Brief Original Article

Antiretroviral drug resistance and HIV-1 subtypes among treatment-naive prisoners in Kelantan, Malaysia

Tengku Ahmad Akram Tengku Mohd Ariffin[1], Suharni Mohamad[2], Wan Nazirah Wan Yusuf[3], Rafidah Hanim Shueb[1]

[1] *Department of Medical Microbiology and Parasitology, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia*
[2] *School of Dental Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia*
[3] *Department of Pharmacology, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia*

Abstract

Introduction: The widespread use of highly active antiretroviral therapy (HAART) and continuous reports of HIV-1 strains developing resistance to these drugs is rather alarming, as transmission of resistant viruses to newly infected persons is possible. This study aimed to determine HIV-1 subtypes and the prevalence of primary mutations associated with antiretroviral (ARV) resistance among treatment-naive prisoners on the east coast of Malaysia.

Methodology: Viral RNA was extracted from plasma samples of 21 treatment-naive prisoners. Protease (PR) and reverse transcriptase (RT) regions were amplified and sequenced. Stanford HIV database algorithms were used for interpretation of resistance, and phylogenetic analysis was performed for subtype assignment.

Results: In the PR gene, no antiviral resistance-associated mutation was detected. For RT-associated mutations, K103N was the most prevalent in sequenced samples (14.3%). Genetic subtyping on the *pol* gene revealed that the majority of the prisoners were infected with subtype CRF33_01B (52.4%).

Conclusion: Continuous surveillance of newly infected individuals is required to help strategize the best antiviral treatment for these patients.

Key words: HIV-1; CRF33_01B; drug resistance; prisoners.

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Introduction

Highly active antiretroviral therapy (HAART) was first introduced in Malaysia in 1997 and was chosen to be the preferred treatment strategy from 2001 onward. This resulted in a remarkable reduction of morbidity and mortality rates in HIV-1 patients. HAART, which consists of a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI), is accessible through clinics in designated government general and district hospitals [1].

Although HAART is beneficial, increasing its use unfortunately leads to the emergence of drug-resistant HIV strains. Poor adherence to ARV treatments further contributes to the high number of resistant HIV strains reported [2]. Subsequently, this could lead to the transmission of ARV-resistant strains of HIV to susceptible individuals and eventually to a less effective first-line regimen.

HIV-1 subtypes reported to circulate in Malaysia thus far include pure subtype B and six recombinant forms: CRF01_AE [3], CRF33_01B [3], CRF48_01B [4], CRF52_01B [5], CRF53_01B [6], and CRF54_01B [7]. However, most of the molecular epidemiology studies of HIV-1 in Malaysia were carried out in Kuala Lumpur, and thus available data pertaining to the prevalence of HIV-1 subtypes and HIV primary drug resistance in various risk groups in other states, including Kelantan, is lacking. Kelantan is located on the northeastern coast of Peninsular Malaysia and shares a border on the north with Thailand. Interestingly, Kelantan has one of the highest numbers of newly reported HIV cases in Malaysia. This study aimed to describe for the first time the HIV-1 genetic diversity and the prevalence of transmitted primary drug resistance of HIV-1 based on sequence analyses of the *pol* gene (PR and RT regions) among treatment-naive prisoners from Kelantan.

Methodology

Study population

Twenty-one plasma samples were collected from treatment-naive HIV-1-infected prisoners in Pengkalan Chepa Prison, Kelantan, between June 2009 and January 2012. Clinical data were obtained from prisoners' medical histories. The study protocol was approved by the Research Ethics Committee (Human), Universiti Sains Malaysia (USMKK/PPP/JEPeM198.3[1]). Informed consent was obtained from all patients prior to sample collection.

Genotypic resistance assessment

The samples were analyzed for HIV-1 resistance mutations in the PR and RT regions. Viral RNA extraction, reverse transcriptase-PCR (RT-PCR), and nested PCR were performed as previously described [8]. Nucleotide sequences of PR and RT regions were edited, aligned, and analyzed for drug resistance using the Stanford HIV Resistance Database (http://hivdb.stanford.edu/).

