Iran J Public Health, Vol. 50, No.8, Aug 2021, pp.1586-1594

Original Article

Evaluation of the Outcome of Antiviral Therapy among Individuals Infected HIV-1 in Suqian District of Jiangsu Province, China

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(Received 18 Sep 2020; accepted 19 Nov 2020)

Abstract

Background: To evaluate the condition of antiviral therapy (ART) for individuals infected HIV-1 in Suqian district of Jiangsu Province, China.

Methods: Altogether, 561 HIV-positive patients who received antiviral therapy in Suqian district in 2019 were recruited. EDTA anticoagulated blood was collected and separated to obtain the plasma samples. Viral load (VL) were tested for evaluating the outcome of ART. Then samples with VL beyond 1000IU/mL were used to conduct the molecular test in order to master the characters of HIV-1 and the prevalence of resistance strains.

Results: VL results showed that the virus in 91.1% of the patients who received continuous antiviral treatment for more than 6 months were effectively inhibited (VL ≤ 1000 IU / ml). Among the 50 patients who failed in the treatment, 46 HIV-1 pol gene sequences were obtained, and the positive rate was 92.0%. The most prevalent strain was CRF_ 07bc (32.6%), and new epidemic strains, such as 67_01B、79_0107、87_cpx, were popular in this district. Drug resistance test results showed that 56.5% of the patients failed in antiviral treatment due to drug resistance, mainly resistant to the national first-line antiviral drug 3TC. Drug-resistant strains were not found in 43.5% of the patients.

Conclusion: ART achieved a satisfied result in Suqian district, but the main cause resulting in ART-failure was resistant, so it is very necessary to enhance the education of adherence prior to the initiation of ART.

Keywords: HIV-1; Prevalence; Drug resistant mutation sites, China

Introduction

Since HIV was discovered in 1981, it has spread rapidly all over the world. Nearly 37 million people have been infected (http://www.unaids.org/), resulting in serious public health problems all over the world. Antiviral therapy (ART) has effectively delayed the disease progress of HIV-infected people and controlled the rapid spread of AIDS by blocking virus replication at different targets at the same time. It has become an important means of global AIDS treatment, prevention and control. Up to now, more than 21 million HIV-infected people in the world have accepted ART (1), which has played a positive role in virus suppression and immune reconstruction in infected people (1).

At present, there are more than 861, 000 reported living HIV-infected /AIDS patients in China (Internal information: progress in prevention and treatment of AIDS, STD and hepatitis C in China in 2019). The number of newly reported cases of HIV infection is increasing year by year, and the seriousness of the AIDS epidemic is growing evidently. In order to achieve the "three 90%" prevention and treatment targets proposed by WHO (1), China has rapidly expanded its treatment in recent years. At present, more than 500,000 HIV-infected people have received free ART, which has contributed to improving the quality of life of AIDS patients, prolonging their life cycle (2,3), and reducing the spread of AIDS (Internal information: progress in prevention and treatment of AIDS, STD and hepatitis C in 2019).

Suqian, located in the north wing of Yangtze River Delta, is the youngest emerging prefecture-level city in Jiangsu Province with a large floating population. The monitoring results of AIDS epidemic showed that there were 190 new HIV infections in Suqian in 2018. A total of 1006 cases of HIV infection were found in the city, 840 cases of HIV infection were followed up and 658 cases were treated with antiviral therapy. The treatment rate was 75.9%, far lower than the national level (Internal information: progress in prevention and treatment of AIDS, STD and hepatitis C in China in 2019). Compared with AIDS prevention and control measures in other regions, the monitoring of AIDS in this region started late, and the understanding of molecular epidemiological characteristics was very limited. Therefore, it is extremely urgent to investigate the molecular epidemiology, monitor the effect of antiviral clinical treatment and learn the emergence and prevalence of drug-resistant strains in this area. In this study, HIV-infected individuals who received antiviral treatment in Suqian in 2019 were taken as the research participants. The antiviral treatment effect was evaluated, the epidemic situation of HIV-1 resistant strains was analyzed, and the occurrence and epidemic situation of HIV-1 resistant strains were learned.

We aimed to provide theoretical guidance for antiviral treatment in this area, improve the antiviral treatment effect, and apply the concept of "treatment for prevention and control".

