Real-time polymerase chain reaction for diagnosis and management of HIV-induced uveitis

Jayapratha R Selvaraj, S Sudharshan, Lily K Therese[1], M K Janani[1], Poongulali Selvamuthu[2], Parveen Rewri[3], Jyotirmay Biswas

Intraocular (IO) inflammation in patients with Human immune deficiency virus (HIV) infection can be due to opportunistic infections, immune recovery uveitis, drugs used in the management or a primary manifestation of HIV itself. We studied the role of RT-PCR for HIV RNA in confirming the diagnosis of HIV induced uveitis and its useful in the management and follow-up of these patients.

Keywords: HIV-induced uveitis, real-time polymerase chain reaction

Intraocular inflammation in patients with human immune deficiency virus (HIV) infection can be due to opportunistic infections, drugs used in the treatment, immune recovery uveitis (IRU), or a primary manifestation of the HIV infection itself. After the introduction of highly active antiretroviral therapy (HAART), while the prevalence of opportunistic infections has decreased, the incidence of IRU, ocular toxic, and allergic reactions have increased. HIV itself can be a cause of intraocular inflammation and there have been very few reports in the literature.[1,2] However, the course of the disease and follow-up findings of the same patients is limited. We studied the role of RT-polymerase chain reaction (PCR) for HIV RNA in confirming the diagnosis of HIV-induced uveitis and its usefulness in the management and follow-up of these patients.

Case Reports

Three patients with age range 21–36 years, HIV disease duration – 1 day–10 years, and CD4 counts 138–412 cells/microlitre and with intraocular inflammation were included.

Access this article online

Quick Response Code:

Website: www.ijo.in

DOI: 10.4103/ijo.IJO_509_18

Department of Uveitis and intra-ocular inflammation, Medical Research Foundation, Sankara Netharalaya, [1]Department of Microbiology Vision Research Foundation, Sankara Netharalaya, [2]YRG Care Hospital, Chennai, Tamil Nadu, [3]Maharaja Agrasen Medical College, Hisar, Haryana, India

Correspondence to: Dr. S Sudharshan, Medical Research Foundation, Sankara Netharalya, 18, College Road, Nungambakkam, Chennai - 600 006, Tamil Nadu, India. E-mail: drdharshan@gmail.com

Manuscript received: 13.04.18; Revision accepted: 08.08.18

Investigations to rule out collagen vascular diseases, autoimmune diseases, and other underlying infective etiology including rheumatoid factor, antinuclear antibody, serum angiotensin converting enzyme, rapid plasma regain (RPR)/treponema pallidum hemagglutination assay (TPHA) tests, enzyme linked immune sorbent assay (ELISA) for toxoplasma, quantiferon tuberculosis gold test, chest radiograph, and mantoux test were done in all patients. Aqueous aspirate was tested for real-time PCR for mycobacterium tuberculosis (MTB), cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), and toxoplasma. Pre- and posttreatment real-time PCR of HIV RNA was carried out using Artus kit (QIAGEN, Hilden Germany) using the Rotorgene Q (QIAGEN, Hiden, Germany) real-time PCR machine. The process included reverse transcription at 50°C for 30 min followed by 50 cycles of initial denaturation at 95°C for 15 min, annealing at 50°C for 60 s and extension at 72°C for 30 s. Pre- and posttreatment real-time PCR HIV loads were tested in all patients. Real-time PCR of HIV RNA from blood was done as part of their systemic workup under care of an AIDS care physician. All the patients were treated with HAART. Blood real-time PCR HIV load values were noted in all patients pre- and posttreatment.

Three patients (four eyes) were included in the study. Age range was 21–36 years. CD4 counts range was 138–412 cells/microlitre. In all patients, aqueous testing for RT PCR for HSV, VZV, CMV, MTB, and toxoplasma were negative. None of the patients had any systemic opportunistic infection. Blood RPR/TPHA, ELISA for toxoplasmosis, and tests for cryptococci were negative. Real-time PCR for HIV RNA was positive in three patients with a range of 121 to 1,64,773 copies/ml. Chest X-ray was normal.

Case 1

A 36-year-old male presented with history of 2 months of gradual diminution of vision in both eyes. On examination, his best-corrected visual acuity in the right eye was 6/6, N6 and in the left eye 6/18, N6. Slit lamp examination showed an anterior chamber reaction of 2 + with vitreous cells 2+. There were no peripheral synechiae. Media was hazy due to vitritis (2+). Disc evaluation was normal. There was no clinical evidence of any infective retinal or choroidal lesion in the posterior segment. Investigations to rule out other causes of uveitis and infective etiology were negative.

