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SCIENTIFIC LITERATURE

RESEARCH ARTICLE

Viral Load in Plasma and HIV-1 DNA in Lymphocytes of Patients Received HAART for 1 to 10 Years in Dehong Prefecture of Yunnan Province, China

Xiaoxia He[1#], Jibao Wang[2#], Jun Yao[1], Yikui wang[2], Maofeng Qiu[1], Jin Yang[2], Shitang Yao[2], Song Duan[2*] and Yan Jiang[1*]

[1]National HIV/HCV Reference Laboratory, National Center for AIDS/STD Control and Prevention, China
[2]Center for Disease Control and Prevention in Dehong Prefecture, Yunnan Province, China

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Correspondence:
Dr. Song Duan&Dr.Yan Jiang, National HIV/HCV Reference Laboratory, National Center for AIDS/STD Control and Prevention and Center for Disease Control and Prevention in Dehong Prefecture, Yunnan Province, China,
Email: jiangyan03@263.net

ABSTRACT

Highly Active Antiretroviral Therapy (HAART) is the most effective treatment for HIV infection, but information on patients that underwent HAART for a long time is unavailable. So, a group of 300 HIV-1 infected adults from Dehong prefecture of Yunnan province who received HAART treatment for 1 to 10 years (30 cases per year) were enrolled. The plasma HIV-1 RNA VL was measured by the branch DNA approach and the in-house Polymerase Chain Reaction (PCR) was used to detect HIV-1 DNA in lymphocytes of individuals with undetectable VLs. Fluorescence probe real-time Polymerase Chain Reaction (PCR) was used to detect HIV-1 DNA in lymphocytes of individuals whose DNA was undetected by conventional in-house PCR. Our results show that individuals in DehongPerfecture that underwent long-term HAART displayed good viral suppression, 94.7% (284/300) of patients had a VL lower than 1000 copies/ml, which exceeded the third 90 of UNAIDS 90–90–90 target. We also show that 98.9% of patients with undetectable HIV-1 RNA in the plasma but tested positive for HIV-1 DNA in lymphocytes. Our results provide a clinical reference point.

Introduction

Dehong Dai and Jingpo Autonomous Prefectures in the Yunnan Province of China are experiencing an Acquired Immune Deficiency Syndrome (AIDS) epidemic. With the introduction of free Highly Active AntiRetroviral Therapy (HAART) in 2004, however, the number of Human Immunodeficiency Virus (HIV) cases, as well as morbidity and mortality, has decreased. Thus, the HIV/AIDS prevention and control program in Dehong Prefecture has clearly benefited those living in Dehong Dai and Jingpo Autonomous Prefectures [1-6].

HAART is the most effective treatment for HIV infection. Although drugs can suppress viral replication, they cannot destroy latently integrated HIV-1 DNA in stable latent reservoirs in infected lymphocytes and tissues [7]. Research has shown that when the plasma Viral Load (VL) is below the detection limit (50 copies/ml), HIV proviral DNA continues to lurk in lymphocytes and undergoes replication [8-11]. Thus, HIV-1 proviral DNA can still cause HIV infection during drug resistance or in the absence of antiviral drugs [12]. Although it does not

distinguish between replication-competent and -defective latent viruses, HIV DNA is a biomarker of HIV reservoirs, which can be defined as all infected cells and tissues containing all forms of HIV persistence that participate in pathogenesis [13].

However, the HAART therapy effect information on patients that underwent HAART for 1 to 10 years is unknown in China. In addition, based on the third 90 of UNAIDS 90–90–90 target is to ensure that 90% of individuals living with HIV receiving antiretroviral therapy have viral suppression. To understand the treatment effects on viral suppression, we collected samples from 300 individuals living in Dehong Prefecture with HIV who underwent HAART for 1 to 10 years and determined whether they had a detectable HIV-1 RNA VL in plasma and HIV-1 DNA in lymphocytes.

Materials and Methods

1. Ethical considerations

We obtained ethical clearance for the use of patient sample material was obtained through the Ethics review committees of National Center for AIDS/STD Control and Prevention, China CDC. Written informed consent for collection of blood was obtained from 300 patients, and the requirement for informed consent was waived.