HIV-1 subtype classification

Nucleotide sequences were aligned using Clustal W in the MEGA 4 version 4.0.2 program (Arizona State University, Arizona, USA). The sequences covered all of PR (HXB2 position: 2253-2549) and most of RT (HXB2 position: 2550-3279). In subtype assessment, phylogenetic analyses were performed by the neighbour-joining (NJ) method using Kimura two-parameter distance estimation method with transition-transversion ratio of 2.0 in 1,000 bootstrap replicates. The recombination form was further analyzed by performing bootscanning analysis with a sliding window of 200 bp, incremental steps of 20 bases, and the Kimura two-parameter model using Simplot 3.2 software (Johns Hopkins University, Baltimore, USA).

Statistical analysis

Fisher's exact test in SPSS version 20 (IBM, New York, USA) was used to evaluate any possible association of HIV-1 subtype with demographic and risk factors.

Sequence data

Sequences generated have been submitted to GenBank under accession numbers KC762591-KC762616.

Results

Patient baseline characteristics

Among the 21 prisoners, 14 (66.7%) were males and 7 (33.3%) were females. All prisoners were Malaysians, but of different ethnicities: 19 (90.4%) were Malays and 1 each was Chinese (1.4%) and Indian (1.4%). The mean age of the prisoners during sample collection was 39.1 years (standard deviation [SD]: 7.8), and most of them were in the 30–39 (n = 7, 33.3%) and 40–49 (n = 8, 38.1%) age groups. The median of viral load at sampling was $10^{4.76}$ (interquartile range [IR]: $10^{4.86}$) RNA copies/mL. The median CD4 count was 325 (IR: 185) cells/mm^3 (Table 1).

Figure 1. Phylogenetic analysis of 1026 bp HIV-1 *pol* sequences (HXB2: 2253-3279) among 21 prisoner samples for subtype characterization. HIV-1 reference subtypes and CRFs retrieved from Los Alamos HIV database (http://www.hiv.lanl.gov/). Reference strains used: subtype B (including Thai B'), CRF01_AE, CRF15_01B, CRF33_01B, CRF34_01B, CRF48_01B, CRF51_01B, CRF52_01B, CRF53_01B, and CRF54_01B. O.CM.91MVP5180.L20571 was included as an outgroup.

