• Clinical Research •

Investigation of the infection route of HIV-associated cytomegalovirus retinitis

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Abstract

● **AIM:** To investigate the etiology of ocular pathogens and to establish the various pathogens present in human immunodeficiency virus (HIV) patients with cytomegalovirus retinitis (CMVR).

● **METHODS:** A total of 17 HIV-infected patients with concomitant eye disorders were enrolled. Patients were divided into CMVR group (10 patients, 18 eyes) and non-CMVR group (7 patients, 9 eyes) based on clinical manifestations and the presence of cytomegalovirus (CMV)-DNA in ocular specimens. The viral load of CMV was assessed using polymerase chain reaction in aqueous humor, vitreous fluid, and peripheral blood samples of patients in the CMVR group. Additionally, peripheral blood $CD4^+$ T cell counts were measured in both groups.

● **RESULTS:** In the CMVR group, the CMV-DNA load in the vitreous and aqueous humor samples was substantially higher than in the peripheral blood samples (P<0.01). CMV-DNA load in the aqueous humor and vitreous samples of the two eyes in the CMVR group was determined to be statistically significant (10 patients, 16 eyes, P=0.018, 0.012). Peripheral blood $CD4^+$ T cell counts in the CMVR group were adversely linked with the CMV-DNA load in both the aqueous humor and peripheral blood (P=0.005, 0.048). Compared with the non-CMVR group, the peripheral blood $CD4^+$ T cell count in the CMVR group decreased significantly (P=0.014). The peripheral blood $CD4^+$ T cell count exceeded 300 cells/μL in 85.71% of non-CMVR patients, whereas it was below 100 cells/μL in 90.00% of the CMVR group. The intraocular specimens of the patients who underwent CMVR testing did not include any additional infections.

● **CONCLUSION:** In HIV-associated CMVR patients, there may exist alternative, yet unidentified, infection pathways for intraocular CMV in addition to the conventional route. The substantial difference in CMV-DNA load between the eyes of most CMVR patients suggests that CMV may originate from different sources in each eye. The proportion of peripheral blood $CD4^+$ T cells in HIV patients is negatively correlated with the quantity of CMV viruses in their eyes. The peripheral blood count of <100 cells/μL indicates a considerable increase in the risk of concurrent CMVR. Multi-ocular pathogen presentations are uncommon in HIV individuals with CMVR.

● **KEYWORDS:** cytomegalovirus; retinitis; acquired immunodeficiency syndrome; human immunodeficiency virus

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INTRODUCTION

Cytomegalovirus retinitis (CMVR) is a prevalent late-stage opportunistic ocular infection in patients with acquired immune deficiency syndrome (AIDS), which has attracted increasing attention from ophthalmologists[1]. The pathogen that causes this condition is called cytomegalovirus (CMV), a double-stranded DNA virus of the herpesviridae family[2]. Studies have found that CMVR usually occurs when the $CD4^+$ T cell count is below 50 cells/μL[3-4]. The majority of immune-competent people do not present with any symptoms from the virus. But in those with a compromised immune system, a primary CMV infection can cause a major immune system deterioration that can substantially increase the risk of mortality and serious complications like retinal detachment (RD), which is the primary cause of blindness, and permanent visual loss[5]. It is reported that the main symptoms of CMVR include blurred vision, floaters, and visual field defects. These conditions are easily overlooked and also bring difficulties to the early diagnosis of CMV in AIDS patients[6]. In addition, although immunotherapy research has made some progress in

recent years, potential adverse reactions and drug resistance in immunocompromised patients are still challenges[7-8].

Individuals with human immunodeficiency virus (HIV)-associated retinopathy are more likely to develop CMVR. CMVR is the most common cause of vision impairment in one or both eyes in HIV-positive patients, with observations made in approximately 20%–40% of cases[9]. After contracting an HIV infection, the body's immune function becomes significantly compromised, leading to the development of opportunistic conditions such as CMV-induced encephalitis, hepatitis, colitis, and intraocular infections. The pathophysiology of HIV-related CMVR is still not entirely understood, however, numerous investigations support the hypothesis that CMV enters the eye through the blood-retinal barrier (BRB), a blood-derived channel, as the etiology of CMVR[10]. Specifically, CMV mainly affects vascular endothelial cells and then retinal pigment epithelium, providing opportunities for viruses to enter retinal tissue and cause retinal necrosis[11-12]. Due to the high species-specific nature of CMV, previous studies usually relied on mouse models[13-14], which aided in providing new insights into the pathogenesis of HIV-related CMVR. Nevertheless, a lot of the causes remain unidentified, and further research is required to validate them. Furthermore, there have been few reports of other infection routes to date, and the majority of this research is based on theoretical conjecture or animal model experiments.

