Study of drug resistance among 78 antiretroviral treatment-naïve patients with HIV-1 subtype B infection in central China

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ABSTRACT: To study the prevalence of drug resistance mutations among HAART (highly active anti-retroviral therapy) naïve subjects with HIV-1 subtype B infection, evaluate the correlation between major mutations and viral loads. Additionally, to investigate the primary resistance spectrum in the central plains of China and provide some guidance for the choice of antiretroviral drugs (ARV). Drug resistance mutations and viral loads were measured in 78 treatment-naïve patients with HIV infection and the results were analyzed with descriptive statistical and multiple statistical analysis. The most common mutations were L63P, V77I and I93L, which belong to minor mutations of the proteinase gene, and none of which had any relation to viral loads. The major mutations, which were mainly K103N and Q151M, were less frequent in China than those in other countries. There was a certain correlation between viral loads and I93IL according to stepwise regression analysis. The incidence of primary mutations among HAART naïve patients was lower in China's central plains than that in other countries, and the most common mutations had no relation to viral loads. Though major mutations affecting choice of ARV are not common in China, they deserve further attention.

Key Words: HIV-1 subtype B, drug resistance, viral load

Introduction

Since the advent of highly active anti-retroviral therapy (HAART), the prognosis for AIDS has significantly improved. However, its appearance was followed by a series of problems, chief among them drug resistance brought about by HAART, that could greatly impact the effects of this therapy and cause difficulty in choosing therapy regimens. The study aimed to analyse the primary drug-resistances among treatment-naïve patients infected with HIV in central plain of China, to detect the prevalence of resistance mutations and the relation to HIV viral load so as to guide the choice of HAART.

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Patients and Methods

Patient selection

Subjects were 78 patients infected with HIV during 1992 to 1994 through commercial blood donation who were all from central China. Genotype test analysis showed that all of the patients were infected with HIV-1 type B. All subjects had received no ARVs previously, had a $CD4^+$ T lymphocyte count of more than 200 cells/μL, and did not have AIDS-associated diseases. Patient samples were collected and analyzed in June 2006.

Detection methods

200 μL of anticoagulated whole blood specimen were collected from each subject. Viral genome RNA was extracted with a QIAamp Viral RNA Mini Kit and amplified by reverse transcription PCR and nested-PCR (as shown in Table 1, two pairs of primers were designed). The products of PCR were purified and then genotyped with a Trugene HIV Genotyping Kit. HIV viral loads were detected by nucleic acid sequence-based amplification (NASBA).

Data statistics

Resulting mutations and HIV viral loads were analyzed with the SPSS13.0 statistical package; this work included descriptive and multiple statistical analysis.

Results

1. *Prevalence of HIV drug resistance*

Different gene mutations of RTIs and PIs were detected among the 78 patients. All of the mutations and their frequencies are summarized in Table 2.

2. *Classification of mutations*

Table 1. The sequences of two pairs of primers

	Sequences	Gene location
Outer-primers	5'-CCTAGGAAAAAGGGCTGTTGGAAATGTGG-3' 5'-AACTTCTGTATGTCATTGACAGTCCA-3'	2011～2039 3303～3328
Inter-primers	5'-ACTGAGAGACAGGCTAATTTTTTAGGGA-3' 5'-CATTTATCAGGATGGAGTTCATA-3'	2068～2095 3243～3265