2. Participants and sampling

A group of 300 HIV-1 infected adults who underwent HAART treatment for 1 to 10 years (30 cases per year) were enrolled. They were from treatment facilities in five counties or cities of Dehong Prefecture (Mangshi, Ruili, Longchuan, Lianghe and Yingjiang), and detailed follow-up information was available for each participant. Venous Blood blood samples (5ml) were collected from each participant and numbered.

3. Testing procedure

After sampling, all All procedures were conducted in accordance with the National Guidelines for HIV Diagnosis (revised in 2015) [13,14] by the technicians who received training by the relevant state department. After blood sampling, centrifuged at 3000 rpm/min for 15 min to separate plasma and lymphocytes concentration liquid. The 300 plasma samples HIV-1 RNA VL was were measured by the branch DNA approach. DNA was isolated from 187 lymphocytes concentration liquid samples and used for conventional in-house PCR to qualitatively detect HIV-1 DNA segments (env/gag/pol). 47 of 187 lymphocytes concentration liquid samples, which VLs were lower than 50 copies/ml and HIV-1 DNA segments were undetectable by in-house PCR, were transported to the National HIV/HCV Reference Laboratory, and then fluorescence probe real-time PCR with more sensitivity was used to quantitatively detect HIV-1 DNA in 47 lymphocytes concentration liquid samples. lymphocytes of cases whose VLs were lower than 50 copies/ml and HIV-1 DNA segments (env/gag/pol) were undetectable by conventional PCR. (Figure 1).

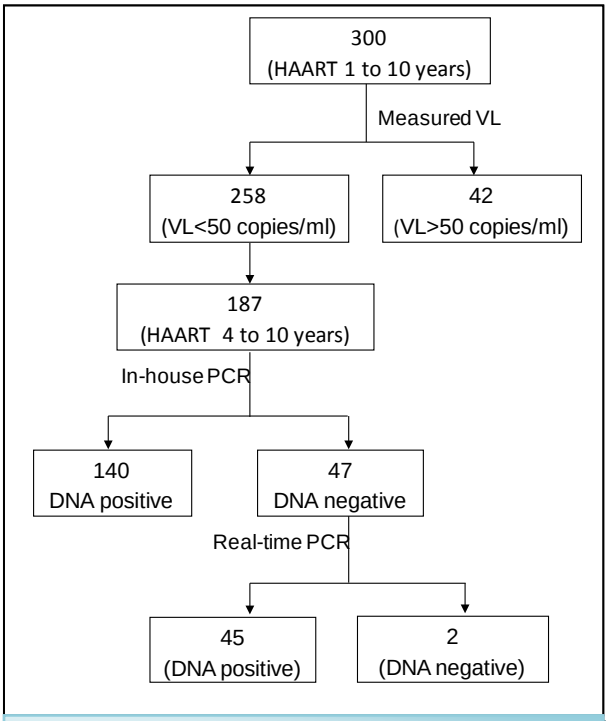

Figure 1: Testing procedure and results.

Plasma HIV-1 RNA VL of 300 patients measured by the branch DNA approach. DNA isolated from lymphocytes of 187 cases that underwent HAART from 4 to 10 years and whose VLs were undetectable (less than 50 copies/ml) was used for conventional PCR to detect HIV-1 DNA segments (env/gag/pol), one of the pol/gag/env fragments positive was defined as DNA positive. Fluorescent probe real-time PCR was used to detect HIV-1 DNA in lymphocytes of 47 cases whose VLs and HIV-1 DNA segments (env/gag/pol) were undetectable by conventional in-house PCR.

4. Reagents and apparatus

The plasma HIV-1 RNA VL was measured with the Quantiplex System 340 bDNAAnalyzer and HIV-1 RNA 3.0 Assay (Bayer, Germany), according to the manufacturer's protocol. The detection limit was 50 copies/ml. A human tissue DNA detection extract kit (Qiagen, Germany) was used to extract DNA from lymphocytes and using in-house PCR to detect HIV-1 DNA segments. A human DNA detection kit (SUPBIO, China) was used to fluorescent probe real-time PCR method.