Currently, fundus examination is the primary method used to diagnose CMVR, and the data on clinical diagnosis rates are still inadequate. For certain challenging cases, the detection of CMV, herpesvirus, and *Toxoplasma gondii (T. gondii)* in aqueous humor or vitreous samples may aid in the diagnosis and differential diagnosis of CMVR[15]. When diagnosing CMVR, positive vitreous CMV DNA or aqueous humor is highly valuable information[6]. The most significant predictor of CMVR is $CD4^+$ T cell count <50 cells/μL[16]. A drop in these cells increases susceptibility to pathogen assaults, leading to a variety of opportunistic infections. Additionally, CMV reactivation may be impacted by the HIV viral load. A study by Xie *et al*[1] found that, although a decrease in $CD4^+$ T cells may not be a risk factor for RD, a high viral load in the early stage of CMVR may pose a risk for secondary RD. Therefore, viral load and $CD4^+$ T cell count are frequently used in clinical practice to evaluate the severity and recovery from AIDS. This work used a prospective research design to identify and analyze the CMV-DNA viral load in the vitreous fluid, aqueous humor fluid, and peripheral blood of HIV-associated CMVR patients to examine the pathogenic source and routes of transmission of intraocular infection. In addition, the immunoglobulin M (IgM) antibodies of *T. gondii*, herpes simplex virus (HSV) 1/2, and the DNA viral loads of HSV-2 and Epstein-Barr virus (EBV) were also investigated in the vitreous humor and blood to determine the possibility of multiple pathogen infections in the eyes of HIV-infected patients. This study aimed to provide a theoretical basis for further elucidating the pathogenesis of CMVR and effective prevention and treatment strategies.

PARTICIPANTS AND METHODS

Ethical Approval The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (approval number: 2023-E742-01). All patients were fully informed about the purpose and significance of the study and agreed to participate after understanding the details. All patients also signed an informed consent form.

Participants The study recruited patients with HIV infection and concomitant intraocular diseases who were admitted to the hospital between August 2016 and January 2019. Based on clinical examination and CMV detection results, the patients were divided into two groups: the CMVR and the non-CMVR groups. Samples of aqueous humor and/or vitreous and peripheral blood were collected from all patients. The samples were tested for $CD4^+$ T cell count, alanine aminotransferase, aspartate aminotransferase, urine routine, renal function, CMV, toxoplasma, HSV, EBV DNA and/or corresponding antibody levels. Additionally, a pulmonary imaging examination was performed.

The diagnosis of HIV infection was based on the "Guidelines for Diagnosis and Treatment of Human Immunodeficiency Virus Infection/Acquired Immunodeficiency Syndrome (2021 edition)" in China[17]. The guidelines required a positive HIV antibody screening test and a positive HIV supplementary test (positive antibody additional test or positive nucleic acid qualitative detection or nucleic acid quantitative detection greater than 500 copies/mL) in peripheral blood. Alternatively, isolation of HIV was also considered.

The inclusion criteria for the CMVR group were: 1) progressive visual impairment; 2) confirmation of HIV-related fundus changes by three experienced ophthalmologists in the research group after mydriasis. These changes should include yellow-white necrotic lesions, retinal hemorrhage, white line-like vessels, and vascular white sheath lesions.

Following the fulfillment of the aforementioned requirements, an additional fundus fluorescein angiography (FFA) examination was carried out to verify the existence of one of the following conditions: 1) a decrease in retinal artery diameter, delay in arterial filling disorder, tortuosity, and dilation of the retinal vein; 2) extensive regions of non-perfusion in the retina, the borders of which exhibit fluorescence staining or leakage; 3) fluorescence masking due to significant retinal hemorrhage; 4) spontaneous fluorescence in the retina.

The following were the inclusion criteria for the non-CMVR group: 1) patients who underwent phacoemulsification and aspiration surgery for cataracts at the First Affiliated Hospital of Guangxi Medical University due to lens opacity requiring increased vision; 2) the absence of aqueous flare, aqueous humor cells, iris adhesion, iris nodules, vitreous inflammation cells, or corneal posterior deposits; 3) conducting slit-lamp examination before and after surgery to verify that the changes in the retina did not meet the inclusion criteria for the CMVR group, three highly qualified ophthalmologists.

The exclusion criteria for the non-CMVR group were as follows: 1) previously diagnosed with CMVR, RD, uveitis, or other vitreoretinal diseases; 2) history of open eye trauma or intraocular surgery.

