

# Factors associated with detection of human immunodeficiency virus in tears: a cross-sectional study

**Yujing Qian**

Beijing Tongren Eye Center, Beijing Key Laboratory of Intraocular Tumor Diagnosis and Treatment, Beijing Ophthalmology and Visual Sciences Key Lab, Beijing Tongren Hospital, Capital Medical University

**Zunyou Wu** (✉ [wuzy@263.net](mailto:wuzy@263.net))

National Center for AIDS/STD Control and Prevention, China CDC <https://orcid.org/0000-0002-0839-4548>

**Sarah Robbins Scott**

National Center for AIDS/STD Control and Prevention, China CDC

**Chao Chen**

Beijing You'an Hospital, Capital Medical University

**Kuifang Du**

Beijing You'an Hospital, Capital Medical University

**Wenbin Wei**

Beijing Tongren Eye Center, Beijing Key Laboratory of Intraocular Tumor Diagnosis and Treatment, Beijing Ophthalmology and Visual Sciences Key Lab, Beijing Tongren Hospital, Capital Medical University

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## Research article

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# Abstract

**Background** Human immunodeficiency virus (HIV) continues to be a global public issue and identifying viral reservoirs is needed in order to better understand the transmission of the disease. We aimed to identify the level of HIV viral load in tears of people living with HIV (PLWH) and study factors influencing their tear viral load. **Methods** This was a cross-sectional study of patients infected with HIV or acquired immune deficiency syndrome (AIDS) at Beijing You'an Hospital, Beijing, China between May 2018 and July 2018. Socio-demographic information and laboratory test results were collected, including tear samples. Data were analyzed using independent sample t-test, Mann-Whitney U test, Wilcoxon test, Chi-square test, and Spearman correlation analysis. **Results** 67 subjects met inclusion criteria and were enrolled in the study, of which, 23.9% had detectable HIV ribo nucleic acid (RNA) in their tears. 53.3% of treatment naïve patients and 0.0% of patients who were on anti-retroviral therapy (ART) had detectable HIV RNA in their tears. Tear viral load was lower in tears than in blood plasma and was significantly correlated with plasma viral load ( $Rho=0.566$ ,  $p<0.001$ ), but negatively correlated with CD4+T cell count, CD4+/CD8+T cell count, and duration of HIV infection ( $Rho=-0.450$ ,  $Rho=-0.464$ ,  $Rho=-0.565$ ,  $Rho=-0.252$ ;  $p<0.001$ ). **Conclusions** HIV-1 RNA is present in tears, but at a lower detection rate and viral load than in blood plasma. This study provides a potential new avenue for the early diagnosis and linkage to care for patients infected with HIV.

## Background

It is widely recognized that human immunodeficiency viruses (HIV) can penetrate into various tissues and exist in many bodily fluids and secretions, such as blood, seminal plasma, cerebrospinal fluid, breast milk, and saliva (1-2). Fortunately, highly active antiretroviral therapy (HAART) cannot only protect the health of infected people through suppression of HIV replication, but also improve the overall health and wellbeing of those infected [3]. However, more and more studies have discovered that the relationship between HIV viral load in blood and bodily fluids is not consistent, as many factors contribute to the detection of HIV ribonucleic acid (RNA) in patients regardless of HAART status (4-7). Thus, further investigations into this phenomenon demand our attention.

Tears play a crucial role in refraction, preventing infection, and maintaining homeostasis of ocular surfaces (8). Studies have demonstrated that HIV-positive patients are more likely to suffer from anterior segment diseases such as dry eye (9-11), compared to the general population without HIV infection. While previous studies have found that it was hard to isolate HIV-1 and detect pro-viral sequences in tears of HIV-positive patients (12), recent research has suggested that patients who underwent long-term HAART with undetectable plasma viral load had detectable HIV-1 viral load in their tears (13). This suggests that the lacrimal gland could be a new reservoir for the HIV virus. However, few available data have reported on the dynamics of HIV in tears. Therefore, our objectives were to measure the level of viral load in tears of people living with HIV (PLWH) and study factors influencing their tear viral load.