A possible diagnosis of HIV-induced uveitis was made based on the increased real-time-PCR HIV viral load, both in blood (261 million copies/ml) and aqueous (13,404 copies/ml). Combination antiretroviral therapy was started under care of an AIDS care physician. The patient was also treated with

Cite this article as: Selvaraj JR, Sudharshan S, Therese LK, Janani MK, Selvamuthu P, Rewri P, *et al*. Real-time polymerase chain reaction for diagnosis and management of HIV-induced uveitis. Indian J Ophthalmol 2018;66:1634-6.

a short tapering dose of topical steroids. At 8 weeks on ART, ocular inflammation had resolved and the aqueous samples were subjected for microbial evaluation, which showed no HIV RNA. Blood real-time PCR values for HIV had reduced to 54,000 copies/ml. At his last follow-up after 1 year, his CD4 counts were 350 cells/µl, with no inflammation and viral load in blood <150 copies.

Case 2

A 21-year-old male presented with painless progressive diminution of vision in left eye for 1 year. He was a known case of HIV detected 1 month back, but not on HAART with CD4 counts of 412/µl. On examination, his best-corrected visual acuity was 6/9, N6 in right eye and 6/12, N6 in left eye. Slit lamp examination showed anterior chamber reaction of 1 + and posterior subcapsular cataract in left eye. The media was hazy due to 1 + vitritis. There was no peripheral synechiae. Fundus examination revealed a normal disk with no evidence of any active retinitis or infective lesion. Slit lamp and fundus examination of right eye were normal. RT-PCR for HIV RNA in aqueous aspirate was 164,773 copies/ml and in the blood sample was 150,729 copies/ml of HIV RNA [Fig. 1]. Considering a possible diagnosis of HIV induced uveitis, combination of anti retro viral therapy (tenofovir+ lamivudine+ efavirenz) , under care of AIDS care physician along with a short course of steroids was started. At 4 months follow-up visit, the patient was asymptomatic with best-corrected visual acuity of 6/7.5, N6 in right eye and 6/9, N6 in left eye with no intraocular inflammation. At 1 year follow-up, blood viral load had reduced to 29,805 copies/ml [Fig. 2] and CD4 counts of 223/µl.

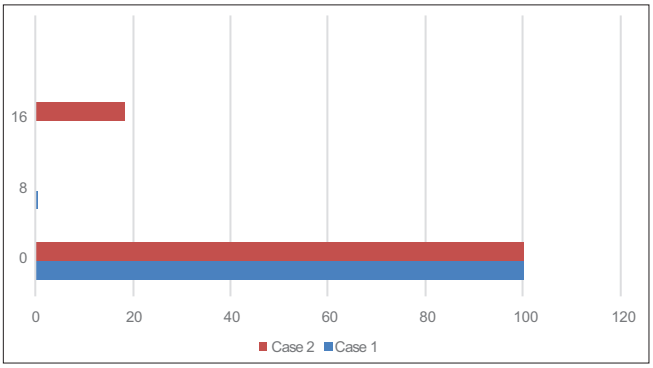
Case 3

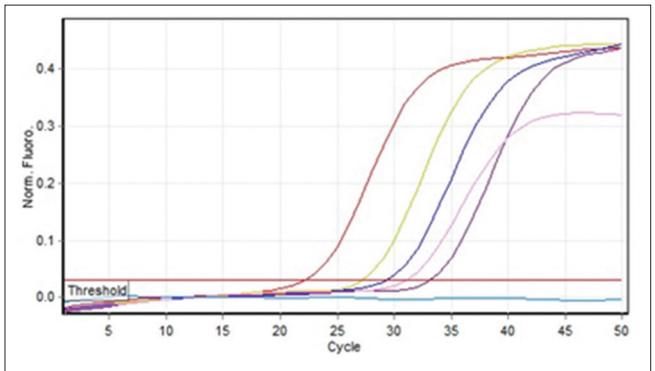
A 29-year-old male, known hemophiliac came with complaints of blurring of vision in both eyes since the past 6 months. His best-corrected visual acuity was 6/7.5, N6 in right eye and 6/6, N6 in left eye. Slit lamp examination showed an anterior chamber reaction of 1 + cells in both eyes. Fundus examination in both eyes was within normal limits. His CD4 counts were 138 µl. Real-time PCR for HIV RNA from the aqueous detected 162 copies/ml and 121 copies/ml in right and left eyes, respectively, and correspondingly in the blood was 29,185 copies/ml. The patient was started on a short course of topical steroids and HAART was continued. At 3 months follow-up visit, he was asymptomatic with fully controlled inflammation and undetectable aqueous viral loads. His best-corrected visual acuity improved to 6/6, N6 in both eyes in both eyes. His CD4 counts were 250/µl and he had no relapses till his last follow-up visit of 1 year with undetectable peripheral blood viral loads of < 150.