Aqueous Humor Samples Before the surgery, the conjunctival sac was flushed, and 0.1 mL of aqueous humor was extracted by puncturing the aqueous humor. The subsequent surgical procedure included routine phacoemulsification, aspiration, and implantation of an intraocular lens for cataracts in the non-CMVR group.

In the CMVR group, the same method was used to collect aqueous humor. However, a vertical puncture of the vitreous cavity was performed 4 mm behind the temporal superior angle of the sclera, and about 0.3 mL of vitreous cavity fluid was slowly extracted.

The aqueous humor and vitreous fluid collected during the operation were sent to our hospital's laboratory for quantitative detection of CMV-DNA, HSV 2-DNA, and EBV-DNA viral loads after surgery. Additionally, HSV 2-IgM and *T. gondii* IgM were detected in the vitreous fluid.

Safety Assessment of Intraocular Fluid Sampling During a month of continuous surveillance, none of the patients experienced any problems following the collection of intraocular fluid specimens, such as medical RD or intraocular septic infection.

Polymerase Chain Reaction Assay The nucleic acid amplification fluorescence detection kit (Sansure Biotech Inc., Hunan, China) was used to perform real-time quantitative polymerase chain reaction (PCR) according to the manufacturer's instructions. The viral loads of CMV-DNA, HSV 2-DNA, and EBV-DNA in aqueous humor, vitreous fluid, and peripheral blood were detected by SPLAN-96P fluorescence quantitative PCR (Sansure Biotech Inc., Hunan, China). Samples with CMV-DNA and HSV 2-DNA levels surpassing 500 copies/mL were classified as positive, whereas those below this threshold were categorized as negative. The EBV-DNA samples were classified as positive when the value was ≥400 copies/mL, and negative when the value was <400 copies/mL.

Statistical Analysis All the data in this study were analyzed using SPSS 23.0 software (IBM Corp., Armonk, NY, USA). Normally distributed data were expressed as mean±standard deviation (SD). The comparison of rates between two groups with a sample size of less than 40 was tested using Fisher's exact probability method. Non-normally distributed metric data were tested using the Kruskal-Wallis rank sum test and the Wilcoxon paired sign rank sum test. The non-normally distributed count data was compared using the Spearman rank correlation coefficient. A statistically significant difference was indicated by $P<0.05$.

RESULTS

Patient Information In this study, 17 patients with HIV infection and intraocular diseases were included after strict screening. The CMVR group consisted of 10 patients with fundus inflammation, totaling 20 eyes. HIV-related CMVR fundus lesions were observed in 18 eyes during ophthalmoscopy. The average age of the 6 male and 4 female members of the CMVR group was 40.09±12.64y. Of them, nine patients were sampled peripheral blood before receiving systemic antiviral therapy for CMV, and one case was sampled following 3d of systemic antiviral therapy. Nine eyes from seven patients with concurrent cataracts made up the non-CMVR group. Patients with lesions HIV-related fundus lesions were eliminated after a thorough examination. With an average age of 68.71±16.49y, the non-CMVR group included 7 males. All patient imaging data, with the exception of one case with obvious radiological characteristics of pulmonary tuberculosis, exhibited no typical radiological manifestations of viral pneumonia after consultation with experts in radiology and respiratory care.

CMV-DNA Load and $CD4^+$ T Cell Count No viral copy was detected in the non-CMVR group (7 patients, 9 eyes) in aqueous humor and vitreous fluid. The results of the CMV-DNA load and $CD4^+$ T cell count in the aqueous humor and vitreous fluids, as well as the peripheral blood of patients in the CMVR group, were shown in Tables 1 and 2.

Nineteen of the ten CMVR patients who were HIV-positive had fundus CMVR abnormalities. In case 2, the patient declined vitreous fluid sampling in the right eye despite showing CMVR changes in both eyes. Case 6 only exhibited CMVR changes in the right eye. Consequently, vitreous fluid samples were taken for this study from 18 eyes in the CMVR group. CMV was detected in the aqueous humor and vitreous fluid of 15 eyes, with a positive rate of 83.33% (15/18). Only 3 cases had CMV-DNA detected in peripheral blood, with a positive rate of 30.00% (3/10). Even in patients with positive CMV-DNA in peripheral blood, the CMV-DNA load in the aqueous humor and vitreous fluid was much higher than that in peripheral blood. There was a statistically significant difference in the CMV-DNA load in the aqueous humor fluid, vitreous fluid, and peripheral blood serum ($P<0.01$). The CMV-DNA

