Diagnostic utility of polymerase chain reaction on intraocular specimens to establish the etiology of infectious endophthalmitis

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PURPOSE. To evaluate the utility of polymerase chain reaction (PCR) on intraocular clinical specimens (aqueous humor [AH] and vitreous fluid [VF]) as an etiologic diagnostic tool relative to microbiological culture methods in infectious endophthalmitis.

METHODS. Conventional bacterial and mycologic cultures and PCR for eubacterial and panfungal genomes were applied for etiologic diagnosis on pairs of AH and VF obtained from 72 patients with clinically established infectious endophthalmitis.

RESULTS. Based on cultures, an infectious etiology was established in 27 (37.5%) of 72 patients. PCR detected infectious etiology in all 72 patients. PCR increased the clinical sensitivity over culture by 62.5% (p<0.0001, McNemar test). The frequency of culture positivity, single infections, and polymicrobial infection varied significantly among the types of endophthalmitis (p<0.0001, chi-square test). PCR detected an infectious etiology in 48 patients and polymicrobial infection in 24 patients. An etiology was established by PCR on 56 (77.8%) AH and 65 (90.3%) VF of the 72 patients and this difference had no statistical significance.

CONCLUSIONS. PCR on intraocular specimens as an etiologic diagnostic tool has been shown to be specific and severalfold more sensitive than cultures and clinically useful. Therefore, PCR may be considered the gold standard to establish the etiology of infectious endophthalmitis. As there is no statistically significant difference in the results of PCR on AH and VF, PCR on AH could be the method of choice considering safety and simplicity of the procedure of its collection (Eur J Ophthalmol 2009; 19: 812-7)

KEY WORDS. Aqueous humor, Infectious endophthalmitis, Polymerase chain reaction, Vitreous fluid

INTRODUCTION

Infectious endophthalmitis is the most severe form of vision-threatening ocular infection resulting from the introduction of an infectious agent into the eye after surgery, trauma, or bacterial keratitis, or may be of endogenous origin. At present, confirmation of the etiology of infectious endophthalmitis is dependent on microbiologic isolation of the infectious agents, but there are several reports suggesting high prevalence of culture-negative endophthalmitis (1-9). With the introduction of polymerase chain reaction (PCR), it appears that DNA of bacterial, fungal, and specific infectious agents can be detected in almost all cases of infectious endophthalmitis. However, the sequencing of these samples has revealed that many of these are caused by previously unrecognized and unculturable bacteria or are polymicrobial (4, 5).

The clinical specimens that are useful in establishing the etiology of infectious endophthalmitis are the intraocular fluids, aqueous humor (AH) and vitreous fluid (VF). The collection of AH is a safe and gentle procedure with fewer complications than vitreous biopsy (6). The objective of the present study was to evaluate the utility of polymerase chain reaction (PCR) on intraocular clinical specimens as

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an etiologic diagnostic tool relative to microbiological culture methods in infectious endophthalmitis.

METHODS

Patients and clinical specimens

Seventy-two patients with clinical evidence of infectious endophthalmitis from whom pairs of AH and VF were collected as diagnostic and/or therapeutic aspirates were included in the study. The patients and the clinical specimens were grouped based on clinical history as 45 post-cataract surgery, 16 post-traumatic, and 11 endogenous endophthalmitis. The 45 patients with postcataract endophthalmitis were further classified as 21 acute, 14 delayed onset, and 10 chronic endophthalmitis based on the delay in the onset of endophthalmitis. The endophthalmitis was classified as acute when there was an onset of the symptoms within 6 weeks of the surgery, as delayed onset when the onset was greater than 6 weeks and within 1 year, and as chronic when the onset was greater than 1 year or if there was a recurrence of the symptoms (7, 8). Almost all the patients included in the study had intraocular antibiotic administration before collection of intraocular fluids. The study adhered to the Declaration of Helsinki guidelines for research involving human subjects.