Participants and Methods

Research participants

Overall, 561 HIV-1 infected individuals recruited for continuous antiviral treatment were taken as research participants. After the informed consent form was obtained from the patients and sty approval by the Ethics Committee of the university, 10 mL of EDTA anticoagulated blood was collected, mixed upside down and centrifuged at 2000rpm for 10 minutes. Then the patient's plasma was separated and stored in 200 ~ 500 L/tube, and placed in a refrigerator at -80℃ to avoid repeated freezing and thawing.

Detection of viral load (VL)

HIV nucleic acid detection kit (Guangzhou Daan) was applied for nucleic acid extraction. Roche CobasZ480 automatic fluorescence quantitative analyzer was applied for load determination.

RNA extraction and amplification reaction procedures and systems

RNA extraction: in order to improve the positive rate of PCR, the samples with virus load of 200 ~ 1000 IU/ml were enriched. The steps were as follows: 500μL plasma samples to be detected were collected and centrifuged at 25,000×g at 4 ℃ for 1 hour. The centrifuge tube was carefully taken out after centrifugation. 300μL supernatant was slowly absorbed and discarded, then the precipitate was suspended and mixed evenly for later use. For samples with viral load ≥1000IU/mL, 200μL plasma samples were extracted normally. MagNA Pure LC2.0 from Roche was applied as the extraction instrument, and MagNA Pure LC Total Nucleic Acid Isolation Kit (Cat No: 3038505001) from Roche was applied. The extraction steps and reagent consumption were carried out according to the instructions, and the final sample elution volume was 50μL.

HIV-1 drug resistance detection method: two rounds of PCR amplification was performed to obtain the target gene of HIV-1 pol region. Protease full-length gene and the first 300 amino acid codons of reverse transcriptase were included. All known drug resistance mutation sites at present were covered in this region. The primers used for detection were listed in the reference (4).

① the first round of RT-PCR reaction system: TaKaRaPrimeScript™ One Step RT-PCR Kit Ver.2 (Code No. RR055A) was applied in this round. The reaction system was PrimeScript 1 Step Enzyme Mix (1 μL), 2×1 Step Buffer (12.5 μL), primer DR-1 (1.5 μL), RNA template (10 μL). The reaction procedure was reverse transcription at 50 ℃ for 32 min, followed by inactivation of reverse transcriptase at 94 ℃ for 2 min. Then 10 cycles were carried out at 94 ℃ for 15s, 53 ℃ for 30 s, and 72 ℃ for 1 min for 30 s (each cycle increased by 5 s). Then 30 cycles were carried out at 94 ℃ for 15 s, 53 ℃ for 30 s, 68 ℃ for 4 min. The first round of reaction was completed at 68 ℃ for 10 min.

② the second round of nested amplification PCR reaction: 1.1×T3 Super PCR Mix (Tsing Ke). The first-round amplification products were used as templates to carry out nested second-round amplification with different primers. The reaction system was 1×T3 Super PCR Mix (43 μL), the first-round amplification product template (5μL) and the detection primer (2 μL). The reaction procedure was pre-denatured at 98 ℃ for 3min, then reacted at 98 ℃ for 10 s, 58 ℃ for 10 s and 72 ℃ for 25 s for 35 cycles, and extended at 72 ℃ for 3 min to complete the second round of reaction. The amplified target gene fragment size was about 1300 bp. The amplified products were detected using 1% agarose gel electrophoresis, and the positive results were sequenced by Sino Geno Max.

Sequencing, HIV-1 drug resistance analysis and phylogenetic tree construction

Sequence splicing and alignment: Contig express in Vector NTI AdvanceTM 11.5 was applied for sequence splicing. Biological analysis software Bioedit was applied for sequence alignment editing. The sequences were submitted to HIV databases for sequence alignment and analysis.

HIV-1 drug resistance analysis and phylogenetic tree construction: the edited sequence was submitted to http://hivdb.stanford.edu/ for drug resistance analysis. Mega6.0 was used for sequence typing and phylogenetic analysis.

Results

Demographic characteristics

It could be seen from the analysis of the epidemic data (Table 1) that most of the infected participants were male, accounting for 85.2% of the recruits, mainly young adults (26-35 yr old) who had been sexually active. Sexual contact was the main route of transmission (90.2%), among which male homosexual transmission is 45.5% and heterosexual transmission was 44.7%. There was no obvious difference between them. With the promotion of the concept of "Treatment after discovery", more and more HIV-1 infected people had received antiviral treatment for more than 2 years.