Table 1 CMV-DNA load and CD4$^+$ T cell count in the CMVR group

Case	Left eye (copy/mL)		Right eye (copy/mL)		Peripheral blood (copy/mL)	CD4$^+$ T cell count (cells/μL)
	Aqueous humor fluid	Vitreous fluid	Aqueous humor fluid	Vitreous fluid		
1	5.10×10^6	8.60×10^7	1.10×10^6	7.00×10^6	7.80×10^5	3
2	8600	1.80×10^7	No detection	No detection	<500[a]	5
3	510	3100	610	3400	<500[a]	15
4	4.20×10^7	1.10×10^8	2.82×10^6	4.30×10^8	<500[a]	17
5	1.60×10^5	1.14×10^6	<500[a]	<500[a]	1.40×10^4	25
6	No detection	No detection	6.20×10^6	6.90×10^7	9000	25
7	3.30×10^4	1.60×10^7	2.00×10^6	3.50×10^7	<500[a]	28
8	<500[a]	<500[a]	<500[a]	9300	<500[a]	50
9	<500[a]	<500[a]	3.50×10^4	7.00×10^4	<500[a]	52
10	2.00×10^4	7.29×10^5	1.55×10^5	5.06×10^6	<500[a]	1244

[a]The detection instrument used had a sensitivity greater than or equal to 500; if it was lower than 500, all statistical data processing was calculated as 499. CMV: Cytomegalovirus; CMVR: Cytomegalovirus retinitis.

Table 2 Comparison of CMV-DNA load between intraocular fluid and peripheral blood in the CMVR group copy/mL

Case	Aqueous humor fluid	Vitreous fluid	Peripheral blood	χ^2	P^a
1	3.10×10^6	4.65×10^7	7.80×10^5	10.40	0.006
2	8600	1.80×10^7	<500		
3	560	3250	<500		
4	2.24×10^7	2.70×10^7	<500		
5	8.02×10^4	5.70×10^5	1.40×10^4		
6	6.20×10^6	6.90×10^7	9000		
7	1.02×10^6	2.55×10^7	<500		
8	499	4899.5	<500		
9	1.77×10^4	3.52×10^4	<500		
10	8.75×10^4	2.89×10^6	<500		

When testing for CMV-DNA, the average of both eyes is calculated if both eyes were sampled. However, if only one eye was sampled, the viral load of the aqueous humor fluid or vitreous humor was calculated, and the arithmetic mean was not calculated. The detection instrument used had a sensitivity greater than or equal to 500; if it was lower than 500, all statistical data processing was calculated as 499. [a]The Kruskal-Wallis rank sum test was performed to compare the viral load of CMV-DNA in the aqueous humor fluid, vitreous humor, and peripheral blood serum.

load in the aqueous humor fluid (higher eye) of all 17 patients (27 eyes) was negatively correlated with the peripheral blood CD4$^+$ T cell count (*rs*=-0.646, *P*=0.005). However, the two had no linear regression relationship (*F*=0.932, *P*=0.350). The CMV-DNA load in the peripheral blood serum of the 17 patients was negatively correlated with the CD4$^+$ T cell count (*rs*=-0.487, *P*=0.048), but, the two groups had no linear regression relationship (*F*=0.597, *P*=0.452). There was no correlation between the CMV-DNA load in the vitreous fluid (higher eye) of the CMVR group patients (10 cases, 18 eyes) and the peripheral blood CD4$^+$ T cell count (*rs*=-0.128, *P*=0.724). In the CMVR group, which consisted of 10 cases and 18 eyes, there was a positive correlation between the copy number of CMV-DNA in the aqueous humor fluid and the vitreous fluid (*rs*=0.899, *P*<0.05). However, there was no linear regression relationship between the two (*F*=1.030, *P*=0.325).

Comparison of Fundus Involvement and CMV Infection in Left and Right Eyes in CMVR Group A total of 20 eyes from 10 patients with HIV infection were investigated. Among these, CMVR foci were found in the fundus of 19 eyes, of which 10 constituted the right eyes (constitutive ratio=52.63%, prevalence rate=50.0%) and 9 constituted the left eyes (constitutive ratio=47.37%, prevalence rate=45.0%). There was no statistically significant difference in the prevalence of fundus involvement between the left and right eyes (*P*=1.00). Of the 18 eyes from which intraocular fluid was collected, 15 were infected with CMV. Among these, seven were the left eyes (constitutive ratio=46.67%, prevalence rate=35.0%), and 8 were the right eyes (constitutive ratio=53.33%, prevalence=40.0%). The difference in the prevalence of CMV infection between the left and right eyes was not statistically significant (*P*=1.00). There were 7:3 as many binocular patients as monocular ones based on clinical presentation. The difference in CMV-DNA load (absolute value) between the two eyes was significantly different in the majority of patients with HIV-combined CMVR. For CMV-DNA in aqueous humor fluid specimens, 50% (4/8) of patients had a difference of 10^5 or more (absolute value) between the high and low-load eyes. For CMV-DNA in vitreous fluid specimens, 75% (6/8) of patients had a difference of 10^5 or more (absolute value) between high and low-load eyes. The detailed information was summarized in Tables 3 and 4.