## Methods

## Study Participants

All patients infected with HIV or acquired immune deficiency syndrome (AIDS) were randomly recruited from the Infectious Diseases Center or Ophthalmology Department of the Beijing You'an Hospital, Beijing, China between May 2018 and July 2018. Eligible participants included all patients with confirmed HIV infection, diagnosed by infectious disease specialists at You'an Hospital. Patients whom were too sick to complete an eye examination or refused to participate were excluded from the study. After screening for eligibility, the study team obtained written and oral consent. Patients meeting inclusion criteria completed an in-person questionnaire delivered by a doctor trained in study ethics.

## Data collection

### *Sociodemographic characteristics*

Information including demographic characteristics (age, sex, race, smoking status, alcohol use), likely mode of HIV transmission (homosexual, heterosexual, injection drug use), date of HIV diagnosis, medical history, co-infections and opportunistic infections, type of HAART regimen used, and duration of HAART treatment, were collected from all participants.

Additionally, participants' laboratory test results, including peripheral blood lymphocyte count and presence of a sexually transmitted infection (STI) were collected. STIs were tested using treponema pallidum particle agglutination assay (TPPA) and rapid plasma reagin circle card assay (RPR). Lastly, plasma HIV-1 viral load was extracted from medical records.

### *Tear collection*

A well-trained ophthalmologist collected tears from patients included in the study in an ultraviolet-disinfected room. 0.2-1.5 milliliters (mL) of tears were collected from the conjunctiva sac directly and stored at -80°C refrigerators at the hospital.

## HIV RNA quantification

The primary outcome of interest was presence and quantification of HIV RNA in tears. Qualitative and quantitative collection of HIV-1 RNA in tears and blood plasma was performed according to manufacturer's instructions using Abbott M2000 Real Time HIV-1 assay (Abbott Molecular, Inc.). HIV RNA results were stratified as TND (target not detected), below 40 copies/mL, or above 40 copies/mL.

## Statistical analysis

Data collected by the questionnaire and blood plasma and tear testing were directly imported into SPSS version 25.0 for Macintosh (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 25.0. Armonk, NY:IBM Corp.). Kolmogorov-Smirnov test was used for normality testing. Normal variables were presented as the mean and standard deviation (SD) and non-normal variables as the median and interquartile range (IQR). T-tests were used to compare the means of normally distributed quantitative

variables, otherwise, a Mann-Whitney U test was used. The nonparametric Wilcoxon test was used to compare tear and plasma viral loads. Chi-square tests were applied to compare qualitative data, such as the detection rate of HIV RNA in tears and blood, and in treatment-naïve patients and HAART-treated patients. Spearman's rho test was computed for measuring associations of tear viral load. The values of blood plasma and tear viral load were transformed to log<sub>10</sub> prior to logistic regression analysis. A value of  $p < 0.05$  was considered as statistically significant.

## Results

### Characteristics of study population

67 patients met inclusion criteria and were included in the study, among which 30 were treatment-naïve patients and 37 were HAART-treated patients; 65 were male; and the mean age was  $35 \pm 9$  years (Table 1). Of all participants, 73.1% were infected due to homosexual contact. Few patients had tuberculosis (1 patient, 1.5%), Hepatitis B (3 patients, 4.5%) or Hepatitis C (1 patient, 1.5%) co-infection. Multiple patients had HIV-related opportunistic infections including cytomegalovirus (34 patients, 50.7%), Pneumocystis (5 patients, 7.5%), herpes simplex virus (11 patients, 16.4%), Candida albicans (1 patient, 1.5%), Epstein-Barr virus (4 patients, 6.0%), and Toxoplasma gondii (1 patient, 1.5%). Of all subjects, the mean CD4+ T cell count was 466 cells/ $\mu$ L [standard deviation (SD) 276 cells/ $\mu$ L] and median CD4+/CD8+ T cell count was 0.48 (range 0-1.87). Treatment-naïve patients had a lower CD4+ T cell count and CD4+/CD8+ T cell count compared to HAART-treated patients (315 vs 589 cells/ $\mu$ L, 0.29 vs 0.57,  $p < 0.001$ , respectively). Of 37 patients who were on HAART, the median duration of infection and treatment were 36 months (range 1-191) and 34 months (range 1-146), respectively.

