Detection of Cytomegalovirus in Vitreous, Aqueous and Conjunctiva by Polymerase Chain Reaction (PCR)

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Objective: *To evaluate the diagnostic value of polymerase chain reaction (PCR) performed on vitreous, aqueous and conjunctiva for the detection of cytomegalovirus in AIDS patients with a clinical diagnosis of cytomegalovirus retinitis.*
Material and Method: *PCR-based assay was used to detect cytomegalovirus DNA in vitreous, aqueous and conjunctival samples from 24 patients with the acquired immunodeficiency syndrome (AIDS) who had untreated clinically diagnosed cytomegalovirus retinitis and from 15 immunocompetent patients, including 11 with retinal detachment, 2 with macular hole and 2 with vitreous hemorrhage.*
Results: *Cytomegalovirus DNA was detected in 16, 9 and 3 of 24 vitreous, aqueous and conjunctival samples, respectively, from patients with AIDS, untreated clinically diagnosis of cytomegalovirus retinitis; and in one patient out of 15 vitreous, aqueous and conjunctival samples from immunocompetent patients with vitreoretinal diseases.*
Conclusion: *The use of PCR in the detection of cytomegalovirus in vitreous, aqueous and conjunctival samples had an equal specificity of 93% and had sensitivity of 67, 37 and 12%, respectively.*

Keywords: *Cytomegalovirus retinitis, Acquired immunodeficiency syndrome, Polymerase chain reaction*

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Cytomegalovirus retinitis is the most common ocular opportunistic infection in patients with the acquired immunodeficiency syndrome (AIDS), occurring in up to one third of the patients[1-4]. Diagnosis of cytomegalovirus retinitis is usually based on clinical findings. However, overlapping funduscopic findings may occur and make it difficult to distinguish from necrotizing retinitis caused by varicella-zoster virus[5], herpes simplex[6] or toxoplasma retinochoroiditis[7] by clinical examination alone. Furthermore, simultaneous infection of the retina by more than one pathogen[8,9] makes it more complicated to have an accurate diagnosis of the disease. Inaccurate or delayed treatment not only causes permanent loss of vision but also exposes the patients to side effects of unnecessary medications.

Polymerase chain reaction (PCR) is a useful diagnostic tool for the detection of small amounts of viral DNA used in vitro amplification of target DNA. It has been performed successfully to detect viral DNA in intraocular fluid (vitreous[10-13] and aqueous[10,14]) and conjunctival samples[15]. Paracentesis to obtain aqueous humor or conjuctival scraping is much easier, safer and less invasive than taking vitreous specimens. The diagnostic value of PCR performed on various samples, however, has yet to be determined.

The authors used a PCR-based assay for cytomegalovirus DNA in vitreous, aqueous and conjunctival samples from acquired immunodeficiency patients with clinically diagnosed CMV retinitis and immunocompetent patients with vitreoretinal diseases and determined the diagnostic value of PCR for the detection of CMV viral DNA in various samples.

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Material and Method

Collection of specimens

Undiluted vitreous, aqueous specimens and conjunctival scraping were obtained from 24 patients with acquired immunodeficiency syndrome (AIDS) who had an untreated clinical diagnosis of cytomegalovirus retinitis and 15 immunocompetent patients with vitreoretinal diseases including 11 with retinal detachment, 2 with macular hole and 2 with vitreous hemorrhage.

After installation of benoxinate (local anesthetic), eyes with newly diagnosed AIDS-related cytomegalovirus retinitis were scraped at the lower palpebral conjunctiva using a sterilized Kimura spatula. Conjuctival scrapings were placed in 1.5 ml of the minimal essential medium (MEM). Under sterile conditions, vitreous specimens were then obtained followed by injection of intravitreal ganciclovir. Anterior chamber taps were performed last. Aqueous humor, 100-150 l, was aspirated through the limbus using a 27-gauge needle attached to tuberculin syringe. Both vitreous and aqueous specimens were placed in sterile tubes. All specimens were stored on ice until they could be transported to the laboratory. The samples were then stored at -70°C.

Anti-HIV tests by ELISA technique were performed on patients with vitreoretinal diseases required retinal surgery. During retinal surgery, conjunctival scrapings and aqueous specimens were also obtained from eyes with non-AIDS-related retinal detachment, macular hole or vitreous hemorrhage. Vitreous specimens were obtained during vitrectomy. All specimens were transported to the laboratory and stored at -70°C. All research specimens were collected with appropriate consent approved by the ethics committee of the Maharaj Hospital, Chiang Mai University.

