ORIGINAL RESEARCH PAPER

Microbiology

Comparison of Culture and Uniplex-Nested PCR in Detecting Post-Operative endophthalmitis

KEY WORDS: endophthalmitis, culture, uniplex-nested PCR, antibiotics

Dr.N.Bharathi Santhose MD. Senior Assistant Professor of Microbiology Coimbatore Medical College Coimbatore -64104.

Mr. Rajiv Sathianarayanan Menon II Year MBBS Student Coimbatore Medical College Coimbatore -64104.

ABSTRACT

Endophthalmitis is a rare but vision-threatening complication that can occur after ocular surgery or trauma or as a consequence of systemic infection. To optimize visual outcome, early diagnosis and treatment are essential. As conventional culture techniques take longer time and lacks sensitivity, polymerase chain reaction (PCR) for detection was evaluated for early diagnosis of postoperative endophthalmitis.14 patients with postoperative endophthalmitis presented to our institution during the study of 3 months were included in the study. The vitreous samples of all patients were subjected to both microbiological culture and Uniplex-Nested PCR testing. Culture was positive in 6 out of 14 specimens (43%) while PCR was positive in 10 out of 14 (71%). Nested PCR proved superior to uniplex PCR, as two of the patients who were negative in uniplex PCR were positive by Nested PCR.

Introduction:

Endophthalmitis is the inflammation of the internal coats of the eye. It is one of the most dreaded complications of any intraocular surgery, especially cataract surgery followed by secondary IOL implantation, penetrating keratoplasty and glaucoma surgeries respectively [15,16]. The incidence of endophthalmitis is high after cataract surgery, 0.02-0.09% [1,2] as it is the most common ocular surgery performed.

Endophthalmitis usually has a poor visual outcome but is variable depending on the causative organism and its virulence [3,17]. The most common bacteria isolated are *Staphylococcus* species, *Streptococcus* species, *Pseudomonas* and *Escherichia coli.* Among fungi, *Aspergillus* species is the most common cause of infection, followed by *Candida* species. Infection by *Staphylococcus aureus* which is less virulent has a better prognosis[4] but 50% of infections caused by *Pseudomonas aeuruginosa* results in evisceration of the eye[5]. Hence, identification of the causative organism plays an important role in the prognosis and treatment of endophthalmitis[6]. Identification of the pathogen allows treatment with specific antibiotics rather than broad spectrum antibiotics which can unnecessarily cause toxic effects[7]. The gold standard technique for identifying pathogens has been gram stain and microbiological culture[8]. But cultures are time consuming and have poor sensitivity and specificity for detecting organisms in aqueous and vitreous humor.[9,10]

Rapid and correct identification of the pathogen causing endophthalmitis and appropriate antibiotic therapy greatly improves the visual outcome. A study conducted by The European Society of Cataract and Refractive Surgeons shows that molecular techniques are much faster and 20% more sensitive in identifying the pathogen than conventional microbiological culture which requires more than 48 hours time to reveal the pathogen[11].

Several Studies [7,13,14] showed that though Eubacterial PCR and culture were equally sensitive for the initial samples but after antibiotic therapy was initiated PCR was 70% sensitive compared to culture which had a sensitivity of 9%. Tarai B et al reported that PCR was found to be superior to culture in the early detection of postoperative fungal endophthalmitis[10]. Chris P. Lohmann in his study showed that PCR was more rapid and sensitive than conventional microbiological methods in detecting delayed postoperative endophthalmitis [18] and thus preventing the need for a vitrectomy in several cases.

Nested PCR uses two sets of primers in successive polymerase chain reactions to prevent amplification of unintended sequences, the error which is common in Conventional PCR . However, no quantitative information about the pathogen load is obtained from nested PCR[12]. This study was conducted to evaluate the usefulness of Uniplex-Nested PCR and its merits over microbiological culture.