**Table 1.** Demographic characteristics of study participants

	Total (n=67)	Treatment-naïve patients (n=30)	HAART-treated patients (n=37)	P-Value
Age (years), ±s	35±9	36±10	35±9	0.853 <sup>b</sup>
Male, n (%)	65 (97.0)	30 (100.0)	35 (94.6)	0.498 <sup>c</sup>
Smoking status, n (%)	14 (20.9)	4 (13.3)	10 (27.0)	0.170 <sup>c</sup>
Alcohol use, n (%)	8 (11.9)	2 (6.7)	6 (16.2)	0.412 <sup>c</sup>
Mode of HIV acquisition, n (%)				0.971 <sup>c</sup>
Homosexual	49 (73.10)	22 (73.33)	27 (73.00)	
Heterosexual	11 (16.40)	5 (16.67)	6 (16.20)	
Injection drug use	2 (3.00)	1 (3.33)	1 (2.70)	
Missing	3 (4.50)	1 (3.33)	2 (5.40)	
Duration of HIV infection (months), M (IQR)	10.00 (0.00- 56.00)	0.00 (0.00-4.50)	36.00 (10.00-85.00)	<0.001 <sup>a</sup>
RPR positive, n (%)	17 (25.40)	12 (40.00)	5 (13.51)	0.974 <sup>c</sup>
TPPA positive, n (%)	34 (50.75)	20 (66.67)	14 (37.84)	0.019 <sup>c</sup>
Co-infection, n (%)	42 (62.69)	22 (73.33)	20 (54.05)	0.105 <sup>c</sup>
LogMAR vision acuity, M (IQR)	0.00 (0.00-0.10)	0.00 (0.00-0.06)	0.00 (0.00-0.10)	0.797 <sup>a</sup>
IOP (mmHg), M (IQR)	13.50 (12.50- 15.50)	13.25 (12.00-15.13)	14.50 (13.25-15.75)	0.037 <sup>a</sup>
CD4+T cell count (cells/μL), ±s	466±276	315±229	589±252	<0.001 <sup>b</sup>
CD4/CD8, M (IQR)	0.48 (0.24-0.62)	0.29 (0.15-0.49)	0.57 (0.44-0.80)	<0.001 <sup>a</sup>
Duration of HAART treatment (months), ±s			43±36	
Current regimen, n (%)				
NRTIs			37 (100.0)	
NNRTIs			27 (73.0)	
PIs			4 (10.8)	
INIs			6 (16.2)	

<sup>a</sup>Mann-Whitney U test, <sup>b</sup> independent samples t-test, <sup>c</sup> Chi-square test

Abbreviations: HIV, human immunodeficiency virus; M (IQR), Median, interquartile range; RPR, rapid plasma regain; TPPA, treponema pallidum particle agglutination; LogMAR, chat used by ophthalmologists and optometrists to estimate visual acuity; IOP, intraocular pressure, mmHG, millimeters of Mercury, HAART, highly active antiretroviral therapy; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; INIs, integrase inhibitors

### HIV-RNA Detection rate and viral load in tears

Of the 67 subjects, 23.9% of patients had detectable HIV RNA in tears (Table 2) compared to almost 63% whom had detectable HIV RNA in blood plasma ( $p < 0.001$ ). Of treatment naïve patients, 53.3% had detectable HIV RNA in their tears compared to 100% of treatment-naïve participants whom had HIV RNA in their blood plasma ( $p < 0.001$ ). Specifically, 20% of treatment-naïve patients had  $< 40$  copies/mL of HIV and 33.3% had  $\geq 40$  copies/mL of HIV RNA detectable in their tears. Of those on HAART therapy, 0.0% of patients had detectable HIV viral load in tears, while HIV RNA was detectable in blood in 32.4% of HAART treated participants.