There was a significant difference in the amount of CMV-DNA present in the aqueous humor and vitreous of eyes with high CMV-DNA load compared to those with low CMV-DNA

Table 3 CMV-DNA load in the aqueous humor fluid between the eyes in the same patient

Case	High CMV-DNA load (H)	Low CMV-DNA load (L)	H/L ratio	Absolute difference
1	5.10×10^6	1.10×10^6	4.64	4.00×10^6
2	8600	No detection	—	—
3	610	510	1.20	100
4	4.20×10^7	2.82×10^6	14.89	3.92×10^7
5	1.60×10^5	499[a]	320.64	1.60×10^5
6	6.20×10^6	No detection	—	—
7	2.00×10^6	3.30×10^4	60.61	1.97×10^6
8	499[a]	499[a]	1	0
9	3.50×10^4	499[a]	70.14	3.45×10^4
10	1.55×10^5	2.00×10^4	7.75	1.30×10^4
Z	-2.366			
P[b]	0.018			

[a]The detection instrument used had a sensitivity greater than or equal to 500; if it was lower than 500, all statistical data processing was calculated as 499. Absolute difference was the difference between high and low CMV-DNA loads in the same eye. [b]Wilcoxon paired sign rank sum test was performed between eyes with high and low CMV-DNA loads. CMV: Cytomegalovirus; H/L: High/low.

Table 4 CMV-DNA load in the vitreous fluid between the eyes in the same patient

Case	High CMV-DNA load (H)	Low CMV-DNA load (L)	H/L ratio	Absolute difference
1	8.60×10^7	7.00×10^6	12.29	7.90×10^7
2	1.80×10^7	No detection	—	—
3	3400	3100	1.10	300
4	4.30×10^8	1.10×10^8	3.31	3.20×10^8
5	1.14×10^6	499[a]	2284.56	1.14×10^6
6	6.90×10^7	No detection	—	—
7	3.50×10^7	1.60×10^7	2.19	1.90×10^7
8	9300	499[a]	18.64	8801
9	7.00×10^4	499[a]	140.28	6.95×10^4
10	5.06×10^6	7.29×10^5	6.94	4.33×10^6
Z	-2.521			
P[b]	0.012			

[a]The detection instrument used had a sensitivity greater than or equal to 500; if it was lower than 500, all statistical data processing was calculated as 499. Absolute difference was the difference between high and low CMV-DNA loads in the same eye. [b]Wilcoxon paired sign rank sum test was performed between eyes with high and low CMV-DNA loads. CMV: Cytomegalovirus; H/L: High/low.

load (*P*=0.018, 0.012, respectively). Nonetheless, there was no significant difference in the quantity of CMV-DNA found in the vitreous fluids (*P*=0.327) or aqueous humor (*P*=0.612) between the left and right eyes.

Peripheral Blood CMV-IgM in CMVR Patients Out of the ten patients in the CMVR group, peripheral blood CMV-IgM testing was conducted on seven patients. The remaining three patients were not tested due to the temporary unavailability of relevant kits in the First Affiliated Hospital of Guangxi Medical University. Five of the seven patients tested were negative for CMV-IgM (71.43%), while two tested positive (28.57%).

Table 5 Peripheral blood $CD4^+$ T cell count in CMVR and non-CMVR groups cells/μL

Case	CMVR	Non-CMVR
1	3	47
2	5	377
3	15	485
4	17	538
5	25	1099
6	25	1299
7	28	1493
8	50	
9	52	
10	1244	

CMVR: Cytomegalovirus retinitis.

Table 6 Comparison of peripheral blood $CD4^+$ T cell count between CMVR and non-CMVR groups mean±SD

Parameters	Number of cases	$CD4^+$ T cell count (cells/μL)
CMVR	10	146.40±385.58[a]
Non-CMVR	7	756.57±535.83

[a]The Levene's test of Chi-square showed that the two samples were not significantly different (*P*=0.096), while the *t*-test indicated a significant difference between the two groups (*t*=-2.767, *P*=0.014). CMVR: Cytomegalovirus retinitis.