Table 2. Gene mutations and their frequencies in 78 patients with HIV infection

Gene mutation	Frequency	Percentage	Gene mutation	Frequency	Percentage
PI mutations:					
A62AV	1	1.3	L63LP	2	2.6
A71AT	4	5.1	L63P	64	82.1
A71AV	3	3.8	L63PS	2	2.6
A71T	5	6.4	L63S	2	2.6
A71V	5	6.4	L63T	2	2.6
D60DE	3	3.8	L90M	1	1.3
D60E	1	1.3	M36I	4	5.1
F63FLPS	1	1.3	M36IM	3	3.8
K70EK	1	1.3	M46I	1	1.3
L10I	3	3.8	V77I	64	82.1
L10IL	4	5.1	V77IV	6	7.7
RT mutations:					
G190A	1	1.3	F116Y	1	1.3
K103KN	4	5.1	F77FL	1	1.3
K103N	9	11.5	I93IL	4	5.1
P236S	1	1.3	I93L	46	59
V106A	1	1.3	K103KR	1	1.3
V106IV	1	1.3	K219N	1	1.3
V179D	2	2.6	K65KR	1	1.3
V179DV	1	1.3	L210LM	1	1.3
Y181C	3	3.8	M184ME	1	1.3
Y181CY	1	1.3	Q151M	3	3.8
Y188L	2	2.6	V118I	4	5.1
F116FHLY	1	1.3	V75IV	1	1.3

Analyzing so many resistance mutations would prove difficult, so mutations were classified by Hierarchical Cluster analysis, the results of which are shown in Figure 1.

3. *Correlation between mutations and HIV viral loads*

The mutations from 68 patients with detectable HIV RNA were classified into 4 classes by Hierarchical Cluster analysis. Class I was L63P, Class II was V77I, Class III was I93L, and Class IV was other mutations. The viral loads of the four groups were analyzed by univariate GLM. The results are shown in Table 3.

Using stepwise regression analysis with LgVL as the dependent variable and the gene mutations as an independent variable yielded the regression equation: LgVL = 4.720 + 0.638 × I93IL. There was statistical significance ($P = 0.023$).

Discussion

Anti-retroviral drug resistance is a major cause of HAART failure. Investigation of the prevalence of resistance is crucial to choosing an appropriate ARV regimen to achieve complete viral suppression, which could in turn reduce mutation accumulation and prevent the spread of resistance strains. Doing so would prove helpful in controlling the disease and reap the full advantages of HAART.

This study revealed that the most common primary mutations were L63P and V77I in treatment-naïve persons with HIV-1 subtype B infection in China's central plains, followed by I93L and other rare mutations. Even though the results were similar to those reported by Si-Xuefeng *et al*. (*1*), there were some differences. The ratios of V77I and I93L were 82.1% and 59.0% respectively, but Si-Xuefeng *et al*. reported respective ratios of 34.15% and 95.73%.

A major resistance mutation present at K103N of the HIV-1 reverse transcriptase gene was found at a higher frequency (11.5%) than as reported abroad (6%) (*2*). While the possibility of a few patients having taken missing doses of HIV medicine could not be excluded, this finding nevertheless warrants attention. The presence of K103N would result in a high level of resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), so the high prevalence of the mutation could reduce the effects of NNRTIs and even accumulate with other mutations to increase the spread of resistance strains and diminish the effects of antiretroviral regimens containing NNRTIs. The high prevalence of K103N also suggested the necessity and urgency of developing new NNRTIs.

Other major mutations are shown in Table 1. The nucleoside reverse transcriptase inhibitor (NRTI) mutation, Q151M, appeared in 3 patients, one of which also had F116Y while another had F116FHLY, V75IV and F77FL mutations, and there was a potential for the development of Q151M complex. Of the major mutations of NNRTIs, Y181C appeared in 3 patients, Y188L in 2 patients, and V179D in 2 patients, respectively. Of the major mutations of protease