Furthermore, of all participants, tear viral load ranged from TND to 13,096 copies/mL and median viral load was generally lower in tears than in blood plasma (0 vs. 156 copies/mL,  $p < 0.001$ ), as seen in Table 2. Additionally, patients not yet initiating HAART-treatment were also found to have a lower median viral load in tears compared to in blood plasma (40 vs. 22,555.5 copies/mL,  $p < 0.001$ )

**Table 2.** Viral load detection levels by the total participant population and treatment status

	Total (n=67)	Treatment-naïve patients (n=30)	HAART-treated patients (n=37)	P-Value
Blood viral load (copies/mL), M (IQR)	156 (0-21539)	22555 (4350.5-52593)	0 (0-40)	$< 0.001^a$
Tear viral load (copies/mL), M (IQR)	0 (0-0)	40 (0-447.5)	0 (0-0)	$< 0.001^a$
Detection rate of blood HIV RNA (%)	62.7	100.0	32.4	$< 0.001^b$
Detection rate of tear HIV RNA (%)	23.9	53.3	0.0	$< 0.001^b$

<sup>a</sup>Mann-Whitney U test, <sup>b</sup> Chi-square test

Abbreviations: HAART, highly active antiretroviral therapy; M (IQR), Median, interquartile range; mL, milliliters; HIV, human immunodeficiency virus; RNA, ribo nucleic acid

### Factors associated with tear viral load in all participants

As seen in Table 3 and Figure 1, viral load in tears and in blood plasma were found to be positively associated ( $Rho= 0.566, p<0.001$ ), meaning that as viral load in tears increased, so would viral load in blood. Tear HIV RNA level, however, was inversely associated with CD4+ T cell count ( $Rho=-0.450, p<0.001$ ), CD4+/CD8+ T cell count ( $Rho=-0.464, p<0.001$ ), duration of HIV infection ( $Rho=-0.565, p<0.001$ ), and intraocular pressure ( $Rho=-0.252, p=0.039$ ), meaning that for every increase in CD4+ T cell count, CD4+/CD8+ T cell count, duration of HIV infection and intraocular pressure, the HIV RNA level in tears decreased. No other variables (e.g. age, CD8+T cell count) were significantly associated with tear viral load.

### Factors associated with tear viral load in treatment-naïve participants

Of 30 treatment-naïve subjects, tear viral load was only associated with duration of infection ( $Rho=-0.389, P=0.033$ ) and logMAR vision acuity ( $Rho=0.422, P=0.020$ ). No other factors (e.g. age, CD4+T cell count, CD8+T cell count, CD4+/CD8+ T cell count, blood viral load, total lymphocyte count, intraocular pressure (IOP), volume of tear samples, dilution multiple) were associated with HIV RNA in tears.

**Table 3.** Factors associated with viral load in tears among all participants and treatment naïve participants

	Total (n=67)		Treatment-naïve patients (n=30)	
	Rho	P-Value	Rho	P-Value
Blood viral load (copies/mL)	0.566	<0.001	0.199	0.292
CD4+T cell count (cells/mL)	-0.450	<0.001	-0.259	0.167
CD4/CD8	-0.464	<0.001	-0.335	0.070
Duration of HIV infection (months)	-0.565	<0.001	-0.389	0.033
IOP (mmHg)	-0.252	0.039	-0.220	0.243
LogMAR vision acuity	0.223	0.070	0.422	0.020
CD8+T cell count (cells/mL)	-0.039	0.756	-0.041	0.830
Total lymphocyte count (cells/mL)	-0.190	0.123	-0.134	0.481
volume of tear samples (µl)	-0.079	0.525	0.125	0.511
dilution multiple	0.112	0.368	-0.084	0.659
Age (year)	0.044	0.725	0.114	0.547

Abbreviations: mL, milliliters, HIV, human immunodeficiency virus; IOP, intraocular pressure; mmHG, millimeters of Mercury; LogMAR, chat used by ophthalmologists and optometrists to estimate visual acuity; µl, microliter

## Figure 1. Viral load in tears and blood plasma among patients by anti-retroviral therapy status.

The scatter diagram summarizes the distribution of patients by viral load status and antiretroviral therapy status. Among 67 subjects, viral load was generally lower in tears than in blood plasma. Among 30 treatment-naïve patients, more than half had detectable HIV RNA in tears, while among 37 patients who were on antiretroviral therapy, 0.0% had detectable HIV RNA in tears.

