Special Focus, Retina, Original Article

Correlation of quantitative polymerase chain reaction with clinical characteristics of patients with viral retinitis

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Purpose: To evaluate the correlation of quantitative real-time polymerase chain reaction (qRT-PCR) to the clinical characteristics of patients with viral retinitis.**Methods:** Retrospective case series. **Results:** Aqueous or vitreous samples of 20 out of 35 eyes showed qRT-PCR positivity for virus etiology (57.14%). Cytomegalovirus (CMV) was most commonly identified in nine eyes (45%). The mean DNA copy number was 2,68,339.65 copies/mL (range: 90–3205397). DNA copy number significantly correlated with the extent of clinical involvement (P = 0.013); however, there was no correlation between DNA copy number and presenting visual acuity (P = 0.31), macular involvement (P = 0.675), optic nerve involvement (P = 0.14), and development of retinal detachment (P = 0.73). There was a significant correlation between the number of DNA copies and the timing of sampling (P = 0.0005). Samples taken earlier in the course of the disease had higher viral copies than later ones. **Conclusion:** qRT-PCR is useful in confirming a viral etiology in over 50% of cases of suspected viral retinitis. It correlates well with the extent of clinical involvement and timing of sampling.

Key words: Acute retinal necrosis, CMV retinitis, real-time quantitative polymerase chain reaction, viral retinitis

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Viral retinitis is caused primarily by the Herpes group of viruses such as herpes simplex virus 1 (HSV 1), herpes simplex virus 2 (HSV 2), varicella–zoster virus (VZV), and cytomegalovirus (CMV). The diagnosis of viral retinitis is usually clinical; however, polymerase chain reaction (PCR) serves as a useful adjunct to confirm the diagnosis. Advances in molecular biology have led to the development of quantitative real-time polymerase chain reaction (qRTPCR) testing, which is performed using fluorescent probes or DNA intercalating dyes that increases in fluorescence with an accumulation of double-stranded PCR product, and this is monitored by fiber-optic fluorimetry. The resulting curve of fluorescence accumulation is used to quantify the viral load in the sample. It is found to be valuable in cases of viral retinitis as it can identify as well as quantify the viral load by DNA copy numbers.[1]qRT-PCR can be useful in prognosticating the disease as well as monitoring response to treatment.

The purpose of our study was to evaluate the correlation of viral PCR DNA copy number in patients with viral retinitis to the presenting visual acuity as well as various clinical characteristics such as the extent of retinitis, macular involvement, optic nerve involvement, development of retinal detachment, and the timing of sampling.

Methods

A retrospective analysis of clinical records of all patients with a clinical diagnosis of suspected viral retinitis from 2017 to 2021 was done. Patient data including age, gender, past ocular, and systemic history were recorded. Ocular data included symptoms at first presentation, visual acuity, grades of anterior and posterior segment inflammation, intraocular pressure, slit-lamp, and fundus findings. Fundus findings included the extent of retinal involvement, optic nerve involvement, macular involvement, and the presence or absence of retinal detachment. The extent of retinal involvement was determined by the number of quadrants involved (each quadrant corresponding to the 3 clock h). Treatment history was also documented. The choice of treatment was based on the course of inflammation and the severity of the disease. Details of retinal detachment surgery, if performed, were also collected. Undiluted aqueous or vitreous samples of the patients were sent for quantitative, real-time PCR analysis for viral etiologies including HSV 1, HSV 2, VZV, and CMV. The lower limits of qRT-PCR with 95% confidence interval (CI) performed in our laboratory is 42.5 copies/mL for CMV, 50 copies/mL for HSV 1, and HSV 2, and 12.725 copies/mL for VZV. Artus kit was used for CMV and VZV and Genosen's kit for HSV. The human immunodeficiency virus was ruled out in all cases using the ELISA technique. The mean logarithm of the minimum angle of resolution (logMAR)

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visual acuity was used for statistical analysis. Spearman's correlation was used to determine if the DNA copy number using qRT-PCR analysis correlated to the extent of retinitis, macular involvement, optic nerve involvement, development of retinal detachment, and the timing of sampling. A *P* value of <0.05 was considered to be statistically significant.