5. Statistical analyses

The SPSS 19.0 software package was used for correlation statistical analyses. The Excel 2007 software package was used for the preparation of tables and histograms. All analyses were conducted with a significance level of 5%, and P-values less than 0.05 were considered statistically significant.

Results

1. Patient characteristics

The age of 300 patients that underwent HAART from 1 to 10 years ranged from 17 to 78 years. The proportion of young adults from 26 to 45 years was 65.67%. The proportion of male (152 cases) and female (148 cases) patients was equal, and 91.67% of patients were infected by heterosexual transmission (Table 1).

2. Determination of the plasma HIV-1 RNA VL

The proportion of patients with an undetectable HIV-1 RNA VL (lower than 50 copies/ml) was 86.33% (259/300), a detectable VL of 50 to 1000 copies/ml was 8.33% (25/300) and a detectable VL higher than 1000 copies/ml was 5.33% (16/300) (Table 1). There was a negative correlationsignificant difference between the number of cases in each treatment time group and the treatment time $\chi^2 = 31.580$, P = 0.025, r = -0.119). The trend equations for the number of cases and the treatment time were as follows: y1(VL<50) = 0.3758x + 23.733 (R^2 = 0.2257), y2(VL=50-1000) = -0.1333x + 3.3333 (R^2 = 0.0382) and y3(VL>1000) = -0.2424x + 2.9333 (R^2 = 0.2377) (Table 1 and Figure 2).

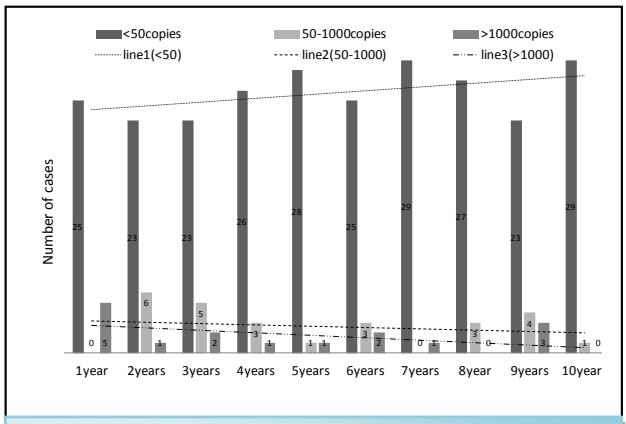

Figure 2: The number of individuals with a detectable plasma HIV-1 RNA VL.

3. Detection of HIV-1 DNA in lymphocytes by conventional PCR

The HIV-1 RNA VL of 187 out of 210 patients who underwent HAART from 4 to 10 years was below the detection limit. Using conventional PCR, the proportion of patients testing positive for HIV-1 DNA was 74.87% (140/187) which means that one of the pol/gag/env fragments was judged as positive. , includingIn addition, 64.29% (90/140) of patients tested positive for gag, 52.14% (73/140) of patients tested positive for env and 45.00% (63/140) of patients tested positive for pol (Table 1). There was a significant difference between the number of patients testing positive for HIV-1 DNA in lymphocytes in each treatment time group There was a negative correlation between the duration of HAART and the number of patients testing positive for HIV-1 DNA in lymphocytes ($\chi^2 = 19.486$, r = -0.204, P < 0.01). The trend equation for the number of positive cases and the duration of HAART were as follows: y= -1.1071x + 24.429 (R^2 = 0.312). The duration of ART was negative correlate to the number of patients positive for pol and gag gene ($\chi^2 = 52.237$, r = -0.360, P < 0.01; $\chi^2 = 17.034$, r = -0.182, P < 0.01) but positive correlate to the number of patients positive for env gene (r = -0.065, P < 0.01). The trend equations were as follows: ypol = -2.2857x + 18.143 (R^2 = 0.4228), ygag= -1.1429x + 15 (R^2 = 0.1441) and yenv= 0.4643x + 11 (R^2 = 0.0765), respectively (Table 1 and Figure 3).