Collection and conventional bacterial and fungal cultures of AH and VF

AH samples (150–200 µL) were collected aseptically in a tuberculin syringe with a 30-gauge needle and uncontaminated VF (200–500 µL) was aspirated by syringe connected to the suction port of the vitreous cutter at the beginning of vitrectomy and these specimens were transported to the microbiology laboratory as described earlier (1). Approximately 20–50 µL of the intraocular fluids (AH and VF) were transferred into sterile vials and stored at −20°C for further processing for PCR. The rest was processed for bacterial (both aerobic and anaerobic) and fungal culture as described previously (2). In brief, the intraocular specimens were inoculated onto blood agar (incubated aerobically at 37°C), chocolate agar (incubated in 10% CO₂ at 37°C), brucella blood agar (incubated anaerobically in Don Whitley Compact anaerobic workstation, Thane, India), brain heart infusion broth, and thioglycollate broth. Sabouraud dextrose agar was used for isolation of fungus, and other aerobic media with no growth at the end of 48 hours were incubated for a period of 10 days to isolate fungus. The isolated microorganisms were identified by standard protocols. Care was taken not to delay the processing of ocular samples; the culture was performed immediately after receipt of the sample (within half an hour post surgery) and PCR was performed within 24 hours of collection.

Polymerase chain reaction for eubacterial and panfungal genomes

Polymerase chain reaction (PCR) for eubacterial and fungal genomes was applied on pairs of intraocular fluids (AH and VF) from the 72 patients respectively. DNA was extracted from the intraocular specimens of AH and VF by Accuprep DNA Extraction kit (Bioneer Inc.) method according to the manufacturer's instructions. PCR was carried out using eubacterial primers targeting 16S rRNA (1) and panfungal genome (9) targeting ITS region as described previously.

Precautions taken to prevent false positivity due to contamination

To prevent contamination, PCR preparation, DNA amplification, and analysis of the amplified product were done in separate laboratories. PCR preparation was performed on a laminar flow workbench with single use aliquots of reagent and dedicated pipettes. Specific negative controls were used to rule out false positives due to endogenous contamination of the enzymes or other reagents used for the PCR.

Analysis of the results

The results of the cultures and PCR on the clinical specimens were analyzed statistically by McNemar test and were considered significant when p value was <0.05. For all other analysis, chi-square test was used.

RESULTS

Of the 72 patients, 27 (37.5%) were culture positives with 24 bacterial and 3 fungal etiology. Polymicrobial infections were not identified by culture.
TABLE I - DETAILS OF THE MICROORGANISMS ISOLATED IN 27 CASES OF CULTURE-POSITIVE ENDOPHTHALMITIS

<table>
<thead>
<tr>
<th>Bacteria/fungus (n)</th>
<th>Acute postsurgical endophthalmitis</th>
<th>Delayed onset postsurgical endophthalmitis</th>
<th>Traumatic endophthalmitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis (2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus epidermidis (9)</td>
<td>6</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus aureus (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Corynebacterium sp (2)</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Alcaligenes faecalis (1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (5)</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus (1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mycobacterium abscessus (1)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus terreus (1)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus niger (1)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fusarium sp (1)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

The details of the organisms isolated with the type of endophthalmitis are provided in Table I. Eighteen (65.75%) of 21 patients with acute postcataract endophthalmitis, 3 (21.4%) of 14 patients with delayed onset postcataract endophthalmitis, and 6 (37.5%) of 16 patients with traumatic endophthalmitis showed positive cultures. The frequency of culture positives in acute postcataract endophthalmitis was significantly higher than in the other groups of patients (p<0.0001, chi square test). Total number of gram-positive bacteria isolated was 17/24 (70.8%) and gram-negative bacteria was 7/24 (29.2%). The difference between the gram-positive and gram-negative bacterial isolates in the study was statistically significant (p=0.0094, chi-square test).

PCR detected infectious etiology in all 72 patients. PCR increased the clinical sensitivity over culture by 62.5% (p<0.0001, McNemar test).