## Discussion

With a better understanding of HIV persistence in various bodily fluids, ophthalmologists are becoming especially interested in the existence of HIV RNA in tears and factors associated with tear viral load. However, few studies have focused on this issue. Our study aimed to gather baseline information regarding the existence of HIV RNA in tears and associated factors, which may lead to presence of viral load in tears. The results of this study can be used to discuss potential HIV reservoirs and inform the integration of a holistic approach to HIV care, not just amongst infectious disease specialists but patients' spectrum of providers.

In this cross-sectional study, 23.9% of participants had detectable HIV RNA in their tears. The presence of tear viral load was also associated with blood viral load, CD4+ T cell count, CD4+/CD8+ T cell count and duration of HIV infection. Furthermore, the detection rate and viral load were lower in tears than in blood and lower in HAART-treated patients than in untreated patients. This is similar to other studies among varying bodily fluids and secretions, such as cerebrospinal fluid, saliva, breast milk, cervicovaginal lavage fluid and semen (14-19). Thus, although the existence of HIV RNA in tears may increase transmission risks for patients and doctors, antiretroviral drugs can penetrate through the blood-ocular barrier and suppress HIV effectively. Additionally, tear collection may be a new, non-invasive way for early diagnosis of HIV and reflect the systematic condition of HIV/AIDS. Particularly, adapting ophthalmology care to incorporate HIV testing may be a new and unique way of finding patients whom otherwise may not be captured in typical screening campaigns. Though the presence of HIV in tears was relatively low, at less than a fourth of participants, initial HIV screening via varying clinical checkups may be one way to increase testing and linkage to care of HIV-infected individuals.

HIV may survive and duplicate in eyes without severe restriction (20). Previous studies have shown that only a small subset of HIV variants could intrude into the eye tissue (21). Therefore, it is of great importance to keep exploring the possibility of HIV reservoirs (22) in eyes and factors associated with the presence of HIV in tears. To identify factors associated with tear viral load in treatment-naïve subjects, we performed various correlation and regression analyses. However, no variables were found to significantly impact viral load except the duration of infection. This lack of association may be the result of the relatively small sample size or the existence of outlier data (Fig. 1). Indeed, when we exclude these four extremes (blood viral load >150000copies/ mL or tear viral load >10000copies/ mL), there is a significant

relationship between tear viral load and blood viral load ( $Rho=0.43$ ,  $p=0.03$ ). This phenomenon was affirmed when we retested the outlier samples, and it was also reported in the study of other bodily fluids (e.g. saliva, cerebrospinal fluid, aqueous humor) (23-27). Considering this is the first study exploring tear viral load in treatment-naïve HIV/AIDS, we still lack sufficient evidence to explain our findings now. Future studies of larger study cohorts are needed to verify our results and establish a more robust association, meanwhile, more histopathology and immunohistochemistry examinations are required to point out the possible mechanism of HIV infection in eyes.

This study had several limitations. This was cross-sectional study, thus we were unable to assess causality. Also, our study was limited by the small sample size and small volume of tear samples for polymerase chain reaction (PCR) analysis. Even though these two parameters did not influence the test results according to statistical analysis, and the Abbott Real Time assay provided a reliable linearly quantified result in diluted samples according to the manufacturer's guidelines, it would be more convincing to use undiluted samples directly. It is well recognized, however, that the Abbott Real Time assay has a wide dynamic range with a significant correlation and good agreement of results when compared to the NucliSENS EasyQ, Cobas TaqMan and bDNA assays, for all HIV-1 subtypes (28-29). More so, the use of a singular assay to determine the viral load in tears may be a limitation, as other studies have observed varying assays to produce different results when HIV RNA is present at low levels (30-32). Thus, further assessment using available tests would be warranted to validate the findings of this study. Lastly, innate inhibitory molecules (33) in tears, such as virus-specific antibodies, soluble proteins, and electrolytes, may limit the accuracy of detecting HIV RNA. Further longitudinal studies, which compare the HIV RNA viral load, drug concentration, and HIV RNA sequences in tears over time, could help inform the development of efficient ophthalmic topical anti-retroviral drops.