Polymerase chain reaction and detection procedures

All assays were performed on unmarked samples. The conjunctival scraping specimens in MEM were centrifuged at 7,000 g for 30 min. The supernatants were removed, leaving approximately 100 l of the precipitates for further processing. The aqueous humor, vitreous specimens and precipitates of conjunctival scraping were extracted by incubating at 100°C for 10 min. The tubes were then placed immediately in an ice bath for at least 5 min before applying 3 l aliquots into separate PCR reaction mixture. Lysate of human embryonic lung fibroblasts (MRC-5) infected with HCMV AD169 served as a positive control, whereas sterile distilled water was used as a negative control in all experiments. Specific primer pairs[16] that corresponded to the second and third exons of the HCMV major immediate-early gene were used. The first primer set amplified a 351 bp product and the nested primer set amplified a 170 bp product. The sequences of primers used for the first round PCR were ACA TCT TTC TCG GGG TTC TCG TTG C and GTC CTC TGC CAA GAG AAA GAT GGA C, and for the nested PCR were TTG AGG GAT TCT TCG GCC AAC TCT G and TCT CCT GTA TGT GAC CCA TGT GCT T.

The PCR reaction mixture of both the first round and nested PCR consisted of PCR buffer (10 mM Tris-HCl, pH 9.0 at 25°C, 50 mM KCl, and 0.1% Triton X-100), 1.5 mM $MgCl_2$, 10% glycerol, 100 M of each dNTP, 1.25 U Taq DNA polymerase (Promega, USA), 0.4 M of each primer, DNA template and sterile distilled water to a final volume of 50 L. The first reaction consisted of 35 cycles of denaturation (94°C, 1 min), primer annealing (65°C, 2 min), and extension (72°C, 1 min) followed by a final cycle at 72°C for 5 min in a Thermal cycler (GeneAmp PCR system 2700). The products of the first amplification were diluted 1:10 with distilled water and 5 L aliquot was transferred to a new 50 L reaction mixture, which included the nested set primers. The solution was incubated for an additional 35 cycles. The amplification products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. Fig. 1 demonstrates the agarose gel electrophoresis obtained in detecting cytomegalovirus DNA by nested PCR technique.

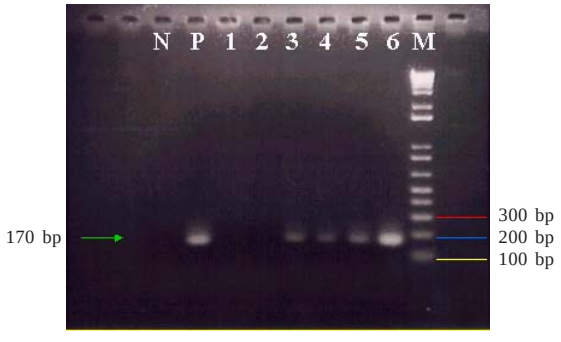

Fig. 1 Demonstrates the agarose gel electrophoresis obtained in detecting cytomegalovirus DNA by nested PCR technique. Lane N = Negative control; P = Positive control; M = DNA Marker; Lane 1 and 2 are negative results; Lane 3, 4, 5 and 6 are positive results

Results

There were 24 AIDS patients who had untreated clinically diagnosis of cytomegalovirus retinitis and 15 immunocompetent patients with vitreoretinal diseases, including 11 with retinal detachment, 2 with macular hole and 2 with vitreous hemorrhage. The mean age of AIDS - related cytomegalovirus retinitis was 33.6 years and that of immunocompetent patients with vitreoretinal diseases 44.6 years.

The authors detected cytomegalovirus DNA in 16 (67%) vitreous, 9 (37%) aqueous and 3 (12%) conjunctival samplings of these 24 eyes of AIDS patients. From 15 vitreous, aqueous and conjunctival scraping specimens from immunocompetent patients with vitreoretinal diseases, cytomegalovirus DNA was detected in 1 (7%) of the three types of samples. No complications from obtaining samples were noted. The PCR-based assay had a specificity of 93% in detecting cytomegalovirus.

Discussion

Several reports have demonstrated the ability of PCR-based assays to detect cytomegalovirus DNA in ocular fluids from AIDS patients with cytomegalovirus retinitis[10,11,13,17-19]. In the study by Fox et al[10] who used 3 aqueous, 5 subretinal fluid and 9 vitreous specimens to detect CMV DNA in patients with clinically diagnosed CMV retinitis, cytomegalovirus DNA was detected by PCR in all specimens. They also investigated 18 normal aqueous and 8 normal vitreous specimens and cytomegalovirus DNA was not detected in any of the aqueous specimens and was weakly positive for CMV in normal vitreous specimens. In our single positive case, because this patient had no signs of retinitis, it could be that these samples were contaminated.

Anterior chamber paracentesis for aqueous specimen exposes a patient to less morbidity than pars plans vitreous biopsy and is the preferred route of biopsy since it is easier to perform and serious side effects are rare. However, the results of the present study are not encouraging in the use of aqueous humor as an appropriate specimen for detecting CMV DNA.

