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Original Article

Ophthalmology Section

PCR Detection and Identification of Bacterial Contaminants in Ocular Samples from Post-Operative Endophthalmitis

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ABSTRACT

Background: Bacterial endophthalmitis is a sight-threatening complication of ocular surgery which requires urgent medical consideration including comprehensive diagnosis. Polymerase chain reaction (PCR) as a sensitive molecular method has been extensively used for detection of microbial species in clinical specimens.

Aim: The aim of this study was to identify the causative organisms of endophthalmitis in our patient population using a procedure based on PCR.

Materials and Methods: Vitreous samples from 32 patients with post-operative endophthalmitis were collected. Total vitreous DNA was extracted and then assessed by agarose gel electrophoresis. Bacterial 16S rRNA gene was amplified from genomic DNA using PCR with a pair of HAD2 universal primers. Library of PCR products from 16S rRNA, cloned into the pTZ57R/T vector. The ligated products were then transformed into *E. coli* DH5α strain and grown in the LB-ampicillin/X-Gal/IPTG plate.

Results: From the total of 32 vitreous samples, 18 specimens were positive, illustrating the presence of bacterial infection (56.4 %). Twelve species including *Escherichia coli*, *Enterobacter cloacae*, *Bacillus subtilis*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamydia trachomatis*, *Staphylococcus aureus*, *Neisseria meningitides*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Bacillus cereus* were identified using BLAST for known 16S rRNA sequences.

Conclusion: Polymerase chain reaction (PCR) accompanied with cloning and sequencing approved to be sensitive and specific. The rapid molecular technique was useful in detection of 12 major microbial species, in infectious endophthalmitis.

Keywords: Bacterial infection, Inflammation, 16S rRNA

INTRODUCTION

Endophthalmitis is an ocular bacterial complication resulting from either endogenous infection such as systemic infectious disorders or exogenous infections including postoperative and post-traumatic [1]. The postoperative endophthalmitis incidence can be in the early phase following the operation (early-onset endophthalmitis) or subsequent stage over a six year period after the surgery (delayed-onset endophthalmitis) [2-4]. It has been reported that the most common form of post-operative endophthalmitis, corresponding for 70% of infectious cases, was endophthalmitis following cataract extraction [5]. The inflammation and infectious lesions as consequences of endophthalmitis lead to incidence of late-onset bleb-related complications in 11% [4] to 57% [6] of cases as well as panophthalmitis and corneal perforation. In addition, the prognosis is worse when diagnosis and treatment are delayed in some bacterial species such as *Staphylococcus aureus* [7]. In this scenario, urgent medical consideration such as rapid and comprehensive diagnosis along with accurate identification of specific pathogens is essential to avoid the inappropriate usage of antimicrobial agents and to prevent the intraocular tissues progressive destruction. Conventional microbiological culture of vitreous and aqueous humors followed by microscopic examinations are considered routine techniques for diagnosis of endophthalmitis. However, it has been reported that bacterial detection sensitivity via the aqueous and vitreous humor microbiological culture is low (25% to 56% of cases) [8-10]. Recently, newer molecular biology methods were developed with the purpose of ameliorating the range of microbiological diagnosis. In this regard, polymerase chain reaction (PCR) has been served as a prosperous technique in detection of pathogens in numerous clinical samples, such as ocular specimens [11-13]. High sensitivity and specificity of PCR-based methods in rapid detection and corroboration of infectious agents make it a high-quality subsequent technique for conventional methods like cultures and smear assays [14]. Bacterial ribosomal RNA gene (16S rDNA) has been previously detected in ocular fluid of infectious endophthalmitis patients [13,15,16].

The present study was designed to identify the causative organisms of endophthalmitis using a procedure based on PCR. After collecting ocular specimens from patients with proved post-operative endophthalmitis we tried to identify bacteria by 16S rRNA sequencing.