Results

Thirty-five eyes of 35 patients were clinically diagnosed with viral retinitis. Aqueous or vitreous samples from these 35 eyes were sent for PCR analysis. Quantitative real-time PCR analysis for viral DNA was positive in 20 of these 35 eyes (57.14%). The clinical characteristics of these 20 patients are described. None of the patients had antiviral treatment before the presentation.

Baseline characteristics are noted in Table 1.

CMV was identified in nine eyes (45%). HSV 1 in four eyes (20%), HSV 2 in four eyes (20%) and VZV in three eyes (15%). The mean DNA copy number was 2,68,339.65 copies/mL (range: 90–3205397). Nine patients (45%) received a course of intravenous acyclovir. All 20 patients (100%) were treated with oral valacyclovir and oral steroids. Three eyes (15%) received intravitreal ganciclovir in addition.

Table 1: Baseline Characteristics

Characteristics	Value
Gender (*n*=20)	
Male	12
Female	8
Age (mean with range)	39.1 (Range 10-68) years
Time between symptoms to sampling (Mean with range)	22.65 (Range 3-60) days
Laterality (*n*=20)	
Unilateral	12
Bilateral	8
Ocular fluid samples (*n*=20)	
Aqueous	14
Vitreous	6

The mean logMAR visual acuity was 1.71 (±1.56) at presentation. There was no correlation between presenting visual acuity and DNA copy number ($P = 0.31$). Only 11 of the 20 patients had a follow-up of greater than 3 months and their final visual acuity was analyzed. The mean logMAR visual acuity of these 11 patients was 1.23 (±0.27) at presentation, which improved to 0.80 (±0.59) at the final visit ($P = 0.03$). There was also no correlation between the DNA copy number and the final visual acuity in these 11 patients ($P = 0.56$).

The percentage of eyes and the number of quadrants involved are shown in Fig. 1. There was a significant correlation between the extent of clinical involvement and PCR DNA copy number ($P = 0.013$). Macular involvement was noted in 13 of the 20 eyes (65%); however, this did not correlate with the PCR DNA copy number ($P = 0.675$). Optic nerve involvement was noted in 9 of the 20 eyes (45%); however, this also did not have any correlation to the PCR DNA copy number ($P = 0.14$).

Eight eyes (40%) developed retinal detachment, of which five underwent retinal detachment repair with silicone oil injection. There was no correlation between