4. HIV-1 DNA in lymphocytes by fluorescent probe real-time PCR

The HIV-1 RNA VL of 47 of 187 patients who underwent HAART for 4 to 10 years, with undetectable HIV-1 RNA VL, and these patients tested negative for HIV-1 DNA By by conventional in-house PCR, these patients tested negative for HIV-1 DNA. However, by fluorescent probe real-time PCR with more sensitivity , however, 95.74% (45/47) of patients tested positive for HIV-1 DNA. The trend equation for the number of positive cases and the treatment time was as follows: $y = 1.1786x + 1.7143$ ($R^2 = 0.39$) (Figure 3).

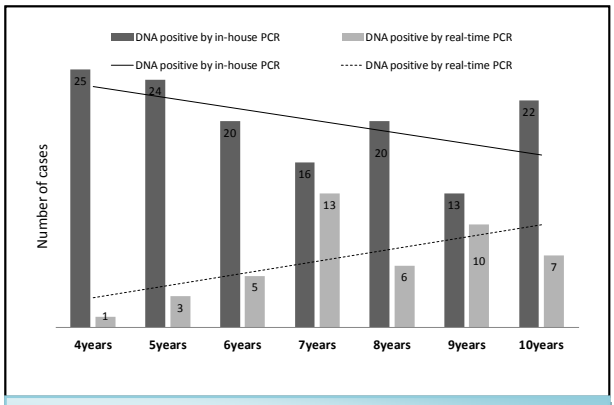

Figure 3: The number of individuals testing positive for HIV-1 DNA in lymphocytes.

5.Special patients information analysis

Gather background information of special patients including 16 patients without viral suppression and 2 patients testing negative for plasma HIV-1 RNA and HIV-1 DNA in lymphocytes. 9 out of 16 patients with a VL higher than 1000 copies/ml had poor adherence to HAART, which included missing several follow-ups. These patients also had HIV-1 antibodies as detected by serological tests. However, among 7 patients with them had good adherence, and 4 patients among had HIV-1 antibodies and were switched to the second-line anti-viral drug LPV. Both 2 patients who negative for plasma HIV-1 RNA VL and HIV-1 DNA in lymphocytes had the presence of HIV-1 antibodies, and they underwent HAART for 5 and 8 years, respectively (Table 2).

Discussion

This is the first study to investigate whether individuals living in Dehong Prefecture with HIV who underwent HAART for 1 to 10 years had detectable plasma HIV-1 RNA VL and HIV-1 DNA in lymphocytes. 94.7% (284/300) of patients had a VL lower than 1000 copies/ml, and 86.0% (258/300) of patients had a VL lower than 50 copies/ml, indicating that these high rates of suppression exceed the UNAIDS 90–90–90 target.Patients that underwent HAART for at least 6 months had a VL lower than 50 copies/ml [15]. However, 5.33% (16/300) of patients that underwent HAART for one year or more had a VL higher than 1000 copies/ml, indicating HAART failure. In addition, a previous study that showed benefits for patients that treatment for more than 5 years [16], but in our study there was no obvious indication of this, which may be related to the small number of cases included in this study.

According to the background information, 9 out of 16 patients with a VL higher than 1000 copies/ml had poor adherence to HAART, which included missing several follow-ups. These patients also had HIV-1 antibodies as detected by serological tests. However, among seven patients with a VL higher than 1000 copies/ml and good adherence, four of them had HIV-1 antibodies and were switched to the second-line anti-viral drug LPV. High VLs may be due to physiological immunity differences in individuals and requires further research. The other three patients who used the first-line drug also had HIV-1 antibodies, suggesting that the suppression failure may be due to inappropriate therapies or drug resistance.

We also found the number of patients with an undetectable VL to escalate with increasing treatment time, that is to say, the longer of HAART duration the HIV-1 VL in plasma was more suppressed. In addition, an earlier study [16, 17] has found viral suppression to be better among women than men, married individuals than unmarried ones and older individuals (age≥26 years) than younger ones (≤25 years). However, among 259 patients with an undetectable VL in this study, the proportions of women (128/259, 49.4%) and men (131/259, 50.6%) were not significantly different, and distributed across all age groups. Inconsistent conclusions may be due to the characteristics of the patients in this study.