Discussion

Opportunistic infections like tuberculosis, CMV, toxoplasmosis, and syphilis are the most common cause of intraocular inflammation in HIV patients. In a large study on ocular lesions in patients with HIV/AIDS of 1000 cases from India from our center, CMV retinitis was the most common ophthalmic opportunistic infection and tuberculosis was the most common systemic opportunistic infection.[3] In our series, none of the

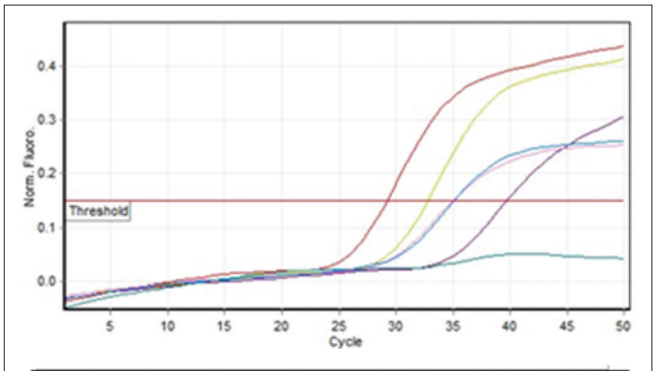
Table 1: Chart showing reduction in the human immune deficiency virus RNA load in the follow-up period





No.	Colour	Name	Type	Ct	Given Conc (IU/ml)	Calc Conc (IU/ml)	% Var
1		S1	Standard	22.18	7142857	10201111	42.8%
2		S2	Standard	27.23	714285	369260	48.3%
3		S3	Standard	29.35	71428	91753	28.5%
4		S4	Standard	33.15	7142	7531	5.4%
5		SN NO 3805/16	Unknown	31.06		29805	
6		NC	NTC				

Figure 1: Real-time PCR of HIV-1 from the aqueous aspirate in the right eye



No.	Colour	Name	Type	Ct	Given Conc (IU/ml)	Calc Conc (IU/ml)	% Var
1		S1	Standard	29.26	7,142,857	7,301,520	2.2%
2		S2	Standard	32.82	714,285	691,130	3.2%
4		S4	Standard	39.71	7,142	7,221	1.1%
5		VRF 2798/13	Unknown	34.98		164,773	
6		VRF 2801/13	Unknown	35.12		150,729	
7		NC	NTC				

Figure 2: Real-time PCR of HIV-1 from the aqueous aspirate in the left eye

patients had any systemic or ocular opportunistic infection at the time of presentation with intraocular inflammation.

HIV patients newly started on HAART can often develop inflammation after an attack of opportunistic infections, especially tuberculosis considering its endemicity in India. This is because of the increase in T-lymphocytes which in turn attack the previously acquired antigens, resulting in an increased inflammatory mediator release. This immune recovery inflammatory syndrome in the eye is IRU. Monitoring the CD4 count and HIV loads in blood and aqueous before and after initiation of HAART is necessary in differentiating the reason for the inflammation. Increase in inflammation while on HAART, with increase in CD4 counts and a corresponding decrease in viral loads, favors the diagnosis of IRU, whereas HIV-induced uveitis is associated with decrease in inflammation after HAART initiation. In our series, patients presented with inflammation despite low CD4 counts and with high HIV loads both in blood and in aqueous. IRU as a possibility was thus excluded.

There have been various hypotheses for HIV itself causing uveitis. HIV replicates in CD4 lymphocytes. Intrathecal persistence of HIV in spite of HAART and reduced plasma viral loads have been demonstrated,[4-7] due to compartmentalization and replication of HIV in central nervous system.