Correlation Between Peripheral Blood $CD4^+$ T Cell Count and CMVR in HIV Patients The results of the patients' peripheral blood $CD4^+$ T cell count in the CMVR and non-CMVR groups were shown in Tables 5 and 6.

As shown in Tables 5 and 6, 80% of the patients (8/10) in the CMVR group had $CD4^+$ T cell count of 50 cells/μL or less. Additionally, 90% of the patients in the same group had a peripheral blood $CD4^+$ T cell count of less than 100 cells/μL. On the other hand, most patients in the non-CMVR group (6/7, 85.71%) had peripheral blood $CD4^+$ T cell counts greater than 300 cells/μL. The difference between the two groups was statistically significant.

Pathogenetic Distribution The PCR analysis was used to detect the DNA viral load of CMV, HSV-2, and EBV. ELISA assay detected *T. gondii* IgM and HSV 2-IgM (Table 7). The results indicated that no viral copies were found in the aqueous humor of the eyes of 7 cases and 9 eyes in the non-CMVR group. Among the intraocular fluids of the eyes of the 10 cases in the CMVR group, 15 eyes were found to be CMV-DNA-positive (15/18, 94.44%), while 3 eyes were CMV-DNA-negative (3/18, 5.56%). All other pathogen antibody results were negative.

DISCUSSION

CMVR is the most common ocular complication in HIV-infected patients, and as the disease continues to worsen,

Table 7 Pathogens in intraocular fluid and peripheral blood of patients in the CMVR group

Pathogenic DNA/IgM antibody	Positive cases (intraocular fluid)	Positive cases (peripheral blood)
CMV-DNA	15 (15/18, 83.33%)	3 (3/10, 30%)
HSV 2-DNA	0	0
EBV-DNA	0	0
TOX-IgM	0	0
HSV 2-IgM	0	0

CMVR: Cytomegalovirus retinitis; CMV: Cytomegalovirus; HSV: Herpes simplex virus; EBV: Epstein-Barr virus; TOX: *Toxoplasma gondii*; IgM: Immunoglobulin M.

extensive retinal necrosis and RD eventually occur. Many patients present to the clinic with extensive involvement of the fundus tissue, making the treatment of the disease extremely challenging. However, the mechanism of CMV infection in the eye is not yet fully understood. It has been reported that CMV mainly damages the endothelial cells of the blood vessels in the eye, which in turn damages the retinal pigment epithelium, creating opportunities for the virus to penetrate the retinal tissue and eventually lead to retinal necrosis[11]. Due to the functional and structural damage of the retina in HIV-infected patients, other retinal tissues are affected along with the blood circulation to form CMVR[10]. However, the current theoretical basis for the mechanism of CMV invasion into the eye is often based on murine CMV-infected murine models, necropsies, and *in vitro* studies. Therefore, in this study, the source of pathogens of intraocular infection was investigated through laboratory examination of intraocular fluid and peripheral blood. At the same time, pathogens in the blood and vitreous body were further detected to determine whether HIV-infected patients had multiple pathogens in the eye, which provided a scientific basis for in-depth understanding of the pathogenesis of CMVR and timely and effective prevention and treatment of CMVR.

$CD4^+$ T cell counts are known to be an important reference for assessing T-lymphocyte function and predicting the progression of HIV infection[18]. Decreased $CD4^+$ T cell count is a significant risk factor for the development of opportunistic diseases such as CMVR[19]. Our study found that HIV-infected patients with low $CD4^+$ T cell counts are at high risk of developing CMVR, an opportunistic infectious ocular disease. The CMV-DNA viral load in the aqueous humor fluid and peripheral blood of patients increased significantly with decreasing peripheral blood $CD4^+$ T cell counts. In the CMVR group, 8 out of 10 patients had $CD4^+$ T cell counts of ≤50 cells/μL, which is consistent with the findings of Tang *et al*[20], that people with $CD4^+$ T cell counts <50 cells/μL are at higher risk of developing CMVR. Additionally, CMVR is gradual and is only diagnosed when a patient complains of eye symptoms followed by an ophthalmic evaluation, too late for optimal treatment outcomes. Therefore, it is essential for patients with low $CD4^+$ T cells, especially less than 200 cells/μL, to receive regular detailed fundus examinations[17], which will help ensure timely diagnosis and treatment and prevent a poor prognosis of visual function. However, in the case report of Keat *et al*[21], an HIV patient who developed CMVR despite a sustained $CD4^+$ T cell count of >200 cells/μL was documented, suggesting that even HIV-infected patients with high $CD4^+$ T cell counts are still susceptible to CMVR. This phenomenon was also observed in this study, one of the participating patients with bilateral CMVR had a $CD4^+$ T cell count of 1244 cells/μL. The patient confirmed that they had not taken any immunizing agents, glucocorticoids, or other medications. This suggests that although the $CD4^+$ T cell count is essential in assessing the risk of developing CMVR, it still has limitations and may need to be supplemented by other tests.