Aims and objectives:

- To isolate and identify the organisms by conventional cultural methods.
- To detect the organisms using universal 16S and 28S DNA primers by Uniplex-Nested PCR.
- To compare the sensitivity of culture and PCR in detecting suspected post-operative endophthalmitis

Materials and methods:

Sample population: All patients who presented to our institution during the study period of three months with clinical symptoms of endophthalmitis and history of ocular surgery in the past 1 year were subjected to laboratory testing by conventional microbiological culture and PCR technique.

Specimen collection: All the patients underwent either vitreous tap or pars plana vitrectomy along with intravitreal injection of antibiotics (Vancomycin, Ceftazidime) and steroids (dexamethasone). Vitreous sample was collected in all patients. In some cases, anterior chamber wash or lens explantation were required as additional procedures and these specimens were also subjected to microbiological culture and PCR testing.

Conventional microbiological culture: A portion of each aspirate is subjected to gram staining and inoculated in the following media: 5% sheep blood agar, Chocolate agar, Brain heart infusion broth, Thioglycollate broth and Sabouraud's dextrose agar. The bacterial or fungal isolates are identified and confirmed by standard microbiological methods.

PCR : The Nucleic acid (DNA) is extracted as per standard protocol. The Extracted DNA is mixed with Primer& Probe and Master mix, then PCR for Eubacteria, Panfungal and nested PCR was run in a sequential manner.

A. Target gene for Eubacteria: 16SrDNA (Universal sequence)

Primer:

I round (Uniplex)

Forward : U1F 5' TTG GAG AGT TTG ATC CTG GCT C 3'
Reverse : rU4 5' GGA CTA CCA GGG TAT CTA A 3'

Product Size: 766 bp

II round Inner Primers:

Forward: U2F 5' GGC GTG CTT AAC ACA TGC AAG TCG 3'
Reverse: rU3R 5' GCG GCT GGC ACG TAG TTA G 3'

Product Size: 470 bp

Table 1: PCR Program for Eubacteria (round I) -Total Number of cycles: 35

SEGMENT	PCR Steps	TEMP ° C
1	Initial denaturation	94 for 5 minutes
2	Denaturation	94 for 30 sec
3	Annealing	60 for 1 minutes
4	Extension	72 for 2 minutes
5	Final extension	72 for 5 minutes

Table 2: PCR Program for Eubacteria (round II) - Total Number of cycles: 25

SEGMENT	PCR Steps	TEMP ° C
1	Initial denaturation	94 for 5 minutes
2	Cyclic denaturation	94 for 30sec
3	Annealing of primers	58 for 1 minutes
4	Synthesis of DNA	72 for 2 minutes
5	Final Extension	72 for 8 minutes

B.Target gene for Panfungal: 28SrDNA (Universal sequence)
Primer:

I round Primers (Uniplex)
Upstream primer: 5'GTGAAATTGTTGAAAGGGAA3'
Downstream primer: 5'GACTCCTTGGTCCGTGTT3'

Amplification product: 259bp

Table 3: PCR program for Panfungal - Total number of cycles: 34

S.No	PCR Steps	Temp and Time
1	Initial denaturation	94 for 5 minutes
2	Denaturation	94 for 30sec
3	Annealing	58 for 1 minutes
4	Extension	72 for 2 minutes
5	Final Extension	72 for 10min

C.NESTED PCR :
Primer :

Upstream primer: 5' GAAAGGGAAGGGCATTTGAT 3'
Downstream primer: 5' GACTCCTTGGTCCGTGTTTC 3'

Amplification product: 214bp

Table 4: Nested-PCR Program - Total Number of cycles: 35

S.No.	PCR Steps	TEMP ° C
1	Initial denaturation	94 for 5mts
2	Denaturation	94 for 30sec
3	Annealing	58 for 45 sec
4	Extension	72 for 30 sec
5	Final Extension	72 for 7mts