Evaluation of the effect of antiviral treatment

Altogether, 561 HIV-1 infected people were recruited in antiviral treatment for more than 6 months. The virus in 91.1% of the patients were effectively controlled, and the VL was controlled below 1000IU/mL. The VL of 85.6% of the infected individuals was less than 250 IU/mL. The antiviral drugs used in this district were national first-line drugs, which were two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor.

Table 1: Analysis of demographic characteristics of recruiting individuals

Basic characteristics of participants		***Number of cases***	***Composition ratio (%)***
Gender	Male	478	85.2
	Female	83	14.8
Age (yr)	<25	66	11.8
	26-35	205	36.5
	36-45	133	23.7
	>46	130	23.2
	Unknown	27	4.8
Transmission route	Homosexual transmission	255	45.5
	Heterosexual transmission	251	44.7
	Intravenous drug abuse	3	0.5
	Mother-to-child transmission	3	0.5
	Unknown	49	8.7
Antiviral treatment time (months)	6-12	105	18.7
	13-24	117	20.9
	25-48	212	37.8
	>48	127	22.6

This therapy has effectively improved the effect of antiviral treatment. Epidemiological findings in patients with viral suppression failure (VL ≥ 1000 IU / mL) revealed that most of these patients did not insist on taking drugs. There were various reasons for quitting the drug, but the main reason was that they could not get drugs in time because they migrated for work.

Analysis of HIV-1 resistance among people who failed in antiviral treatment

HIV-1 resistance was detected in 51 infected individuals who failed antiviral treatment. The results showed that HIV-1 resistant strains were detected in 26 infected patients, and the drug resistance rate was 56.5%. The drug resistance analysis of different types of inhibitors is shown in Table 2.

Table 2: Drug resistance of different types of inhibitors

Inhibitor type	***Number of drug-resistant cases***	***Composition ratio (%)***
PIs	0	0
NRTIs	0	0
NNRTIs	4	15.4
NRTIs+NNRTIs	22	84.6

* Note: PIs: protease inhibitors; NRTIs: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors.

NRTIs+NNRTIs indicated cross resistance to two inhibitors at the same time

The individuals were mainly resistant to NRTIs and NNRTIs inhibitors at the same time. In addition to the low resistance barrier of antiviral drugs, the main factor may be that the infected people cannot maintain good compliance during treatment. Among the mutations related to HIV-1 resistance (Fig. 1), the mutations closely related to nucleoside reverse transcriptase inhibitors were mainly K65R (30.8%) and M184V/I (30.8%). They can lead to moderate to high drug resistance against the first-line antiviral drugs ABC and 3TC. The mutations closely related to non-nucleoside reverse transcriptase inhibitors were mainly K103N (19.2%), Y181C (23.1%) and G190A/S (23.1%). Once these mutations appear, they can directly lead to severe tolerance of the virus to EFV and NVP.