HIV-induced uveitis, although a diagnosis of exclusion, can be confirmed with the help of PCR which is an important tool to detect infective etiology in uveitis. Confirmation of the same could be done with real-time PCR techniques which can quantify the same and correlates prognosis. Harper *et al*. showed a 61% true positivity and 25% true negativity in demonstrating infectious agents in aqueous samples by PCR.[8] Sudharshan *et al*. reported 4% prevalence of anterior uveitis in an AIDS population.[3] It has been demonstrated that HIV-induced uveitis was observed in 6 out of 56 HIV patients who presented with uveitis.[9] PCR testing of aqueous aspirate has reportedly altered the treatment plan in posterior uveitis.[8,10] HIV infection in the eye and positive HIV RNA in intraocular fluids are associated with high-plasma HIV RNA loads.[11-14]

Our study has demonstrated by real-time PCR the corresponding decrease in HIV copies in aqueous and blood with resolution of inflammation, after initiation of HAART [Table 1]. There are very few reports on proven cases of HIV-induced uveitis based on PCR confirmation.[9] In HIV patients where a complete history and review of systems is inconclusive in establishing the cause for intraocular inflammation, a high intraocular fluid: plasma HIV RNA load ratio establishes the diagnosis of HIV-induced uveitis. Demonstration of a decreasing trend of the HIV load in aqueous tap may have prognostic value.

Conclusion

In contrast to IRU where the HAART increases the symptoms and flares up the inflammation, HAART brings down the inflammation in HIV-induced uveitis. RT PCR for HIV RNA in HIV-induced uveitis will not only serve as a diagnostic tool but can be a guide to treatment.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Rosberger DF, Heinemann MH, Friedberg DN, Holland GN. Uveitis associated with human immunodeficiency virus infection. Am J Ophthalmol 1998;125:301-5.
2. 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.
3. Sudharshan S, Kaleemunnisha S, Banu AA, Shrikrishna S, George AE, Babu BR, *et al.* Ocular lesions in 1,000 consecutive HIV-positive patients in India: A long-term study. J Ophthalmic Inflamm Infect 2013;3:2.
4. Persidsky Y, Poluektova L, Immune privilege and HIV -1 persistence in the CNS. Immunol Rev 26;213:180-94.
5. De Luca A, Ciancio BC, Larussa D, Murri R, Cingolani A, Rizzo MG, *et al.* Correlates of independent HIV-1 replication in the CNS and of its control by antiretrovirals. Neurology 2002;59:342-7.
6. García F, Niebla G, Romeu J, Vidal C, Plana M, Ortega M, *et al.* Cerebrospinal fluid HIV-1 RNA levels in asymptomatic patients with early stage chronic HIV-1 infection: Support for the hypothesis of local virus replication. AIDS 1999;13:1491-6.
7. Strain MC, Letendre S, Pillai SK, Russell T, Ignacio CC, Günthard HF, *et al.* Genetic composition of human immunodeficiency virus type 1 in cerebrospinal fluid and blood without treatment and during failing antiretroviral therapy. J Virol 2005;79:1772-88.
8. Harper TW, Miller D, Schiffman JC, Davis JL. Polymerase chain reaction analysis of aqueous and vitreous specimens in the diagnosis of posterior segment infectious uveitis. Am J Ophthalmol 2009;147:140-700.
9. Kunavisarut P, Sirirungsi W, Pathanapitoon K, Rothova A. Clinical manifestations of human immunodeficiency virus-induced uveitis. Ophthalmology 2012;119:1455-9.
10. Rothova A, de Boer JH, Ten Dam-van Loon NH, Postma G, de Visser L, Zuurveen SJ, *et al.* Usefulness of aqueous humor analysis for the diagnosis of posterior uveitis. Ophthalmology 2008;115:306-11.
11. 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.
12. Ciulla TA, Schnizlein-Bick CT, Danis RP, Frank MO, Wheat LJ. Comparison of intraocular to plasma HIV-1 viral burden in patients with cytomegalovirus retinitis. Am J Ophthalmol 1999;127:221-3.
13. Hsu WM, Chiou SH, Chen SS, Shyong MP, Ho CK, Chen SJ, *et al.* The HIV RNA levels of plasma and ocular fluids in AIDS patients with ophthalmic infections. Ophthalmologica 2004;218:328-32.
14. Biswas J, Sudharshan S. Anterior segment manifestations of human immunodeficiency virus/acquired immune deficiency syndrome. Indian J Ophthalmol 2008;56:363-75.