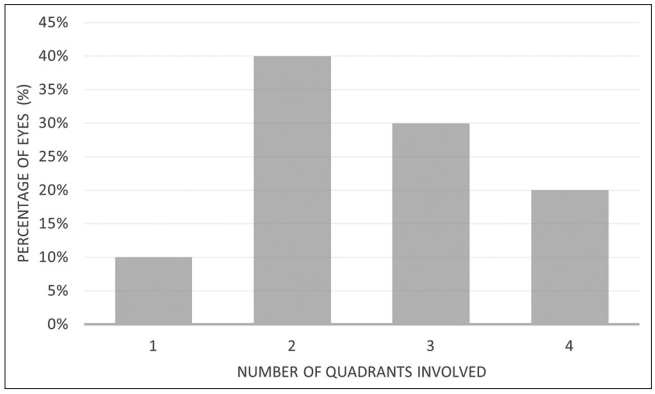

Figure 1: Percentage of eyes in number of quadrants involved

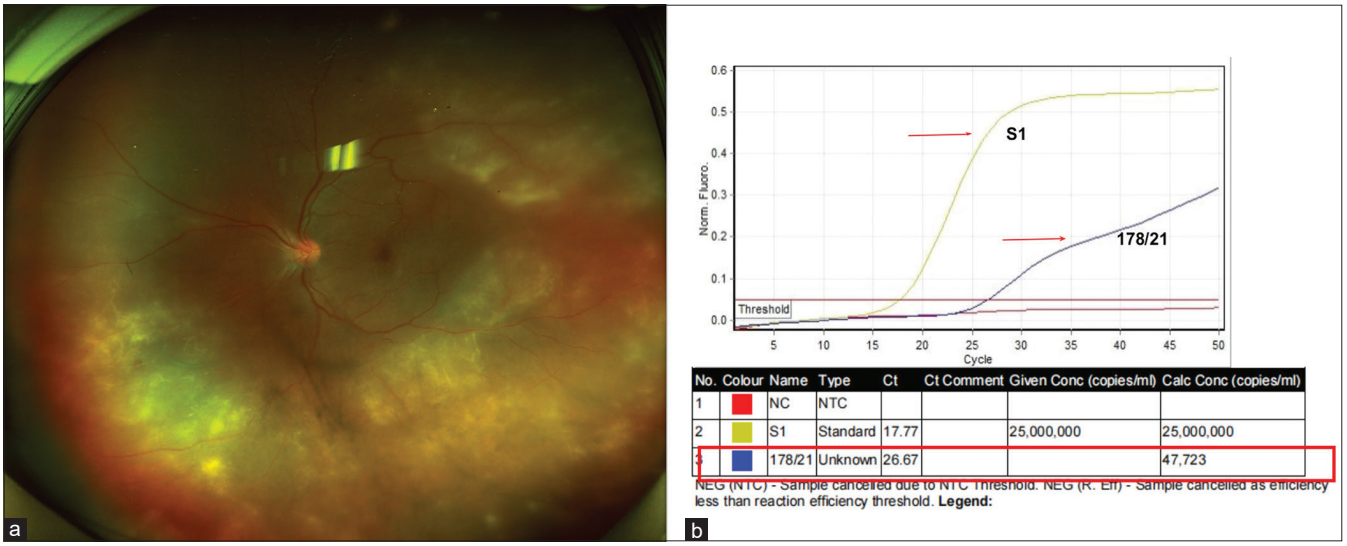

Figure 2: (a) Left eye of a patient showing acute retinal necrosis in three quadrants of the retina without macular or optic nerve involvement. (b) Real-time quantitative PCR of the aqueous sample of this patient identified HSV-1 virus with a DNA copy number of 47,723 copies/mL

the number of DNA copies and the development of retinal detachment ($P = 0.73$).

We also correlated the DNA PCR copy number to the timing of sampling, that is, the timing between symptoms to presentation when the sample was taken and found a significant correlation ($P = 0.0005$). Earlier sampling showed higher viral DNA copy numbers.

Discussion

Our study showed that the DNA copy number correlates to the extent of clinical involvement in cases of viral retinitis, and the timing of sampling plays an important role. PCR plays an adjunctive role in the diagnosis of viral retinitis. qRT-PCR is a more accurate method due to its ability to quantify the viral genome copies. In our study, CMV was most commonly detected in 45% of the eyes, which were PCR positive for viral etiology. This is in contrast to previous studies where VZV and HSV are more common; however, CMV-associated acute retinal necrosis has been reported previously.[2] Only one patient in our series was immunocompromised secondary to chemotherapy for acute lymphoblastic leukemia. qRT-PCR was able to confirm viral etiology in 57% of the suspected viral retinitis cases. According to a study by Santos *et al*.[3] vitreous humor is a better source for molecular diagnosis of posterior infectious uveitis compared to aqueous humor. A majority of samples in our study were from aqueous humor due to ease of collection, and hence, viral detection could have been missed in some cases.