It is reported among the patients with the VL lower threshold of 50 copies/ml, HIV-1 DNA in lymphocytes

Table 1: Background information on 300 patients and the testing results.

	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years	9 years	10 years	Total n (%)
Sex											
Male	17	16	10	14	13	22	15	15	12	18	152 (50.67)
Female	13	14	20	16	17	8	15	15	18	12	148 (49.33)
Age											
15-25	3	1	3	7	4	4	2	1	2	0	27 (9.00)
26-35	14	13	9	13	7	15	10	5	14	1	101 (33.67)
36-45	11	7	10	6	9	7	12	10	8	16	96 (32.00)
>45	2	9	8	4	10	4	6	14	6	13	76 (25.33)
Area											
Mangshi	12	14	19	12	11	19	12	16	14	4	133 (44.33)
Ruili	3	3	0	6	1	3	8	10	3	6	43 (14.33)
Yingjiang	4	1	0	2	0	2	0	2	0	0	11 (3.67)
Longchuan	5	9	11	7	13	1	6	1	7	11	71 (23.67)
Lianghe	1	3	0	2	5	5	4	1	6	9	36 (12.00)
Others	5	0	0	1	0	0	0	0	0	0	6 (2.00)
Marital status											
Divorced/Widowed	6	8	5	3	7	6	9	4	2	7	57 (19.00)
Single	5	3	1	5	1	4	3	1	4	1	28 (9.33)
Married	19	19	24	22	22	20	18	25	24	22	215 (71.67)
Education level											
Illiteracy	9	7	7	5	5	4	2	5	8	3	55 (18.33)
Primary	8	17	13	13	9	10	15	9	9	14	117 (39.00)
Junior high school	10	6	9	8	14	12	7	12	10	9	97 (32.33)
High school	1	0	0	2	2	4	4	2	2	2	19 (6.33)
College or above	2	0	1	2	0	0	2	2	1	2	12 (4.00)
Nation											
Han	11	17	11	13	16	17	21	12	15	12	145 (48.33)
Others	19	13	19	17	14	13	9	18	15	18	155 (51.67)
Infection route											
Blood	0	0	0	0	0	0	0	2	6	4	12 (4.00)
Sexual	25	29	29	30	30	27	29	27	23	26	275 (91.67)
Injection	5	1	1	0	0	3	1	1	1	0	13 (4.33)
VL in plasma (copies/ml)											
<50	25	23	23	26	28	25	29	27	24	29	259 (86.33)
50-1000	0	6	5	3	1	3	0	3	3	1	25 (8.33)
>1000	5	1	2	1	1	2	1	0	3	0	16 (5.33)
in-house PCR											
DNA positive[1]				25	24	20	16	20	13	22	140 (75.87)
pol positive				20	14	4	3	16	6	0	63 (45.00)
gag positive				11	17	11	10	10	12	19	90 (64.29)
env positive				17	14	12	9	0	4	17	73 (52.14)
DNA negative[1]				1	4	5	13	7	10	7	47 (25.13)
Real-time PCR											
DNA positive[2]				1	3	5	13	6	10	7	45 (95.74)
DNA negative[2]				0	1	0	0	1	0	0	2 (4.26)

DNA positive[1] means that one of the pol/gag/env fragments was judged as positive. Otherwise, the results were judged as DNA negative[1].
DNA positive[2] means that the quantitative real-time PCR results were judged as positive. Otherwise, the results were judged as DNA negative[2].

was detected in approximately 90% of patients, and the amount of HIV-1 DNA in lymphocytes showed a significant negative correlation with the duration of the undetectable VL [18, 19]. And the amount of cellular HIV-1 DNA was halved when the VL was below the detection limit for 47 months [20]. In our study, among 187 patients who underwent HAART from 4 to 10 years with a VL below the detection limit, 98.9% (185/187) of patients had HIV-1 DNA in lymphocytes, including 75.68% (140/185) of patients screened by conventional in-house PCR and 24.32% (45/185) by fluorescent probe real-time PCR. Statistical analysis showed that the number of HIV-1 DNA positive patients by conventional PCR decreased by the treatment duration, whereas the number of HIV-1 DNA positive patients by fluorescent probe real-time PCR increased with the duration of HAART. These results indicate that the amount of HIV-1 DNA in lymphocytes decreased with the duration of HAART and the continuous newly generated lymphoid cells during HAART. Only a method with higher sensitivity was useful in these cases. There are a variety of methods to detect latent HIV-1 DNA in

