demonstrate the sequencial evolution of mutations. In addition, the population-based sequencing required that the coselection of NRTI cross-resistance mutations be analysed by the clonal sequencing to confirm their existence in the same genome. It might also be difficult to interpret the effect of d4T and AZT on the selection of resistance pattern because of the bias in the sample size of patients on these drugs. The absence of data on plasma viral load and duration of mono/dual therapy prior to HAART are among the other limitations in this study.

In resource-limited settings where ART options are limited, any rational approach to drug selection requires that NRTI drugs with the highest potential for cross-resistance should be used later in therapy, rather than earlier. Furthermore, when thymidine analogues are used as first-line regimen, they should be accompanied by viral monitoring to detect failure, followed by genotyping to avoid accumulation of cross-resistance mutations. Otherwise, non-thymidine analogues should be used in order to preserve thymidine analogues for second-line therapy. This study emphasizes the importance of strengthening the development of technologies for affordable viral load monitoring with genotyping.

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Table 1

Treatment history and demographic details of HIV-1-infected patients failing therapy in group A and group B (*n*=228)

Characteristic	Group A, *n*=126	Group B, *n*=102
Mean age, years (SD)	34 (10)	00 (/)
Gender		
Male, *n* (%)	94 (75)	87 (85)
Female, *n* (%)	32 (25)	15(15)
Mode of HIV transmission[a]		
Heterosexual, %	96	97
Blood transmission, %	3	2
Mother-to-child transmission, %	1	1
$CD4^+$ T-cell count		
Median pre–HAART, cells/μl (lQR)	148(12–998)	146(13–663)
Median at failure, cells/μl (IQR)	197(24–757)	152(18–999)
Median time to failure, months (IQR)	37.5 (3–82)	43.0 (2–100)
HAART regimen		
AZT+3TC+NVP/EFV, *n* (%)	48 (38)	38 (37)
d4T+3TC+NVP/EFV, *n* (%)	78 (62)	50 (49)
ddl+3TC+NVP/EFV. n(%)	0(0)	14(14)

[a]Mode of transmission was obtained from the YRG CARE natural history database. AZT. zidovudine; ddl, didanosine; d4T, stavudine; EFV, efavirenz; HAART, highly active antiretroviral therapy; HIV-1; HIV type-1; IQR. interquartile range; NVP. nevirapine; 3TC, lamivudine.

Table 2
Patterns of RT resistance mutations in group A and group B

RT drug resistance mutation	Group A, *n*=100	Group B. *n*=100
NRTIs		
NAMs		
K65R	5	11
L74V	8	11
T69d	1	1
T69N/D	14	13
151 Q-complex	8	9
M184V	75	73
E44D7V118I	21	21
TAMs		
M41L	27	49[a]
L210W	3	8
T215Y	16	33[a]
T215F	12	12
D67N	40	40
K70R	26	26
K219E/Q	15	17
NRTI cross resistance mutations[b]	49	64
NNRTIs		
L1001	3	3
K103N	27	41
V106M	8	12
V106A	0	6
V108I	8	12
Y181C	37	41
Y181I/L/V	5	1
Y188L/H/C	9	11
G190A	28	30
G190/E/S	1	1
V179D	1	1
P225H	2	3
A98G	17	11
M230L	1	1
K101E/P	7	15
E138K	3	2
F227L	2	2
ETR-associated mutations[c]		
<3, %	85	92
≥3, %	–	2

[a] $P<0.05$.

[b] Nucleoside reverse transcriptase inhibitor (NRTl) cross-resistance mutations include Q151M, thymidine analogue mutations (TAMs; 215Y) and a deletion at position 69.

[c] Etravirine (ETR) resistance mutations as per the International AIDS Society–USA 2008. NAMs, nucleoside analogue mutations; NNRTl. non-nucleoside reverse transcriptase inhibitors; RT. reverse transcriptase.