MATERIALS AND METHODS

Sample Collection

This study was approved by the Medical Ethics Committee of Mashhad University of Medical Sciences and written consent was obtained from patients for their participation in experimental procedures. Vitreous samples from 32 patients (one sample from each patient) with post-operative endophthalmitis were collected. The patient population age ranged between 20 to 80 y with most of them above the age of 50 y. Patients already on antibacterial therapy were not included in the study. The endophthalmitis was approved by an ophthalmologist based on typical clinical features such as pain, deterioration of vision, conjunctival injection, and severe inflammation of anterior and posterior segments of eye. All eyes were sampled in an identical manner. Sampling was conducted under the sterile operating conditions. Viterous aspiration was done slowly using a sterile syringe [17,18]. The specimens were collected in pre-sterilized microfuge tubes and stored at -20˚C for further analysis.

DNA Extraction

Total vitreous DNA was extracted using QIAamp DNA minikit (Qiagen, CA, USA) in accordance to the manufacturer's instructions.

DNA Quantification

The quality of extracted DNA was assessed by agarose gel electrophoresis. The concentration of isolated DNA was also determined with a Nanodrop ND 2000 at 260 nm (Thermo Scientific, USA).

PCR Amplification

Bacterial 16S rRNA gene was amplified from genomic DNA isolated from the vitreous samples using PCR with a pair of HAD2 universal primers [18,19]: F 5' ACTCCTACGGGAGGCAGCAGT 3' and R 5'GTATTACCGCGGCTGCTGGCA 3'. The PCR reactions were carried out in a total volume of 25 µl containing 2.5 µl of 10× PCR buffer (CinnaGene, Iran), 25 mM $MgCl_2$, 10 mM of dNTP, 2 µl of DNA template (50-500 ng) , 0.1µM of each primer, 1.0 U of Taq DNA polymerase. The denaturation step was carried out at 95̊C for 5 min, followed by 25 amplification cycles of 95°C for 1 min, 54°C for 30 sec, and 72°C for 1 min, with the final extension step of 20 min at 72°C. PCR products were examined on 2% agarose gels prepared in 1 x Tris-Borate-EDTA (TBE) buffer and visualized by ethidium bromide staining under uv-light.

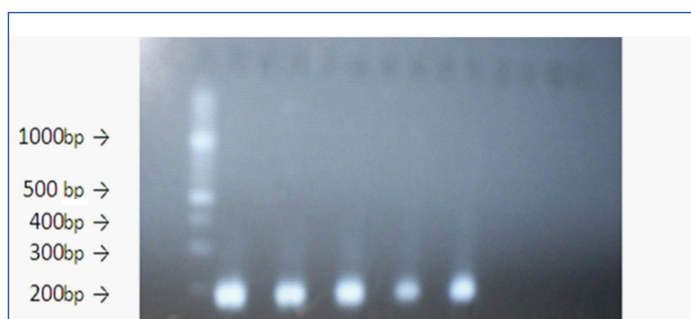
Cloning, DNA Sequencing and Sequence Analysis

Library of PCR products from 16S rRNA, cloned into the pTZ57R/T vector (MBI Fermentas). The ligated products were then transformed into *E. coli* DH 5A strain and grown in the LB-ampicillin X-Gal/IPTG plates. Plasmid purification was carried out from 30 colonies with transformed recombinant vector using plasmid purification kit (Sigma-Aldrich). After extraction of plasmid, PCR with conditions as described above was performed. Finally, the PCR products were sequenced and identified by blasting against those available in the Gen-Bank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Our PCR results showed that from the total of 32 post-operative endophthalmitis vitreous samples, 18 specimens were positive, illustrating the presence of bacterial infection (56.4 %). The quality of PCR products, as assessed by agarose gel electrophoresis, is shown in [Table/Fig-1].

After cloning and sequencing, bacterial species were identified by blasting known 16S rRNA sequences. Identified species were: *Escherichia coli* (3 cases), *Enterobacter cloacae* (3 cases), *Bacillus subtilis* (4 cases), *Neisseria gonorrhoeae* (3 cases), *Streptococcus pneumoniae* (3 cases), *Haemophilus influenzae* (3 cases), *Chlamydia trachomatis* (2 cases), *Staphylococcus aureus* (3 cases), *Neisseria meningitides* (3 cases), *Staphylococcus epidermidis* (3 cases), *Pseudomonas aeruginosa* (2 cases), *Bacillus cereus* (4 cases) [Table/Fig-2].