## Conclusions

According to the results of this study, HIV-1 RNA is present in tears, but at a lower detection rate and viral load than in blood plasma. Tear viral load is positively associated with plasma viral load while it is negatively correlated with CD4 cell count, with HAART treatment reducing viral load in tears. This study provides more insight into the HIV viral dynamics in eyes and opens up new avenues of early diagnosis, intervention, and prognosis of the disease.

## Abbreviations

AIDS: Acquired immune deficiency syndrome

ART: Anti-retroviral therapy

HAART: Highly active antiretroviral therapy

HIV: Human immunodeficiency virus

INIs: Integrase inhibitors

IOP: Intraocular pressure

IQR: Interquartile range

NNRTIs: Non-nucleoside reverse transcriptase inhibitors

NRTIs: Nucleoside reverse transcriptase inhibitors

PCR: Polymerase chain reaction

PIs: Protease inhibitors

PLWH: People living with HIV

RNA: Ribo nucleic acid

RPR: Rapid plasma regain

SD: Standard deviation

STI: Sexually transmitted infection

TND: Target not detected

TPPA: Treponema pallidum particle agglutination assay

## **Declarations**

### **Ethics approval and consent to participate**

This study was reviewed and approved by the institutional review board of the National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention. All participants signed written informed consent to participate in the study and allow their de-identified records to be included in this analysis.

### **Consent for publication**

All participants included in the study provided written, informed consent

### **Availability of data and materials**

The datasets used during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests

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## Contributions

YJQ participated in the study design, organized sample inventory and data, performed experiments and statistical analyses, drafted the manuscript and prepared tables and figures. ZYW helped organize the manuscript. SRS drafted the manuscript and helped edit all iterations. CC and KFD participated in the study design and data organization. WBW participated in the study design and helped edit the manuscript. All authors read and approved the final manuscript.

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## References

- [1] Liuzzi G, Chirianni A, Clementi M, Bagnarelli P, Valenza A, Cataldo PT, et al. Analysis of HIV-1 load in blood, semen and saliva: evidence for different viral compartments in a cross-sectional and longitudinal study. *AIDS*. 1996;10:F51-6.
- [2] Stekler JD, Ure G, Dragavon J, Chang M, Coombs RW. Detection of HIV RNA in dried blood spots and oral fluids. *AIDS*. 2017;31:1191-3.
- [3] Benzekri NA, Sambou JF, Ndong S, Tamba IT, Faye D, Diallo MB, et al. Prevalence, predictors, and management of advanced HIV disease among individuals initiating ART in Senegal, West Africa. *BMC Infect Dis*. 2019;19:261.