Table2: Background information on special patients

ART time	VL(copies/mL)	Adherence	ART regimen	ELISA(S/CO)	WB band
(16 patients without viral suppression)					
1 year	27022	Missing 2 times	LPV+3TC+TDF	27.33	P17.P24.P31.P39.gP41.P51. P66.gP120.gP160
1 year	13732	good	TDF+LPV+3TC	25.88	P17.P24.P31. gP41.P51. P66.gP120.gP160
1 year	3861	Lost 2 times	LPV +TDF+3TC	28.78	P24. gP160
1 year	470000	good	3TC+ LPV +AZT	26.73	P17.P24.P31. gP41.P51.P55.P66.gP120.gP160
1 year	7511	Missing14times	EFV+3TC+TDF	19.64	P17.P24.P31.gP41.P51. P66.gP120.gP160
2 years	47000	good	EFV+3TC+AZT	28.51	P17.P24. gP41.P51.P55.P66.gP120.gP160
3 years	2134	Transfer out 1	EFV+3TC+AZT	19.53	P17.P24.P31.P39.gP41.P51. P66.gP120.gP160
3 years	124999	Transfer out 1	EFV+3TC+TDF	18.36	P17.P24. gP41.P51.P55.P66.gP120.gP160
4 years	148334	good	3TC+EFV+TDF	22.28	-
5 years	13896	good	LPV +3TC+AZT	28.98	P24.P31. gP41.P51. P66.gP120.gP160
6 years	6182	Lost 2 times	NVP+3TC+AZT	25.53	P31. gP41.P51. P66.gP120.gP160
6 years	13405	Missing 2 times	3TC+ LPV +TDF	25.43	P24.P31. gP41.P51. P66.gP120.gP160
7 years	1271	good	LPV +3TC+TDF	23.70	P24.P31. gP41.P51. P66.gP120.gP160
9 years	1173	good	NVP+3TC+AZT	-	P17.P24.P31.P39.gP41.P51.P66.gP120.gP160
9 years	13178	Transfer out 2	3TC+ LPV +TDF	-	P24.P31.gP41. P66.gP120.gP160
9 years	62,469	Missing 2 times	TDF+EFV+3TC	-	P24.P31. gP41.P66.gP120.gP160
(2 patients testing negative for plasma HIV-1 RNA VL and HIV-1 DNA in lymphocytes)					
5 years	<50	Missing 5 times	EFV+3TC+TDF	26.91	P24. gP41.P51. P66.gP120.gP160
8 years	<50	Missing 7 times	EFV+3TC+TDF	-	P17.P24.P31.P39.gP41.P51.P55.P66.gP120.gP160

– means that was not able to do testing. TDF (Tenofovir), TC (lamivudine), EFV (efavirenz), NVP (nevirapine), AZT (azidothymidine) are first-line drugs, LPV (Lopinavir) is a second-line drug; Adherence was defined as actively undergoing HAART.

reservoirs, and we opted for fluorescent probe real-time PCR (detection limit was 50 copies/106 cells) based on the conditions. However, the fluorescent probe real-time PCR failed to determine whether the HIV-1 genome could produce virus infection. Interestingly, there were two patients who underwent HAART for 5 and 8 years that had an undetectable VL and HIV-1 DNA and the presence of HIV-1 antibodies. It might be due to the sensitivity of the detection method, the quality of the specimens or differences in individuals, and there may be a hope of functional cure. Eradicating HIV in individuals undergoing HAART remains a daunting challenge for the scientific community, must be accompanied by comprehensive virological assays to further research these two patients.

In conclusion, our results show that patients in Dehong Prefecture that underwent long-term HAART have good effect on viral suppression as long as the adherence to HAART treatment. However, the patients have maintained undetectable plasma HIV-1 RNA for prolonged periods of time also could be detected HIV-1 DNA in lymphocytes.

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