This study found that compared to peripheral blood samples, CMV-DNA copies were significantly higher in atrial or vitreous fluid samples from patients with CMVR. In addition, the study also found that 70% of patients had no detectable CMV-DNA in their peripheral blood in the CMVR group, which was consistent with the results of Mao *et al*[22]. This suggests that detecting CMV-DNA viral load in the blood has limited value in diagnosing CMVR and should be evaluated with other tests. When HIV-infected patients show signs of fundus lesions, it is crucial to consider the possibility of CMVR, even if CMV-DNA is not detected in their peripheral blood. As in our study, only 2 out of 7 patients (28.57%) in the CMVR group tested positive for peripheral blood CMV-IgM, indicating that about two-thirds did not have recent CMV bloodstream infections. However, their intraocular fluids had very high viral loads of CMV-DNA, while peripheral serum CMV-DNA was undetectable. This suggests that the onset of CMVR in this group of patients cannot be fully explained by the theory that CMV is latent in other body parts and then transfers into the eye with viremia. Therefore, we have reason to suspect that CMV in the intraocular fluid of CMVR patients does not originate exclusively from the bloodstream. Studies have shown that lymphatic vessels exit in the eyes and may be involved in overall aqueous humor drainage and intraocular pressure regulation[23-24]. Koina *et al*[25] found that lymphatic vessel endothelial hyaluronan receptor-1 ($LYVE\text{-}1^+$) macrophages were lined up in $CD34^-$ lumens in the developing choroid, suggesting that $LYVE\text{-}1^+$ macrophages may be involved in choroid hyaluronan metabolism or contribute to the temporary formation of lymphatic vessels under inflammatory conditions. CMV was also found in lymph nodes[26]. All of these findings suggest that the lymphatic system is one way the virus can reach the eye. In addition, Voigt *et al*[27] pointed out that corneal

tissue cultures of mice with CMV infection showed signs of mouse cytomegalovirus (MCMV) reactivation, suggesting that corneal tissue may be a target tissue for CMV and a potential location of CMV latency. Shimizu *et al*[28] also found that CMV can replicate in trabecular meshwork cells, which are located at the junction of corneal and scleral tissues and communicate with the intraocular lumen, suggesting that the trabecular meshwork may be a potential site for the entry of CMV into the eye. It can be seen that in addition to the intraocular route involving the bloodstream, CMV has other ways to enter the eye, such as the lymphatic system.

In the present study, it was found that there was a significant difference in viral load in the intraocular fluid between the eyes of those with CMVR in both eyes. The difference between eyes with high and low CMV-DNA loads in the aqueous humor and vitreous fluids in the CMVR group was 10^5 or more in 50% (4/8) and 75% (6/8) of the patients, respectively. Meanwhile, three patients had CMVR fundus lesions in both eyes, but CMV-DNA viral load was detected in the intraocular fluid in only one eye and not in the other eye. This reflects the asymmetry of CMV invasion in both eyes, suggesting that ophthalmologists should not diagnose CMVR in one eye alone, but in combination with other methods to rule out interference from other diseases, such as immune reconstitution uveitis, HIV retinopathy. Some studies have showed that 20% of individuals with CMVR initially only have problems with one eye, and within six months, the second eye also develops CMVR, which they believe is caused by the virus spreading from the affected eye to the contralateral eye through blood flow[29-30]. Therefore, the uneven onset of CMVR in both eyes or the degree of BRB disruption in both eyes could also be contributing factors to the variation in CMV-DNA virus loads in the intraocular fluid of both eyes. Xu *et al*[31] also found that the viral load of CMV-DNA in aqueous humor of CMVR patients was positively correlated with the size of CMVR lesions, that is, when the lesions of CMVR were relatively mild, it was difficult or even impossible to release the virus from the lesions. In addition, the significant difference in loads between the two eyes also suggests that the two eyes may have different sources of CMV. For example, Smith *et al*[32] reported a case of acute retinal necrosis in the other eye of a patient suffering from herpesvirus keratitis in one eye, and PCR confirmed the presence of HSV in the vitreous fluid of the necrotic eye. This indicates that CMV is also neurophilic and corneal invasive, and its pathogenesis may be similar to acute retinal necrosis derived from bilateral HSV[33], with fundus lesions in the contralateral eye forming CMVR after monocular infection.