Abe *et al*.[4] first reported in 1998 that the number of VZV copy numbers using conventional, semi-nested PCR in 12 eyes of 11 patients correlated to the final visual acuity. A few case reports have found that serial qRT-PCR DNA copy numbers can be useful to monitor a patient's response to treatment. Cottet *et al*.[5] described a case of HSV-2 acute retinal necrosis (ARN), in which a reduction of viral load was noted after treatment. Asano *et al*.[6] also demonstrated a correlation between viral load in ocular specimens and the clinical course of the disease in three patients with ARN. Yin *et al*.[7] reported a case of progressive outer retinal necrosis (PORN), in which the presence of VZV DNA correlated with the clinical exacerbations of the disease. Tran *et al*.[8] described 11 eyes with HSV-2 related ARN and found that 86% (6/7) with ≥9 clock h of retinitis had final visual acuities of 20/200 or worse. Calvo *et al*.[9] in 2016 determined in 14 patients with ARN that quantitative DNA copy number ≥ 5.0×10^6 mL was associated with at least 5 clock h of retinal involvement, worse visual acuity, and development of retinal detachment. In our study, although DNA PCR copy number correlated with the extent of clinical involvement, it did not correlate with the visual acuity at presentation or the final visual outcome. This is similar to a study by von Hofsten *et al*.[10] where they found no correlation between high viral load and worse visual outcomes and probably signified early testing. They suggested that the timing of the sample is essential as viral load early in the disease process is high and decreases with time. In our study also, we found that earlier sampling showed higher copy numbers, that is, viral load. This possibly signifies that in the early course of the disease, there is higher active viral replication and hence detection of higher viral load on PCR testing, which decreases as the disease burns out or responds to treatment. Rao *et al*.[11] reported a case of CMV anterior uveitis where the RT-PCR copy number was higher during initial presentation and again during recurrence suggesting that copy number is related to higher active viral replication.

Fig. 2a shows a patient with acute retinal necrosis of the left eye with three quadrants involved. The qRT-PCR of this patient's aqueous sample showed HSV-1 virus with a DNA copy number of 47,723 copies/mL [Fig. 2b].

We also looked at the correlation of DNA PCR copy numbers to the macula and optic nerve involvement. There was no correlation between PCR copy number and the macula or optic nerve involvement. Macula and optic nerve involvement can be the cause of reduced visual acuity, which also did not correlate with the PCR copy number in our study. To our knowledge, there is no previous study that has correlated macula and optic nerve involvement with DNA PCR copy number.

Forty percent of the eyes progressed to retinal detachment in our series. In a study by Calvo *et al*.[9] 57% (eight eye) developed retinal detachment. Although the authors reported a significant association between high PCR DNA copy number and retinal detachment, the *P* value of 0.08 was not statistically significant. Similarly, we also did not find any correlation between the DNA PCR copy number and the development of retinal detachment. Early and aggressive treatment for viral retinitis can reduce the chances of progression to retinal detachment.

PCR copy number signifies high viral replication. Prompt and appropriate management can give good visual outcomes, and hence the PCR copy number does not necessarily have to correlate with final visual acuity in appropriately treated patients. A serial DNA PCR copy number during the course of management of the disease can give a better idea about the response to treatment and correlation to final visual acuity. It can also detect resistance to antiviral treatment by detecting cases with a prolonged initial plateau of viral load.[12]

This study has some significant limitations. These include the retrospective nature of the study and the small sample size. As this is a retrospective analysis, there is no clear reason as to why the same patients had aqueous sampling and some had a vitreous sampling. The small sample size also did not allow for subgroup analysis to detect if aqueous or vitreous samples detected higher yields. Subgroup analysis of different viruses also could not be done due to the small sample size. The time from the onset of symptoms to presentation varied between patients, ranging between 3 and 60 days, and this may have had some impact on the viral load. Another limitation is that serial qRT-PCR was not done in these patients, which would have helped to monitor treatment response, determine the number of antivirals needed for resolution, as well as determine final visual acuity and establish a prognosis. Only three viruses were tested as they are commonly reported and viruses such as Epstein–Barr virus, dengue virus, and chikungunya virus, which could also cause retinitis were not tested, which could be a reason for negative results.

Conclusion

Real-time quantitative PCR is useful in confirming a viral etiology in over 50% of cases of suspected viral retinitis and it correlates well with the extent of clinical involvement. The timing of sampling is important. CMV is the most common

virus detected in cases of viral retinitis in our series contrary to other studies.

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Conflicts of interest

There are no conflicts of interest.

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