- [4] Stekler J, Sycks BJ, Holte S, Maenza J, Stevens CE, Dragavon J, et al. HIV dynamics in seminal plasma during primary HIV infection. *AIDS Res Hum Retroviruses*. 2008;24:1269-74.
- [5] Mujugira A, Coombs RW, Heffron R, Celum C, Ronald A, Mugo N, et al. Seminal HIV-1 RNA detection in heterosexual African men initiating antiretroviral therapy. *J Infect Dis*. 2016;214:212-5.
- [6] Pavlinac PB, Hawes SE, Gottlieb GS, Gaye A, N'Diaye CF, Critchlow CW, et al. HIV shedding in the oral cavity: an assessment of HIV type, immunovirologic, demographic and oral factors. *Sex Transm Infect*. 2012;88:45-50.
- [7] Balamane M, Winters MA, Dalai SC, Freeman AH, Traves MW, Israelski DM, et al. Detection of HIV-1 in saliva: implications for case-identification, clinical monitoring and surveillance for drug resistance. *Open Virol J*. 2010;4:88-93.
- [8] Di Zazzo A, Micera A, De Piano M, Cortes M, Bonini S. Tears and ocular surface disorders: Usefulness of biomarkers. *J Cell Physiol*. 2019;234:9982-93.
- [9] Nizamuddin I, Koulen P, McArthur CP. Contribution of HIV Infection, AIDS, and Antiretroviral Therapy to Exocrine Pathogenesis in Salivary and Lacrimal Glands. *Int J Mol Sci*. 2018;19:2747.
- [10] Agrawal R, Balne PK, Verapan A, Au VB, Lee B, Loo E, et al. A distinct cytokines profile in tear film of dry eye disease (DED) patients with HIV infection. *Cytokine*. 2016;88:77-84.
- [11] Balne PK, Agrawal R, Au VB, Lee B, Loo E, Tong L, et al. Dataset of longitudinal analysis of tear cytokine levels, CD4, CD8 counts and HIV viral load in dry eye patients with HIV infection. *Data Brief*. 2017;11:152-4.
- [12] Mueller AJ, Klauss V, Gürtler L, Geier S. Infrequent detection of HIV-1 components in tears compared to blood of HIV-1-infected persons. *Infection*. 1992;20:249-52.
- [13] Han Y, Wu N, Zhu W, Li Y, Zuo L, Ye J, et al. Detection of HIV-1 viruses in tears of patients even under long-term HAART [Letter]. *AIDS*. 2011;25:1925-7.
- [14] Anderson AM, Muñoz-Moreno JA, McClernon DR, Ellis RJ, Cookson D, Clifford DB, et al; Prevalence and correlates of persistent HIV-1 RNA in cerebrospinal fluid during antiretroviral therapy. *J Infect Dis*. 2017;215:105-13.
- [15] Pilcher CD, Shugars DC, Fiscus SA, Miller WC, Menezes P, Giner J, et al. HIV in body fluids during primary HIV infection: implications for pathogenesis, treatment and public health. *AIDS*. 2001;15:837-45.
- [16] Shepard RN, Schock J, Robertson K, Shugars DC, Dyer J, Vernazza P, et al. Quantitation of human immunodeficiency virus type 1 RNA in different biological compartments. *J Clin Microbiol*. 2000;38:1414-8.