In this investigation, CMV-DNA levels in the vitreous of individuals with CMVR were higher than those in the aqueous humor of the same eye. This may be the result of the CMV virus having its original source in the vitreous fluid and ending up in the aqueous humor. Due to the vitreous body's proximity to retinopathy, after CMV infection occurs in the retina, the virus can enter the vitreous body and move from the posterior segment of the eye to the anterior segment. However, the atrial water had a higher kinetic mobility than the vitreous fluid, making it relatively difficult for CMV to proliferate and accumulate in the atrial water. Moreover, the immune microenvironment in the aqueous humor and vitreous cavity is different[34], which may provide two conditions for CMV proliferation in the eye. Nevertheless, it cannot be excluded that the difference in viral load between the anterior and posterior segments of the eye is related to the differences in CMV permeability to the blood-atrial fluid and BRB. At present, most studies on HIV-related blood-ocular barriers focus on BRB[35-36], while there are few domestic and foreign studies on whether HIV and CMV infection can affect the blood-atrial water barrier.

HSV-2, EBV, and CMV belong to the Herpesviridae family and can cause intraocular infections[37-38]. *T. gondii* is the second most common pathogen after CMV and can cause opportunistic ocular infections in HIV-infected individuals[39]. The presence of these pathogens in the body increases the risk of opportunistic infections in HIV-infected patients. HIV-infected patients with significantly compromised immune function may also be at risk of simultaneous intraocular infection by these pathogenic microorganisms. In this study, multiple intraocular infections in HIV-infected patients were analyzed through laboratory examination of intraocular fluid and peripheral blood. The results showed that CMV, HSV-2, and EBV were not found in aqueous humor fluid and peripheral blood of patients in the non-CMVR group, and *T. gondii* IgM was negative, which was consistent with the findings of Keorochana *et al*[40]. In their reports, the PCR characterization of aqueous humor fluid was 100% negative in 58 cataract patients with no immunodeficient status. Additionally, our study also pointed out that the intraocular fluid of the CMVR group had no HSV-2 and EBV viruses except CMV, and HSV-IgM and *T. gondii* IgM were negative. Given that occurs primarily in the early stages of infection, as HIV disease worsens, the patient's cellular immunity may also be compromised, making it difficult, if not impossible, to generate antibodies against HSV-2 and *T. gondii*[41]. Coupled with the specific immune amnesty state of the vitreous cavity and subretinal space, an immune response is unlikely to be induced even in the presence of intense stimulation of the posterior segment of the eye by foreign antigens. This means that detection of pathogen IgM in vitreous fluid and peripheral blood cannot accurately reflect the status of intraocular infection in HIV patients[42].

According to Gangaputra *et al*[43], intraocular opportunistic infections caused by non-CMV pathogens are quite rare in HIV-positive individuals—approximately one-tenth of CMVR. Therefore, in order to assess the intraocular infection of HSV-2 and *T. gondii* in detail, it is necessary to detect the IgG of the corresponding pathogens in the intraocular fluid and peripheral blood, and to calculate the Goldman-Witmer coefficients for supplementation[42].

Our study still has some important limitations. First, this study found that people with HIV rarely develop intraocular multiple pathogen infections, but because there are so few sample cases, it is currently impossible to determine with accuracy if intraocular multiple pathogen infections are present in HIV-positive patients. Second, the patients involved in the study were from a single center, and the results were not representative. Additionally, prospective study design increases the likelihood of selection bias, and missing patient information often limits the analysis. Therefore, in the future, it is necessary to carry out multi-center clinical epidemiological studies with larger sample size and more sample data, and conduct more in-depth statistical analysis of similar laboratory indicators in order to make exactly our findings.

In conclusion, patients with HIV-associated CMVR may have unknown routes of infection in addition to the traditional route, where CMV in the bloodstream enters the eye through the compromised BRB. The significant differences in CMV-DNA loads between the eyes of the majority of the CMVR group of patients suggest that there may be a different source of CMV in the eyes of these patients. When peripheral blood $CD4^+$ T cell counts in HIV patients were less than 100 cells/μL, the incidence of concurrent CMVR rose considerably, and there was a negative correlation seen between peripheral blood $CD4^+$ T cell counts and intraocular CMV virus levels. Intraocular multiple pathogen infections were rare among HIV patients. These findings can help establish a theoretical framework to improve clinicians' in-depth understanding of the pathophysiology and occurrence and development of CMVR, determine the severity and prognosis of patients as soon as possible, timely adjust the treatment plan, and cooperate in diagnosis and treatment, so as to effectively reduce the probability of ocular complications while maintaining low morbidity and mortality.

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