[Table/Fig-1]: The DNA agarose gel demonstrating PCR samples for amplification of the 16S rRNA of the isolates

Bacteria	Number of cases
Escherichia coli	3
Enterobacter cloacae	3
Bacillus subtilis	4
Neisseria gonorrhoeae	3
Streptococcus pneumoniae	3
Haemophilus influenzae	3
Chlamydia trachomatis	4
Staphylococcus aureus	3
Neisseria meningitides	3
Staphylococcus epidermidis	3
Pseudomonas aeruginosa	2
Bacillus cereus	4

[Table/Fig-2]: Identified species from the vitreous samples of patients presenting with a clinical diagnosis of post-operative endophthalmitis

DISCUSSION

Infectious endophthalmitis represents a real medical emergency requiring quick intervention to prevent the intraocular tissues progressive destruction. Therefore, detection of infectious agents from endophthalmitis should be done through a rapid and accurate procedure. Although the use of intraocular specimen including aqueous humor and vitreous fluid culture is the method of choice for identification of microbiological spectrum of infectious endophthalmitis [20], but even under the standard laboratory circumstances, only 60–70% (in Iranian setting much lower) of endophthalmitis cases have yielded positive results [21]. Negative results of culture studies can be due to several reasons such as sample volume limitation and antibiotics application prior to sampling [14]. The above practical considerations lead to the expansion of culture-independent diagnostic methods in infectious diseases such as endophthalmitis. Polymerase chain reaction (PCR) is one of the most sensitive, specific and rapid molecular techniques which have been extensively used for detection of microbial species in clinical specimens. In order to specify the accurate causative organism of endophthalmitis, the present investigation has been carried out on vitreous samples using PCR. Vitreous samples has been shown to have higher efficacy in cultures of endophthalmitis organisms in comparison with aqueous [22,23]. Therefore, small required vitreous sample and negligible numbers of bacteria are the potential advantage of PCR technique in detecting of bacteria in infectious endophthalmitis [13]. As mentioned above, antibiotics usage prior to sampling may lead to false results in the culture dependent diagnostic methods. It has been recently reported that the efficacy of PCR in detection of vitreous samples bacteria from patients with previous antibiotics administration is more than bacterial cultures [15]. In the present study vitreous samples from 32 patients with post-operative endophthalmitis were collected, the total DNA was extracted and used as a template for amplification of the 16S rRNA sequence. Considering, a wide range of bacteria are responsible for infectious endophthalmitis [24,25] and using primers to detect them is not a rational approach, we used universal primers coding for the conserved regions of 16S rRNA gene in a wide variety of bacteria. In preliminary PCR experiments 18 specimens showed a band in agarose gel in a correct fragment size, illustrating the presence of bacterial infection (56.4 %). Fungal infections may have caused endophthalmitis in 14 remaining PCR-negative samples [26]. BLAST analysis of PCR products indicated that the vitreous samples contained 12 bacterial species. From these, five species were found to be positive and the rest were negative in Gram staining experiments. *Bacillus subtilis* and *Bacillus cereus* detected in four cases consists of the most prevalent species in the samples. Ghasemi Falavarjani et al., have used the culture method to evaluate the microbiological spectrum of infectious endophthalmitis in Iran.

In their investigation the most common organism was Coagulase-negative *staphylococcus* [27]. In another study which was conducted in 2012, *Staphylococcus, Streptococcus*, *Bacillus cereus* and *Pseudomonas aeruginosa* were detected in Iranian endophthalmitis specimens [28]. To our knowledge, the present investigation is the first study identifying endophthalmitis isolates using PCR technique in Iran. Compared with some previous studies [29-32] the current study led to the identification of some different isolates including *B. subtilis*, *N. meningitides*, *C. trachomatis* and *N. gonorrhoeae* in Iranian endophthalmitis vitreous samples.

CONCLUSION

Polymerase chain reaction (PCR) accompanied with cloning and sequencing is one of the most sensitive, specific and rapid molecular techniques in detection of microbial species in clinical specimens like infectious endophthalmitis. The results of this investigation illustrated the presence of some bacterial species including *Escherichia coli*, *Enterobacter cloacae*, *Bacillus subtilis*, *Neisseria gonorrhoeae*, *Streptococcus pneumonia, Haemophilus influenza, Chlamydia trachomatis*, *Staphylococcus aureus*, *Neisseria meningitides*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Bacillus cereus* in vitreous endophthalmitis specimens.

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