Of the 72 patients positive by PCR, 48 patients had single infection, with 46 bacterial and 2 fungal, and 24 had polymicrobial infection with both the genomes detected in their clinical specimens. The frequency of the single infections and polymicrobial infection varied significantly among the types of endophthalmitis (p<0.0001, chi square test). While 19 (90.5%) of 21 patients with acute postcataract endophthalmitis showed single infections, 14 (87.5%) of 16 patients with traumatic endophthalmitis showed polymicrobial infections. The distribution of single and polymicrobial infections with regard to the type of endophthalmitis is shown in Table II.

In the culture-positive group, a positive culture was obtained with AH in 11/27 (40.7%) patients and VF in 19/27 (70.4%) patients. There was a trend of both the intraocular fluids becoming culture positives in a significant number of patients with p=0.0551. A positive PCR result in the culture-positive group was obtained with AH in 24/27 (88.9%) patients and VF in 25/27 (92.6%) patients. The difference between AH and VF with regard to PCR in the culture-positive group was not statistically significant (p=0.99). In the culture-negative group, a positive PCR was obtained with AH in 32/45 (71.1%) and VF in 40/45 (88.9%) patients. The difference between AH and VF with regard to PCR in the culture-negative group was not statistically significant (p=0.0652) (Tab. III).

Of the 24 patients with polymicrobial infections, discrepancies in detection of microbes were observed between the intraocular fluids of 16 patients. The details of the type of endophthalmitis in the 16 patients showing discrepancies in detection of microbes are shown in Table IV. Of the 72 patients, etiology was established by PCR on 56 (77.8%) AH and 65 (90.3%) VF, and this difference had no statistical significance.

DISCUSSION

Conventional culture methods applied to intraocular clinical specimens from infectious endophthalmitis have shown low sensitivity in detection of an infectious agent (4, 5, 8, 10). A variety of reasons have been attributed for the low culture positivity in infectious endophthalmitis, such as
TABLE II - DETAILS OF SINGLE AND POLYMICROBIAL INFECTIONS (AS DECRYPTED BY POLYMERASE CHAIN REACTION RESULTS) WITH REGARD TO THE TYPE OF ENDOPHTHALMITIS IN 72 PATIENTS

<table>
<thead>
<tr>
<th>Group</th>
<th>Culture results</th>
<th>Type of endophthalmitis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Traumatic endophthalmitis</td>
<td>Postoperative endophthalmitis</td>
<td>Endogenous endophthalmitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acute</td>
<td>Delayed</td>
<td>Chronic</td>
<td></td>
</tr>
<tr>
<td>Single infections</td>
<td>Culture positives</td>
<td>02</td>
<td>16</td>
<td>01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Culture negatives</td>
<td>-</td>
<td>03</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Polymicrobial group</td>
<td>Culture positives</td>
<td>04</td>
<td>02</td>
<td>02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Culture negatives</td>
<td>10</td>
<td>-</td>
<td>01</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE III - RESULTS OF CULTURE AND POLYMERASE CHAIN REACTION (PCR) ON INTRAOCULAR FLUIDS OF 72 PATIENTS

<table>
<thead>
<tr>
<th>PCR positives</th>
<th>Culture positives (27)</th>
<th>Culture negatives (45)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AH positive</td>
<td>VF positive</td>
<td>AH and VF</td>
</tr>
<tr>
<td>AH</td>
<td>2*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VF</td>
<td>0</td>
<td>3*</td>
<td>0</td>
</tr>
<tr>
<td>AH and VF</td>
<td>6</td>
<td>19§</td>
<td>3*</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>16</td>
<td>03</td>
</tr>
</tbody>
</table>

*Intraocular fluid of 1 patient showed polymicrobial infection.
†Intraocular fluid of 2 patients showed polymicrobial infection.
‡Intraocular fluid of 4 patients showed polymicrobial infection.
§Intraocular fluid of 5 patients showed polymicrobial infection.
¶Intraocular fluid of 10 patients showed polymicrobial infection.