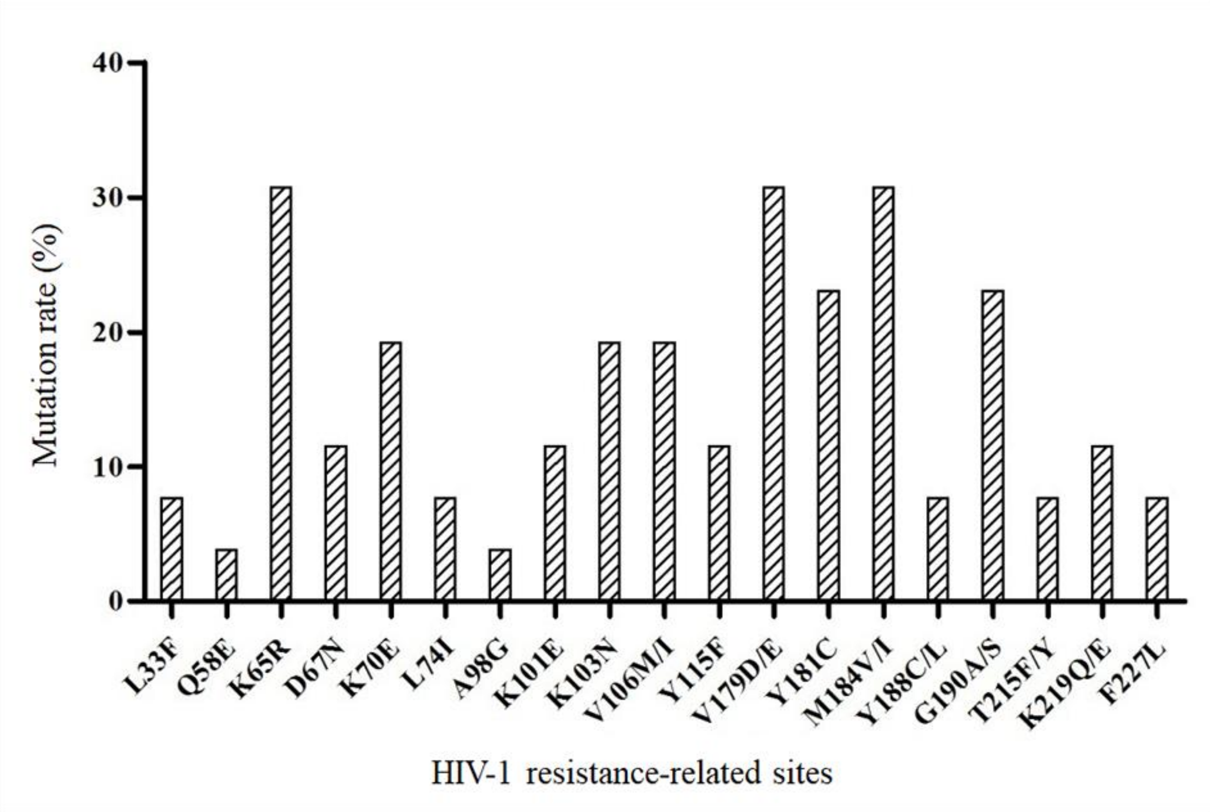

Fig. 1: Analysis of HIV-1 drug resistance mutation

Analysis of HIV-1 epidemic strains among people who failed in antiviral treatment

The 46-pol gene sequences of HIV-1 were aligned head to tail, and the phylogenetic tree was constructed by Mega6.0. The results of phylogenetic tree (Fig. 2) showed that 8 subtypes and 3 cases of URFs appeared and prevailed in this district. Among which, 01_AE accounted for 19.6% (9 cases), 07_BC accounted for 32.6% (15 cases), 08_BC accounted for 6.5% (3 cases), 67_01B accounted for 19.6% (9 cases), 79_0107 accounted for 4.4% (2 cases), 87_cpx accounted for 2.2% (1 case), B accounted for 6.5% (3 cases), and C accounted for 2.2% (1 case). The three URF sequences were all from HIV-1 infected people in Sihong district, and their recombination breakpoint analysis is shown in Fig. 3, 4 and 5. The above results indicate that the epidemic strains of HIV-1 in this area are diversified.