The effectiveness of conjunctival swabs in the diagnosis of CMV retinitis has been evaluated previously[21,22]. However, the presented data do not support the use of conjunctival scrapings as a reliable method for detecting CMV retinitis. CMV DNA was detected in only 12% of patients with clinically diagnosis of CMV retinitis and this sensitivity of PCR-assay is too low to have clinical utility for the diagnosis of CMV retinitis. The authors' failure to detect CMV DNA in the vitreous, aqueous or conjunctival specimens was possible due to a number of reasons: Firstly, inadequate tissue samplings; secondly, low sensitivity of the PCR-based assay; and thirdly, misdiagnosis of CMV retinitis.

Although the diagnosis of cytomegalovirus retinitis is usually based on clinical findings, atypical presentations of retinitis or simultaneous infection of retina by more than one pathogen in AIDS patients may make it difficult to make an accurate diagnosis. Viral DNA detection by PCR may be helpful in such situations. To expose the patients to minimal risks from obtaining samples, the authors have studied the ability of PCR-based assays in detecting CMV DNA from various samples, and the present data do not support the use of aqueous humor or conjunctival scraping as a reliable source in detecting CMV DNA. Vitreous biopsy is the preferred route for the diagnosis of cytomegalovirus retinitis. The benefit of PCR-based assay in the diagnosis of cytomegalovirus retinitis should be balanced with the risk associated with obtaining the sample.

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การตรวจหาเชื้อไวรัส Cytomegalovirus ในน้ำวุ้นลูกตา, น้ำในช่องหน้าลูกตาและสารคัดหลั่งจากเยื่อบุตาโดยวิธี Polymerase Chain Reaction (PCR)

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วัตถุประสงค์*: เพื่อตรวจหาเชื้อ cytomegalovirus ซึ่งเป็นสาเหตุหลักของการติดเชื้อในจอประสาทตาของผู้ป่วยเอดส์จากน้ำวุ้นลูกตา, น้ำในช่องหน้าลูกตาและสารคัดหลั่งจากเยื่อบุตาของผู้ป่วยโดยเทคนิค polymerase chain reaction และหาความจำเพาะของวิธี polymerase chain reaction ในการตรวจหาเชื้อ cytomegalovirus จากน้ำวุ้นลูกตา, น้ำในช่องหน้าลูกตา และสารคัดหลั่งจากเยื่อบุตา*

วัสดุและวิธีการ*: ผู้ป่วยเอดส์ที่มีการอักเสบของจอประสาทตาที่เข้าได้กับการติดเชื้อ cytomegalovirus ที่เพิ่งได้รับการวินิจฉัยเป็นครั้งแรก จำนวน 24 ราย และ ผู้ป่วยด้วยโรคจอประสาทตาจากสาเหตุต่าง ๆ ที่ไม่ได้เป็นโรคเอดส์แต่จำเป็นต้องได้รับการผ่าตัดภายในลูกตา จำนวน 15 ราย โดยเป็นผู้ป่วยโรคจอประสาทตาหลุดลอกจำนวน 11 ราย โรคจอรับภาพเป็นรูจำนวน 2 ราย และผู้ป่วยเลือดออกในน้ำวุ้นลูกตาจำนวน 2 ราย จะได้รับการเก็บน้ำวุ้นลูกตา, น้ำในช่องหน้าลูกตา และสารคัดหลั่งจากเยื่อบุตา เพื่อนำไปตรวจหาเชื้อ cytomegalovirus โดยเทคนิค polymerase chain reaction*

ผลการศึกษา*: มีการตรวจพบ CMV DNA โดยวิธี polymerase chain reaction จากน้ำวุ้นในลูกตา, น้ำในช่องหน้าลูกตาและสารคัดหลั่งจากเยื่อบุตาของกลุ่มผู้ป่วยเอดส์ที่มีการอักเสบของจอประสาทตาที่เข้ากันได้กับการติดเชื้อ cytomegalovirus จำนวน 16, 9 และ 3 ราย ตามลำดับจากผู้ป่วยทั้งหมด 24 ราย และตรวจพบ CMV DNA โดยวิธี polymerase chain reaction จากน้ำวุ้นในลูกตา, น้ำในช่องหน้าลูกตาและสารคัดหลั่งจากเยื่อบุตาของกลุ่มผู้ป่วยโรคจอประสาทตาจากสาเหตุต่างๆที่ไม่ได้เป็นโรคเอดส์ 1 รายจากผู้ป่วยทั้งหมด 15 ราย*

สรุป*: ค่าความจำเพาะของเทคนิค polymerase chain reaction ในการตรวจหาเชื้อ cytomegalovirus จากน้ำวุ้นลูกตา, น้ำในช่องหน้าลูกตาและสารคัดหลั่งจากเยื่อบุตามีค่าเท่ากันคือ 93% และค่าความไวในการตรวจหาเชื้อ cytomegalovirus จากน้ำวุ้นลูกตา, น้ำในช่องหน้าลูกตา, และสารคัดหลั่งจากเยื่อบุตามีค่าเท่ากับ 67, 37 และ 12% ตามลำดับ*