AH = aqueous humor; VF = vitreous fluid.

small sample size, sequestration of bacteria on solid surfaces (e.g. intraocular lens, lens remnants, and capsule) leading to low numbers in the liquid sample, the use of antibiotics prior to sampling, the fastidious nature of some of the organisms which cause intraocular infection, and presence of uncultivable microbes (4, 5, 8, 10). The present study compared the results obtained with culture and PCR for eubacterial and panfungal genomes on pairs of AH and VF from 72 patients with clinical diagnosis of infectious endophthalmitis, and the results confirmed the common view that PCR was a more sensitive technique than culture method in establishing the etiology of infectious endophthalmitis (4, 11, 12). The low percentage of culture positives in the study may be attributable to the collection of intraocular samples from patients who had intraocular antibiotic administration, which led to the low number of viable organisms that prevented growth on culture media. Almost all the patients included in this study were referred to our hospital from ophthalmologists from different parts of India after a course of treatment. Previous studies have also shown that intravitreal treatment with antibiotics followed by collection of vitreous may reduce the isolation rate from 38.2% to 9% (11). Use of quantitative PCR in samples other than intraocular fluids have suggested that most of the culture-negative PCR-positive cases result due to the presence of small number of bacteria in the samples (13).

In the present study, the rate of culture positivity differed significantly with respect to the type of endophthalmitis. Culture negativity predominated in endogenous endophthalmitis and chronic postcataract endophthalmitis compared to other types of endophthalmitis in the study.
Diagnostic utility of PCR in infectious endophthalmitis

<table>
<thead>
<tr>
<th>Culture results</th>
<th>Type of endophthalmitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traumatic endophthalmitis</td>
</tr>
<tr>
<td>Culture positives</td>
<td>3</td>
</tr>
<tr>
<td>Culture negatives</td>
<td>8</td>
</tr>
</tbody>
</table>

The reason for such an association is not known but may depend on presence of uncultivable microbes in the samples (5). It is also shown that presence of certain gram-negative bacteria requires specific culture techniques for successful isolation, which may be responsible for culture negativity (4).

The cultures did not have the potential to detect a polymicrobial infection in this study, which is not in agreement with a previous study performed at the same center (14). Another study suggested that molecular assays are superior to conventional cultures in identifying mixed infections (2). Though mixed infections with bacteria and fungus have been reported in post-traumatic endophthalmitis (15), in this study, such mixed infections were encountered even in postcataract and endogenous endophthalmitis. Occasional studies have shown the presence of such mixed infections in patients with postcataract endophthalmitis (16, 17).

Studies by Okhavi et al (4) and Chiquet et al (11) confirmed the superiority of PCR compared with culture of vitreous samples after intraocular antibiotic administration. A recent study by Seal et al (12) showed that PCR may be superior to culture in laboratory diagnosis of postoperative endophthalmitis.

In this study, a separate control group of patients was not included, since the PCRs were applied on intraocular fluids (n=190) collected from patients with noninfectious cataract during standardization. The rate of positivity for eubacterial PCR in the control group was 3.7% and for the panfungual PCR 1.05% (data not shown).

A review by Okhavi et al (18) suggests that the positivity of PCR in culture negatives may not be excluded as false positives. These studies including the present study indicate that PCR for detection of the infectious agent on intraocular fluids should be considered as the gold standard, particularly because only small volumes of intraocular clinical samples are available and a high degree of sensitivity is required, which is important in the context of immediate institution of specific treatment.

This study further suggested that PCR on AH may be a good enough sample for detection of eubacterial genome, while VF may be essential for the detection of panfungual genome. The former view is contradictory to previous studies, which suggest VF to be superior to AH for the detection of bacterial genome (4, 11), but these studies were limited only to postoperative endophthalmitis. In the present study, the type of endophthalmitis was not restricted to either postoperative/traumatic or endogenous endophthalmitis.

This study clearly showed that PCR on AH was equally sensitive to that on VF for detection of microbial genome in both culture-positive and culture-negative specimens. These findings are significant because the anterior chamber tap is a simpler and safer office procedure compared with diagnostic vitreous aspiration. Hence, the anterior chamber tap could be the method of choice in the diagnosis of bacterial endophthalmitis when a highly sensitive molecular technique such as PCR is applied.

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