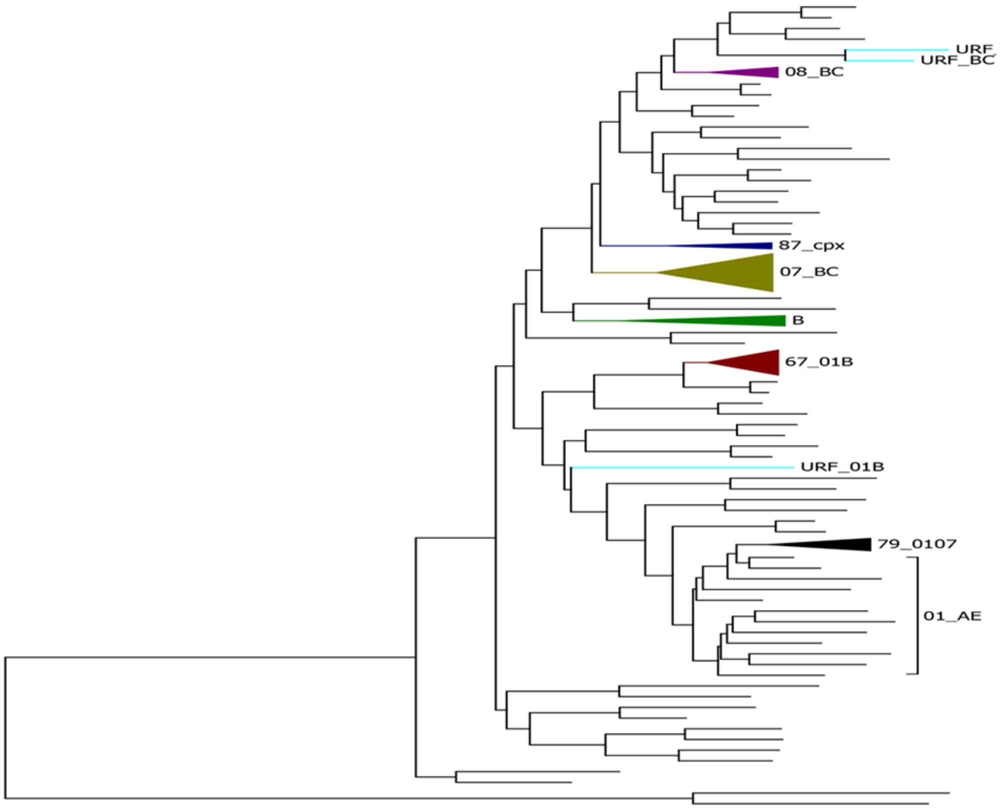

Fig. 2: Phylogenetic tree analysis of HIV-1 epidemic strains

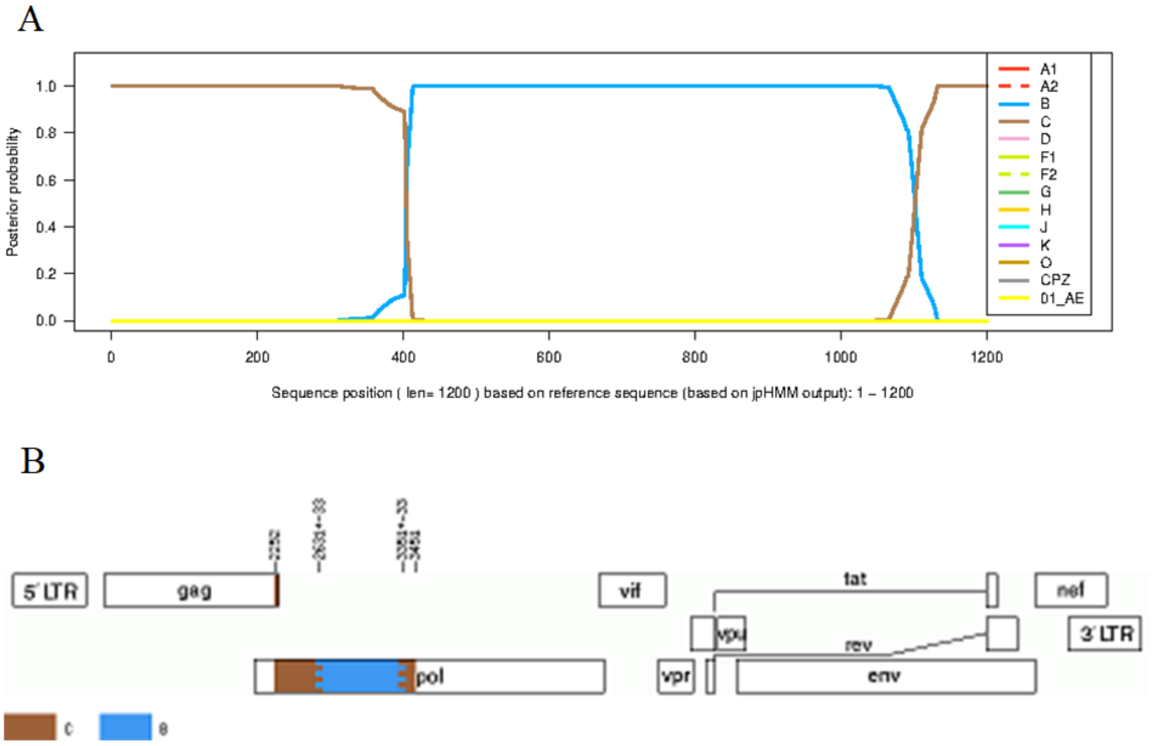

Fig. 3: Analysis chart of recombination breakpoint of D019 HIV-1 strain (URF_BC)