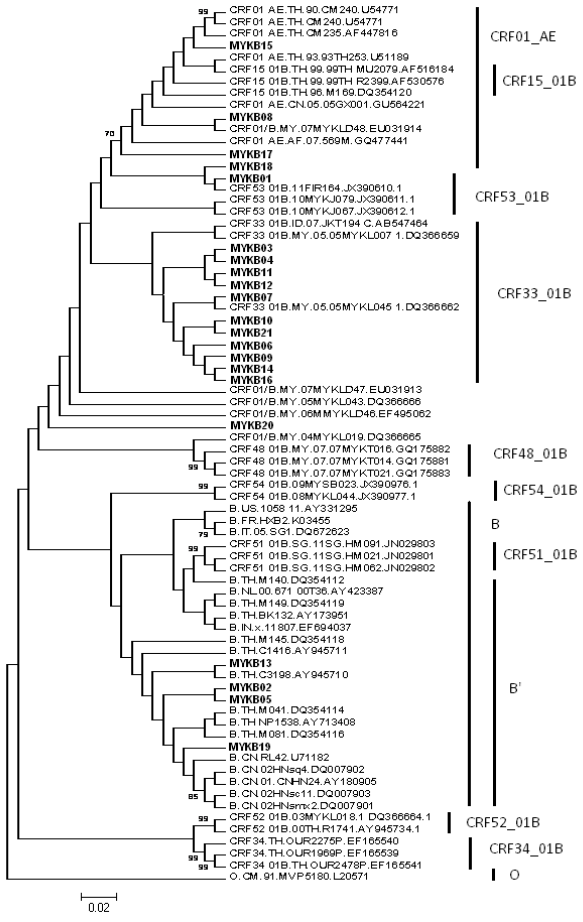

Table 1. Baseline characteristics of treatment-naive HIV-1 infected prisoners

Route of transmission	Injecting drug use n (%) (Total = 12)	Sexual contact n (%) (Total = 8)	Unknown n (%) (Total = 1)	Total n (%)
Gender:				
Male	12 (100)	2 (25.0)	-	14 (66.7)
Female	-	6 (75.0)	1 (100)	7 (33.3)
Ethnicity:				
Malay	11 (91.7)	7 (87.5)	1 (100)	19 (90.4)
Chinese	-	1 (12.5)	-	1 (4.8)
Indian	1 (8.3)	-	-	1 (4.8)
Age groups (years)				
20-29	1 (8.3)	2 (25.0)	-	3 (14.3)
30-39	4 (33.3)	3 (37.5)	-	7 (33.3)
40-49	4 (33.3)	3 (37.5)	1 (100)	8 (38.1)
≥50	3 (25.0)	-	-	3 (14.3)
Viral load (copies/mL)				
$\leq 10^4$	4 (33.3)	-	-	4 (19.1)
10^4-10^5	5 (41.7)	6 (75.0)	1 (100)	12 (57.1)
$\geq 10^5$	3 (25.0)	2 (25.0)	-	5 (23.8)
CD4 count (cells/mm^3)				
≤250	2 (16.7)	1 (12.5)	-	3 (14.3)
250-500	7 (58.3)	6 (75.0)	1 (100)	14 (66.7)
≥500	3 (25.0)	1 (12.5)	-	4 (19.0)
Number of RAMs	2 (16.7)	1 (12.5)	-	3 (14.3)
Subtypes				
CRF33_01B	7 (58.3)	4 (50.0)	-	11 (52.4)
CRF01_AE	1 (8.3)	3 (37.5)	-	4 (19.0)
B	3 (25.0)	1 (12.5)	-	4 (19.0)
CRF53_01B	1 (8.3)	-	-	1 (4.8)
URF CRF01/B	-	-	1 (100)	1 (4.8)

RAMs: resistance-associated mutations

Table 2. HIV-1 genotypes and drug resistance analyses in treatment-naive prisoners in Kelantan

Sample ID	Viral load (copies/mL)	CD4 count (cells/mm^3)	Subtype	NRTI-resistant mutation	NNRTI-resistant mutations
MYKB01	$10^{5.01}$	348	CRF53_01B	-	-
MYKB02	$10^{4.59}$	598	B’	-	K103N
MYKB03	$10^{5.11}$	293	CRF33_01B	-	-
MYKB04	$10^{4.51}$	492	CRF33_01B	-	-
MYKB05	$10^{3.21}$	325	B’	-	-
MYKB06	$10^{3.74}$	389	CRF33_01B	-	K103N, Y188L
MYKB07	$10^{4.84}$	200	CRF33_01B	-	-
MYKB08	$10^{4.65}$	579	CRF01_AE	-	-
MYKB09	$10^{4.98}$	438	CRF33_01B	-	-
MYKB10	$10^{3.75}$	314	CRF33_01B	-	-
MYKB11	$10^{4.55}$	300	CRF33_01B	-	-
MYKB12	$10^{5.33}$	214	CRF33_01B	-	-
MYKB13	$10^{4.61}$	282	B’	-	-
MYKB14	$10^{4.81}$	595	CRF33_01B	-	-
MYKB15	$10^{4.90}$	269	CRF01_AE	-	-
MYKB16	$10^{4.76}$	297	CRF33_01B	-	-
MYKB17	$10^{4.83}$	287	CRF01_AE	-	-
MYKB18	$10^{5.28}$	447	CRF01_AE	M41L, T69N, L74I, M184V, T215Y	K103N, Y181C
MYKB19	$10^{3.78}$	633	B’	-	-
MYKB20	$10^{4.27}$	358	CRF01_AE/B	-	-
MYKB21	$10^{5.59}$	222	CRF33_01B	-	-

NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; Note: PI-resistant mutation was not observed

The major mode of transmission was intravenous drug use (IDU) (57.1%), followed by sexual contact (38.1%). The majority (85.7%) of male prisoners acquired HIV infection through IDU ($p < 0.001$), while 85.7% of female prisoners acquired it through sexual contact ($p < 0.001$).

Fisher's exact test analysis revealed no significant association between HIV-1 subtypes and gender, race, and route of transmission ($p > 0.05$) (data not shown).

Drug resistance profile

Only three prisoners (14.3%) were resistant to at least one ARV drug; one prisoner was resistant to both NRTIs and NNRTIs, and the other two prisoners were resistant to NNRTIs only. Resistance to PI drugs, however, was not observed. In the RT region, one prisoner had multiple NRTI resistance-associated mutations: M41L, T69N, L74I, M184V, and T215Y (Table 2). For NNRTI resistance-associated mutations, K103N was the most prevalent (n = 3, 14.3%), followed by V179T, Y181C, and Y188L, with a prevalence of 4.8% (n = 1) each. The total number of resistance-associated mutations (RAMs) to NNRTIs and NRTIs was six and five, respectively.