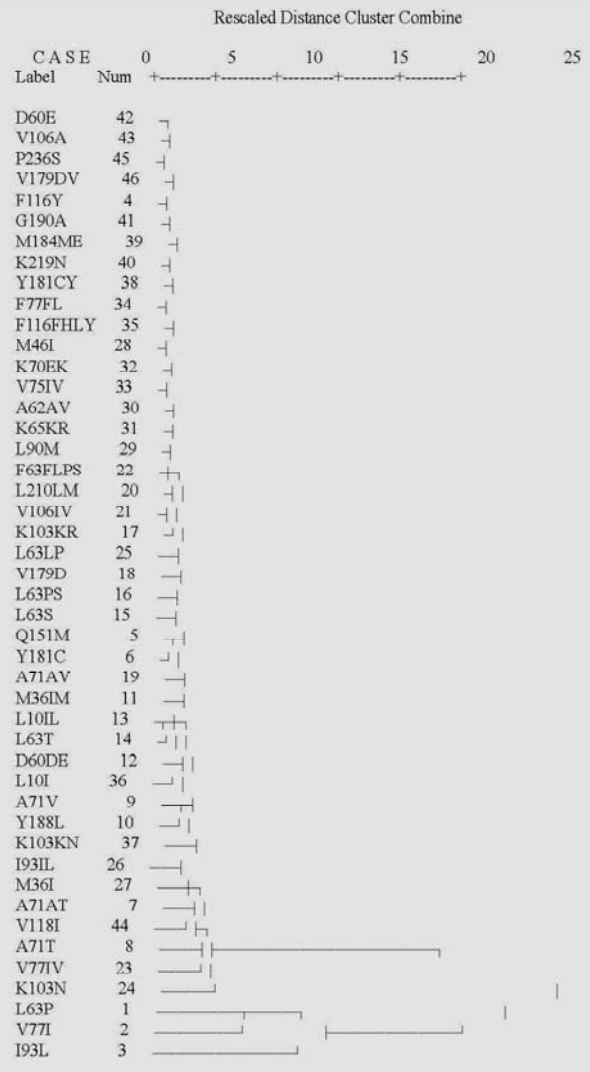

Figure 1. Hierarchical cluster analysis of gene mutations in 78 HIV-infected individuals (Dendrogram using Average Linkage (betwenen groups)).

Table 3. The correlation between gene mutations and logarithm of HIV viral loads (LgVL)

Mutation type	Number	LgVL ($\overline{X} \pm s$)	*P* value
1	54	4.746 ± 0.553	0.324
2	55	4.688 ± 0.541	0.256
3	42	4.737 ± 0.527	0.122
4	39	4.857 ± 0.494	0.003

inhibitors (PIs), M46I and L90M appeared in 1 patient. The low level prevalence of other major mutations besides K103N had less impact on the choice of HAART regimen.

The prevalence of major HIV mutations was still lower in China than that in other countries (*3*). The differences were greater particularly for prevalences of thymine-associated mutations (TAMs) and major protease-associated mutations, which maybe related to the short history of HAART use in China. In addition, frequency of the Q151M complex is higher than that of TAMs in NRTIs mutations in China. When choosing appropriate ARV regimens, the resistance prevalence domestically and abroad should serve as a reference, and especially for resource-limited regions.

The detected mutations were classified into 4 classes by Hierarchical Cluster analysis. Classes I and II had a high prevalence in HIV-1 subtype B-positive patients. Class III, I93L, had a moderate prevalence, and Class IV, which included other mutations, had a low prevalence. Another clustered method combined classes I and II into just one class. L63P, V77I, and I93L were all minor resistance mutations of PIs. Whether the high prevalence of mutations could affect the choice of PIs is still being studied in China. The correlation between the four classes of mutations and the logarithm of HIV viral loads (LgVL) was analyzed using univariate GLM analysis. The results showed that only class IV had a distinguished statistical significance in correlation to LgVL independently ($P = 0.003$). However, there was no statistical significance to the correlation between LgVL and the three mutations of L63P, V77I, and I93L. This fact suggests that the three mutations instead of other rare mutations were unable to decrease the HIV viral loads, so they may not affect the replication and adaptability of HIV. The results of stepwise liner regression analysis showed that LgVL was 0.638 higher in patients with the I93IL mutation. Further research on the clinical significance of this mutation should be done since the current study included few patients with I93IL.

To conclude, the mutations of HIV could not only result in resistance to ARV drugs but also affect the replication capacity of HIV. There was a direct correlation between HIV viral loads and the prognosis of the disease. If the replication capacity of the mutants was less than the wild type virus, the viral loads would remain at a lower level. Thus, ARV drugs would be a better choice when resistance is inevitable. If the replication capacity was greater, however, such drugs would accelerate the progress of the disease. The current study found that these common mutations did not increase viral load but they did have the potential to develop into cumulative mutations. In contrast, some rare mutations such as I93IL could increase HIV viral loads, which would then accelerate the progression of AIDS.

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