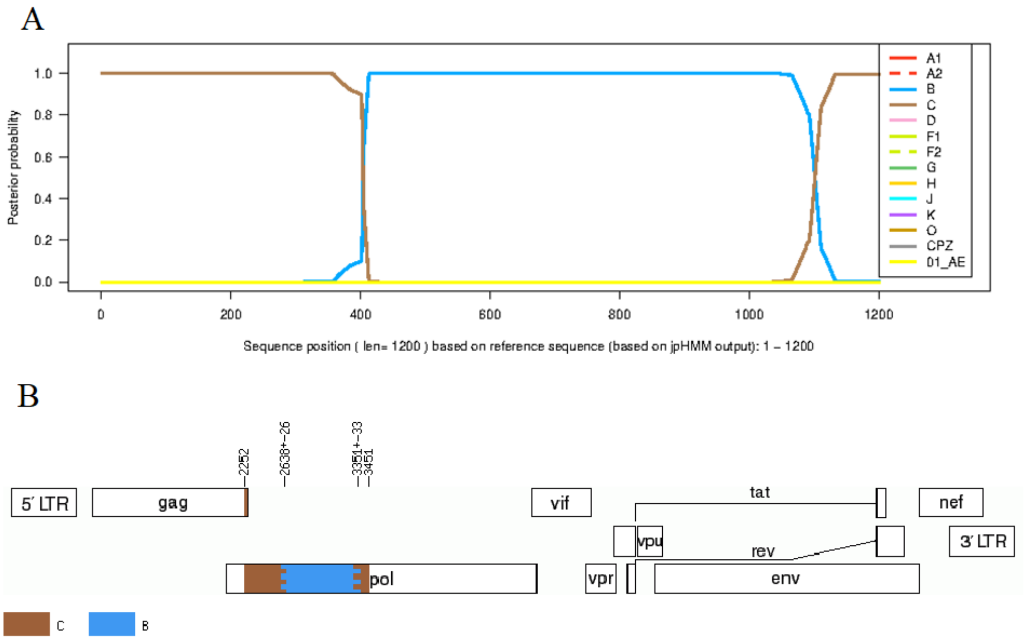

Fig. 4: Analysis chart of recombination breakpoint of D020 HIV-1 strain (URF_BC)

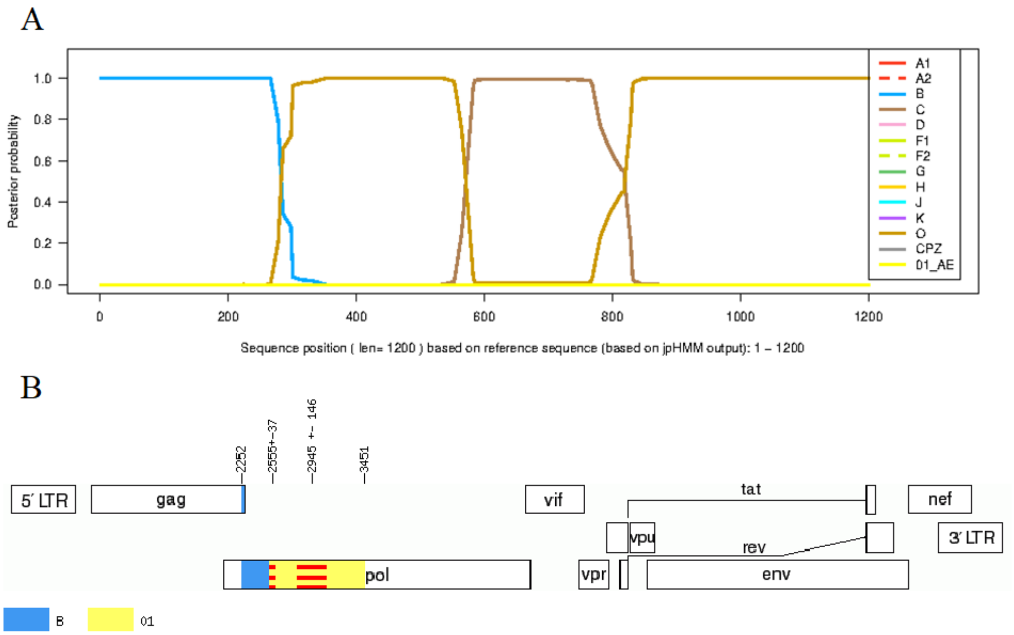

Fig. 5: Analysis chart of recombination breakpoint of D095 HIV-1 strain (URF_BC)