Results:
During the study period of 3 months, fourteen patients presented to our institution with suspected postoperative endophthalmitis. The mean age of the patients was 62 years (range 32-85) with 6 men and 8 women. The mean duration from the time of intra-ocular surgery to diagnosis of endophthalmitis was 28 days (range, 1 day to 200 days). Among the 14 patients, 4 were under treatment for diabetes. The initial procedure in 10 patients was vitreous tap with intravitreal injection of vancomycin ceftazidime and dexamethasone while the other 4 underwent pars plana vitrectomy along with intravitreal injection of vancomycin and dexamethasone. Also, 6 patients required anterior chamber wash and 1 required an IOL explantation in addition to the initial procedure. 6 patients required more than two intraocular procedures. ceftazidime Of the 14 patients included in the study, culture was positive in 6 (43%) while PCR was positive in 10 (71%). Among the specimens positive by culture, 5 were vitreous samples and only one was an aqueous sample. All specimens with positive culture were also positive by PCR. However PCR was also positive in four cases with negative microbiological culture. One patient (subject 9) who showed Aspergillus species in culture was also positive in PCR for panfungal DNA. Two of the Uniplex PCR negative specimens were positive by Nested PCR. Of the 6 patients who were positive by culture, 2 tested positive for Pseudomonas aeuruginosa, 1 tested positive for Staphylococcus aureus, 1 was positive for Aspergillus terreus, 1 was positive for Nocardia and 1 was positive for Streptococcus pneumoniae. Table 5 depicts the culture and PCR results of the fourteen patients included in our study.

Table 5: Comparison of Culture and PCR Results

S.No.	Culture	Organism	PCR	
			Uniplex	Nested
1	Positive	Staphylococcus aureus	Eubacteria positive	Not done
2	No growth	-	Eubacteria positive	Not done
3	No growth	-	Negative	Negative
4	Positive	Pseudomonas aeuruginosa	Eubacteria positive	Not done
5	Positive	Pseudomonas aeuruginosa	Eubacteria positive	Not done
6	Positive	Nocardia	Positive	Not done
7	No growth	-	Eubacteria positive	Not done
8	No growth	-	Negative	Negative
9	Positive	Aspergillus terreus	Negative	Panfungal positive
10	No growth	-	Eubacteria positive	Not done
11	No growth	-	Negative	Negative
12	Positive	Streptococcus pneumoniae	Eubacteria positive	Not done
13	No growth	-	Negative	Negative
14	No growth	-	Negative	Panfungal positive

Discussion:
The aim of this study was to compare PCR with traditional microbiological culture for pathogen identification in postoperative endophthalmitis. Culture was positive in 6 out of 14 specimens while PCR was positive in 10 out of 14. All culture positive specimens were positive by PCR also. In our study, Nested PCR proved superior to uniplex PCR, as two of the patients who were negative in uniplex PCR were positive by Nested PCR.

Table 6: comparison between the current study and previous studies

Study	Number of eyes	Culture	PCR
Current	14	6 (43%)	10 (71%)
Anand et al	43	24 (56%)	32 (74%)
Seal et al	29	14 (48%)	19 (65%)

The results of our study are similar to that of these studies. A study conducted by Anand et al 19 showed a sensitivity of 56% by culture and a sensitivity of 74% by PCR. A study conducted by Seal et al 11 showed 48% sensitivity by culture and 65% by PCR. Several studies have reported the superior sensitivity of Nested PCR. Bharathi et al in their study showed that Uniplex and Multiplex PCR had a sensitivity of 54% while Nested PCR had a sensitivity of 64% and hence that Nested PCR is more sensitive than Uniplex PCR 20. This was also seen in our study were two of the patients who were negative by Uniplex-PCR were positive by Nested-PCR.

The limitations of our study were the small sample size and the lack of DNA sequencing done for pathogen identification. The main fallacy of conventional PCR is the possible false-positives due to

sample contamination This can now be overcome with the advent of Real-Time PCR (RT-PCR) that quantitatively records the pathogen DNA which may help in ruling out false positive. Lalitha et al reported the superior sensitivity and specificity of RT-PCR in detecting postoperative endophthalmitis[21] with a sensitivity of nearly 70%.

Conclusion:

The results of our study show that molecular diagnostic techniques like polymerase chain reaction are faster and have better sensitivity compared to conventional microbiological techniques. NESTED-PCR is superior to other conventional PCRs and may improve the specificity of pathogen identification in endophthalmitis.

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