Role of polymerase chain reaction in current ophthalmology practice

Nicey Roy Thomas, Kumar Saurabh and Rupak Roy

Introduction
Polymerase chain reaction (PCR) is a technique involving enzymatic amplification of nucleic acid sequences in repeated cycles of denaturation, oligonucleotide annealing and DNA polymerase extension. It selectively amplifies a single or few copies of a piece of DNA, thereby generating millions or more copies of a particular DNA sequence. It is thus also described as ‘molecular photocopier’ in a test tube. PCR was invented by Kary Mullis of USA in 1983 for which he was awarded the Nobel Prize in chemistry in 1993.1

Advantages of PCR are that it can be performed on a very small amount of tissue and almost any tissue or body fluid can be used. The sensitivity of the technique is high. Generally, PCR can detect 10–100 viral genomes which is less than the amount of genomes which is required to form 1 plaque in viral culture.2

In ophthalmology practice, the samples for PCR are usually obtained from conjunctival swab, tear fluid, corneal epithelial scrapings, anterior chamber or vitreous aspirate. The choice of collecting sample should be guided by disease suspicion. Fifty microliters of aqueous and 100–300 μl of vitreous aspirate are sufficient for diagnostic purpose. Specimens should be aseptically transferred to a sterile, capped tube (i.e. a 1.5-ml microfuge tube) and quick-frozen on dry ice or in liquid nitrogen.

Indications of PCR3–8

a In diagnosing Acanthoemeba keratitis and in the detection and serotyping of viral genomes in patients with adenoviral infections.
b Infectious endophthalmitis: To differentiate bacterial from fungal aetiology.
c Viral retinitis: To detect Herpes simplex virus, Varicella Zoster virus or Cytomegalovirus as causative organism.
d In the diagnosis of Ocular toxoplasmosis and Ocular tuberculosis.
e Masquerade syndrome: To detect IgH gene rearrangements and provide a helpful adjunct for the diagnosis of B-cell lymphoma in the eye.
f HLA typing in non-infectious endophthalmitis.

PCR lab in Sankara Nethralaya Kolkata
PCR was introduced in Sankara Nethralaya, Kolkata in the year 2012. It was set up with the expertise of the L&T Microbiology research centre, Vision Research Foundation in Chennai. Currently, it is the only centre in Eastern India providing state-of-the art PCR diagnostic facilities to both our in-house patients and surrounding referral hospitals. Presently, our lab provides facilities to detect Eubacterial genome, Panfungal genome, P Acnes genome, HSV,VZV,CMV, HIV1 and 2, Toxoplasmosis, Mycobacterium Tuberculosis (MPB64 gene) and for HLA B27 typing. For infectious endophthalmitis panel, the eubacterial genome (targeting the 16s rRNA gene), Propionibacterium acnes (targeting the 16s rRNA gene) genome and the panfungal genome (28Srna gene) are tested.

Our experience with PCR in Infectious Endophthalmitis
At Sankara Nethralaya Kolkata, we did a study to analyse the efficacy of PCR in Endophthalmitis isolates. We conducted a retrospective analysis of consecutive post-cataract surgery endophthalmitis patients treated at a Sankara Nethralaya, Kolkata between 2012 and December 2015. The data about clinical features, investigations, treatment, and outcome were obtained from the medical records. All patients had undergone comprehensive ophthalmic examination which included recording of best corrected visual acuity (BCVA) with Snellen’s distance visual acuity chart, slit lamp examination and fundus evaluation. In eyes with no view of fundus, ultrasonography was performed to assess the status of posterior segment.

Aqueous tap (0.05 to 0.1 ml) was performed as the first step in the microbiological analysis of endophthalmitis. It was performed in the outpatient department at the first visit of the patient. Vitreous sample was obtained for analysis only in patients who underwent pars plana vitrectomy. To identify the bacterial and fungal isolates, samples were first analysed with Gram’s stain, 10% potassium hydroxide (KOH) mount, Giemsa stain and Ziehl–Neelsen stain. Further samples were inoculated into blood agar, chocolate agar, Sabouraud’s dextrose agar, thioglycolate medium, brain–eart infusion agar and Lowenstein–Jensen agar. All samples were analysed with PCR to identify Eubacterium, Panfungal, and P Acnes genome.
One hundred and thirty two eyes with post-cataract surgery endophthalmitis were included in the study. Seventy (53%) patients were male and 62 (47%) were female. The mean age of patients was 58.87±12.27 years (range 18–90 years). Gram’s stain was positive in 21 (15.9%) and negative in 111 (84.1%) eyes. KOH mount showed fungus in five (3.8%) eyes and was inconclusive in 127 (96.2%) eyes. PCR was positive 118 (89.4%) and negative in 24 (18.2%) eyes. A total of 95 (72%) were eubacterium genome positive and 23 (17.4%) were panfungal genome positive. Overall, 51 (38.6%) eyes were culture positive and remaining 81 (61.4%) were culture negative. Gram negative bacilli (27; 51.9%) were the commonest isolate, followed by Gram positive cocci (10; 19.2%), fungus (9; 17.3%), and Gram positive bacilli (6; 11.5%). Staphylococcus epidermidis (8; 15.4%) and Bacillus cereus (6; 11.5%). The isolated fungi were Aspergillus fumigatus (3), Fusarium (2), Candida albicans (1), Candida lipolytica (1), Scedosporium apiospermum (1), and Paecilomyces (1).

In the literature review, it was noted that microbial culture was effective in identifying the causative organism in a limited number of cases. This may mean that newer diagnostic modalities like PCR need to be utilized for timely identification of microorganism and institution of targeted antimicrobial therapy. The present study is the first to report the diagnostic role of eubacterial and panfungal genome PCR in post-cataract surgery endophthalmitis in Eastern Indian scenario. PCR positivity (81.8%) was significantly higher than culture positivity (38.6%) in our study. Hence in patients with symptoms of endophthalmitis but negative culture, a positive PCR helped us in diagnosing it as endophthalmitis. This may mean that rather than conventional staining techniques, it is the PCR which would help us in pinpointing the diagnosis in endophthalmitis with negative culture reports and start early treatment. Since conventional culture techniques are time-consuming and have variable yield, PCR techniques can be a significant tool in the armamentarium of ophthalmologists for the timely management of endophthalmitis.

References