Discussion

Antiviral therapy has been widely used for AIDS prevention and control. However, with the development of antiviral therapy, the problem of HIV drug resistance has gradually become prominent and has become a key issue in AIDS prevention, control and treatment. HIV drug resistance is caused by the interaction of virus, drug and host. Under the pressure of drug selection, HIV drug-resistant strains were screened out and gradually developed into dominant strains. The clinical manifestations were decreased drug sensitivity and/or unsatisfactory virological response, which ultimately affected the therapeutic effect of ART. There are many reports of ART failure caused by drug resistance (5). For example, drug resistance monitoring in some ART areas showed that about 50% of patients have experienced immunological failure, and then HIV drug-resistant strains appear and spread rapidly. In some areas, the prevalence rate of drug-resistant strains against first-line drugs has exceeded 50% (6). The newly diagnosed population's transmissible drug resistance has also shown a rapid upward trend (7). The spread and prevalence of HIV-resistant strains have brought unprecedented difficulties to the prevention and control of AIDS. It is of great guiding significance for AIDS prevention and control to find out the prevalence of HIV-1 strains and learn the drug resistance of people who fail in antiviral treatment in areas where antiviral treatment is carried out.

In this study, 561 HIV-1 infected individuals who received antiviral treatment in Suqian in 2019 were comprehensively evaluated. The main route of HIV-1 infection in this area is sexual contact, in which male homosexual transmission and heterosexual transmission are equally divided (45.5% and 44.7%, respectively). It also proved why the infected population in this area is mainly male young adults. This result is also consistent with the occurrence and epidemic characteristics of AIDS in China in recent years. In the follow-up AIDS prevention and control, the key target was the young men. The propaganda of AIDS was strengthened, and a correct view of marriage and love was established. The correct use of condoms in sexual behavior and self-protection were still the contents of constant propaganda. In addition, the rich and diverse epidemic strains are also a remarkable feature in this area. Although Suqian is a new city, among the more than 500 infected people who were followed up, 8 epidemic strains and 3 URF strains were found. The prevalence of many strains may be mainly transmitted through sexual contact with the infection, especially male homosexual sex. The characteristics of this population are high mobility and unstable sexual partners, thus passing through the "bridge" of this population. The economy of this region is developing relatively rapidly, and emerging enterprises are rising rapidly, which requires a large number of foreign workers to solve the local labor shortage. The influx of a large number of people breeds the soil for the introduction and popularity of new HIV-1 strains. Therefore, it is a long way to go for the local administrative departments to strengthen effectively the publicity and education of AIDS among the foreign population.

Through the detection of VL, it could be seen that the antiviral treatment in this area has achieved remarkable and satisfactory results. This achievement comes from the compliance education and drug supervision and management of infected people before treatment. Another achievement was the goal of effectively suppressing 90% of the viruses among the three "90%" who received antiviral treatment in AIDS prevention and control. However, two parts cannot be ignored among the people who fail in antiviral treatment. First, the long-term adherence education in the process of antiviral treatment is unremitting, as the emergence of drug-resistant strains were not found in more than 40% of the failed patients. The patients' compliance was poor or they have not taken antiviral drugs at all. That is the biggest possibility of less effectively inhibited virus. How to make these infected people recognize antiviral treatment and have hope for antiviral treatment is a difficult problem for medical staff. The second is the drug resistance among the people who have been treated with antiviral drugs. Among those who have failed to treat, 56.5% of the infected people have detected drug-resistant strains, indicating that the most likely reason why the virus of these infected people is not effectively inhibited is drug resistance. There are many reasons for drug resistance, but poor compliance is the main reason (8-10). Maintaining good compliance before and during treatment is the key to preventing drug resistance. At present, the drugs applied in AIDS treatment in

China are very limited, and once drug resistance occurs, there would be a dilemma of no follow-up drug selection or paying for treatment costs by itself. In addition, once HIV-1 resistant strains spread and become popular, it will be more difficult to prevent and control AIDS.

Conclusion

In this study, an overview of the antiviral treatment of HIV-1 infected people in Suqian was obtained, and a general situation of HIV-1 epidemic strains in this area was obtained. These results may provide theoretical guidance and support for AIDS prevention and control in this area, and provide a key goal for future prevention and control.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

Thanks for the guidance of the whole army AIDS testing center of the Academy of Military Medical Sciences in the experimental program and result analysis.

Competing interests

The authors declare that they have no competing interests.

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