Subtype distribution

Based on phylogenetic analysis of the *pol* gene (PR-RT region), CRF33_01B was the most prevalent subtype among the prisoners; 52.4% (n = 11) harbored this subtype of HIV-1 (Figure 1). This was followed by subtype B' (Thai strain) (n = 4, 19.0%), CRF01_AE (n = 4, 19.0%), CRF53_01B (n = 1, 4.8%), and CRF01_AE/B unique recombinant form (URF) (n = 1, 4.8%). Interestingly, CRF33_01B also predominated in both prisoners who acquired infection through injecting drugs (58.3%, 7/12) and those who acquired it through sexual contact (50.0%, 4/8). Moreover, CRF33_01B also predominated in both genders; 57.1% of males and 37.5% of females harboured this subtype.

Discussion

The present study reveals that RAMs exist in treatment-naive HIV-1 infected persons; similar findings have been reported by others [9-14]. The prevalence of RAMs in the present study (14.3%) is in agreement with studies done in Italy (12.5%) and the United States (16.0%) [9-10]. However, in this study, the drug resistance assessment was limited to the *pol* region since entry and integrase inhibitors are not widely used in HIV patients in Malaysia.

Mutations conferring high resistance to NRTIs were found in an HIV-1 sequence isolated from a female prisoner, MYKB18. This sequence bore thymidine analogue mutations (TAMs) M41L and T215Y, and 69 insertion complex (T69N), causing a high level of resistance to six NRTI drugs except tenofovir [15]. The female prisoner acquired this highly resistant virus from her husband. However, since there were no further data regarding treatment and ARV mutation status of prisoner MYKB18's husband, the actual source of these transmitted drug resistance mutations was unknown.

RAMs for NNRTIs were detected in three prisoners: K103N in prisoner MYKB02, K103N and Y188L in prisoner MYKB06, and K103N and Y188C in prisoner MYKB18. However, these prisoners harboured different subtypes; MYKB02 had subtype B', MYKB06 had CRF33_01B, and MYKB18 had CRF01_AE (Table 2). K103N mutation was the most common RAM presented among treatment-naive patients. This finding corroborates several other studies that reported that K103N was highly prevalent among NNRTI-associated resistance mutations [11-12] or among RAMs in treatment-naive patients [13-14]. K103N mutation causes high resistance to nevirapine and efavirenz [16], and thus limits the available choice of the best drug combination in first-line treatment for patients.

Recombinant form CRF33_01B, which was first reported in Kuala Lumpur in 2006, was found to be the major HIV-1 subtype circulating among prisoners in this study. Previously, it was shown that CRF01_AE was the most prevalent subtype in Malaysia [3]. However, in 2007, a rapid expansion of CRF33_01B in Kuala Lumpur was observed, suggesting a possible replacement of predominant subtypes in near future [17]. Interestingly, in 2012, Mohamad *et al.* (2012) [8] reported that 44.4% of pediatric patients harboured subtype CRF33_01B. The following year, CRF33_01B was found to be highly prevalent among drug users (71%) [18]. The finding of this present study further substantiates the observed shifting trend of the current predominant HIV-1 subtype from CRF01_AE to CRF33_01B in various risk groups in Malaysia. It is interesting to note that CRF53_01B, which was first reported to have emerged in Kuala Lumpur in 2012, was also found in this study (albeit in one prisoner), suggesting possible geographical expansion of this particular subtype.

Conclusions

The present study reported for the first time the transmitted drug resistance mutation among treatment-naive prisoners in Malaysia. Drug resistance to NNRTIs was identified in 3/21 (14.3%) isolates – subtype B', CRF01_AE, and CRF33_01B. The study also found that CRF33_01B was the most common subtype among treatment-naive prisoners in Kelantan.

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Corresponding author

Rafidah Hanim Shueb
Department of Medical Microbiology & Parasitology
School of Medical Sciences, Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan, Malaysia
Phone: +6097676255
Fax: +6097676289
Email: hanimshueb@gmail.com

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