- [17] Fiore JR, Suligoj B, Saracino A, Di Stefano M, Bugarini R, Lepera A, et al. Correlates of HIV-1 shedding in cervicovaginal secretions and effects of antiretroviral therapies. *AIDS*. 2003;17:2169-76.
- [18] Kantor R, Bettendorf D, Bosch RJ, Mann M, Katzenstein D, Cu-Uvin S, et al. HIV-1 RNA Levels and antiretroviral drug resistance in blood and non-blood compartments from HIV-1-infected men and women enrolled in AIDS Clinical Trials Group Study A5077. *PLoS One*. 2014;9:e93537.
- [19] Fujikawa LS, Salahuddin SZ, Palestine AG, Masur H, Nussen-blatt RB, Gallo RC. Isolation of human T-lymphotropic virus type III from the tears of a patient with the acquired immunodeficiency syndrome. *Lancet*. 1985;2:529-30.
- [20] Kunavisarut P, Sirirungsi W, Pathanapitoon K, Rothova A. Clinical manifestations of human immunodeficiency virus-induced uveitis. *Ophthalmology*. 2012;119:1455-9.
- [21] Williams-Wietzikoski CA, So ID, Bull ME, Samleerat T, Pathanapitoon K, Kunavisarut P, et al. Genetic analyses of HIV env associated with uveitis in antiretroviral-naïve individuals. *AIDS*. 2017;31:1825-30.
- [22] Pathanapitoon K, Riemens A, Kongyai N, Sirirungsi W, Leechanachai P, Ausayakhun S, et al. Intraocular and plasma HIV-1 RNA loads and HIV uveitis. *AIDS*. 2011;25:81-6.
- [23] Christo PP, Greco DB, Aleixo AW, Livramento JA. Factors influencing cerebrospinal fluid and plasma HIV-1 RNA detection rate in patients with and without opportunistic neurological disease during the HAART era. *BMC Infect Dis*. 2007;7:147.
- [24] Kazmi SH, Naglik JR, Sweet SP, Evans RW, O'Shea S, Banatvala JE, et al. Comparison of human immunodeficiency virus type 1-specific inhibitory activities in saliva and other human mucosal fluids. *Clin Vaccine Immuno*. 2006;13:1111-8.
- [25] Ikeno R, Yamada E, Yamazaki S, Ueda T, Nagata M, Takagi R, et al. Factors contributing to salivary human immunodeficiency virus type-1 levels measured by a Poisson distribution-based PCR method. *J Int Med Res*. 2018;46:996-1007.
- [26] Kunavisarut P, Sirirungsi W, Pathanapitoon K, Rothova A. Clinical manifestations of human immunodeficiency virus-induced uveitis. *Ophthalmology*. 2012;119:1455-9.
- [27] Rothova A, Schneider M, de Groot-Mijnes JD. Human immunodeficiency virus-induced uveitis: intraocular and plasma human immunodeficiency virus-1 RNA loads. *Ophthalmology*. 2008;115:2062-4.
- [28] Gomes P, Carvalho AP, Diogo I, Gonçalves F, Costa I, Cabanas J, et al. Comparison of the NucliSENS EasyQ HIV-1 v2.0 with Abbott m2000rt RealTime HIV-1 assay for plasma RNA quantitation in different HIV-1 subtypes. *J Virol Methods*. 2013;193:18-22.
- [29] Katsoulidou A, Rokka C, Issaris C, Haida C, Tzannis K, Sypsa V, et al. Comparative evaluation of the performance of the Abbott RealTime HIV-1 assay for measurement of HIV-1 plasma viral load on

genetically diverse samples from Greece. *Virology*. 2011;8:10.

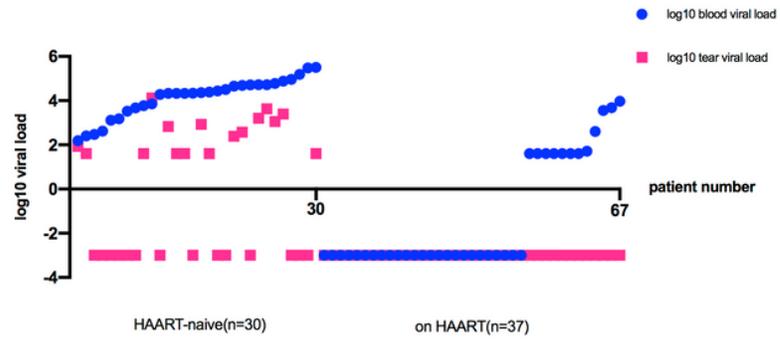
[30] Amendola A, Marsella P, Bloisi M, Forbici F, Angeletti C, Capobianchi MR. Ability of two commercially available assays (Abbott RealTime HIV-1 and Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 Version 2.0) to quantify low HIV-1 RNA levels (<1,000 copies/milliliter): comparison with clinical samples and NIBSC working reagent for nucleic acid testing assays. *J Clin Microbiol*. 2014;52:2019-26.

[31] Garner W, White K, Szwarcberg J, McCallister S, Zhong L, Wulfsohn M. Concordance of HIV-1 RNA values by Amplicor and TaqMan 2.0 in patients with confirmed suppression in clinical trials. *Clin Infect Dis*. 2016;62:929-34.

[32] Lalama CM, Jennings C, Johnson VA. Comparison of three different FDA-approved plasma HIV-1 RNA assay platforms confirms the virologic failure endpoint of 200 copies per milliliter despite improved assay sensitivity. *J Clin Microbiol*. 2015;53:2659-66.

[33] Shugars DC. Endogenous mucosal antiviral factors of the oral cavity. *J Infect Dis*. 1999;179:S431-5

## Figures



**Figure 1**

Viral load in tears and blood plasma among patients by anti-retroviral therapy status.