Diagnosis of Endophthalmitis with amplification of syndrome specific signature genes by Syndrome Evaluation System: A Retrospective Analysis

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ABSTRACT

Background: To evaluate the microbiological concordance of clinically diagnosed endophthalmitis cases using isolation of genetic material of the causative agent from either aqueous or vitreous fluid and simultaneous amplification of syndrome specific signature genes.

Methods: Thirteen cases clinically diagnosed as endophthalmitis at tertiary centre of Vitreo-Retina care, in whom isolation of genetic material of the causative agent from either aqueous or vitreous fluid and simultaneous amplification of syndrome specific signature genes termed as Syndrome evaluation system (SES) was carried out were included in this retrospective analysis. The vitrectomy samples or Aqueous taps were sent to for SES analysis and results were obtained within 24 hours of vitrectomy and results considered for deciding treatment.

Results: 7 out of 13 cases were positive for bacteria or fungi or virus when tested on SES. Out of Seven positive two were also culture positive and concordant. Three out of these seven positives did not improve in visual acuity while two cases improved and in two cases there was deterioration in visual acuity noted. Six out of the 13 cases SES did not detect any organisms. It can also be seen that 5 out of six negatives improved in visual acuity with treatment.

Conclusion: SES was an accurate, quick and reliable diagnostic method in endophthalmitis whose sensitivity was much higher than culture and Gram’s staining. This diagnostic technique can help in administering targeted therapies at a much earlier stage and hence improve the overall outcome of the endophthalmitis cases.

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INTRODUCTION

Endophthalmitis is the bacterial or fungal infection of the vitreous and/or aqueous humors\[1\]. This condition is usually categorized based on several aspects including clinical course (acute or chronic), aetiology (infectious or non-infectious), route of entry of the causative agent (exogenous - noted following eye surgery or penetrating eye trauma or endogenous - occurring due to bacteremic or fungaemic seeding of the eye) and organism(s) involved (bacteria, fungi, parasites and rarely, viruses)\[2\]. Prompt and accurate diagnosis is vital to guide the initial therapy and minimize damage as disorders such as bacterial endophthalmitis can often progress rapidly, while fungal endophthalmitis can worsen with the empiric use of corticosteroids (without specific antimicrobial therapy)\[3\].

Diagnosis of endophthalmitis relies on clinical symptoms as well as laboratory tests\[1\]. Gram stain of the aspirate of aqueous and/or vitreous is commonly followed, which often is not highly sensitive and is considered helpful only when positive for organisms. Vitrectomy cultures although highly reliable, often have poor concordance when comparing anterior chamber culture with vitreous isolates \[2, 4\]. Polymerase chain reaction (PCR), used to amplify deoxyribonucleic acid (DNA) sequences, has been reported to have a vital role in the diagnosis of endophthalmitis\[2\]. However, there is a need for multiple PCR tests in order to point to the aetiological agent in a given case of endophthalmitis \[5\]. The major obstacle for performing multiple PCRs to arrive at a diagnosis in an algorithmic approach is the paucity of clinical specimen, aqueous or vitreous. Moreover, a false-positive rate of 5% has been noted with PCR tests reported hitherto, which in turn resulted in cautious use of molecular diagnostics for endophthalmitis\[6\]. Hence is the requirement of high specificity built into the methodology.

Syndrome evaluation system (SES), a patented technology involving isolation of genetic material of the causative agent from relatively small volume of either aqueous or vitreous fluid and simultaneous amplification of syndrome specific signature genes of all probable organisms, followed by syndrome specific hybridization, allows for simultaneous detection of all probable pathogens in a single test and can prove to be a valuable tool for accurate and rapid identification of the causative microorganisms in endophthalmitis in a short time.\[7\] . Multiplex amplification of genes in SES confers high sensitivity to this molecular diagnostic tool while syndrome specific hybridization conferred specificity to the test.

This retrospective analysis of 13 continuous cases clinically diagnosed as endophthalmitis was performed to compare the microbiological concordance of results of SES test with those of culture, Gram’s stain and Calcofluor white staining for its sensitivity and concordance.

MATERIALS AND METHODS

Thirteen cases clinically diagnosed as endophthalmitis at Aditya Jyoth Hospital, Mumbai between January 2009 and October 2013, in whom SES evaluation was carried out were included in this retrospective analysis. Initial evaluation of these patients were conducted using culture, Gram’s stain and Calcofluor white staining. The SES evaluation was advised on the day of vitrectomy in cases where no clinical improvement was noted. The vitrectomy samples or Aqueous taps were sent to XCyon lab in Bangalore wherein SES analysis was done and results were conveyed to the surgeon within 24 hours after the vitrectomy. The treatment in these 13 cases was then decided based on the SES results.


a) **Nucleic acid extraction**: Nucleic acid was extracted from the standard strains using commercial columns (Qiagen, USA) as per the procedure specified in the instruction manual provided by the manufacturer.

b) **Nucleic acid amplification**: Nucleic acid amplification was standardized in a 50µl volume containing 4 mM magnesium chloride, 0.2 mM deoxynucleoside triphosphates, 50 to 300 nM concentration of each primer set and 1U of Taq polymerase (ABI, USA). The initial denaturation step was carried out at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 45 seconds, annealing at 60°C for 45 seconds and extension at 72°C for 45 seconds in a thermal cycler (Bio-Rad, UK).

c) **Hybridization**: Signature gene sequences chosen as probes for each of the pathogen were commercially synthesized (Metabion Inc., Germany). 20µM of probes for each of the pathogen were transferred onto a pre-determined position on the SES platform according to the templates. The SES platform comprised of a plastic frame mounted on a charged membrane on to which probes were arrayed at predetermined positions For each gene amplified a single probe was used for hybridization. The whole process from Gene Extraction to results takes 7 hours.
Table 1: Results of diagnostic evaluation of samples obtained.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample</th>
<th>SES</th>
<th>Culture</th>
<th>Gram’s Staining</th>
<th>Calcofluor Staining</th>
<th>Vision before treatment</th>
<th>Vision after 3 months of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vitreous humor</td>
<td>Gram Positive Bacteria</td>
<td>Negative</td>
<td>negative</td>
<td></td>
<td>PI Pr</td>
<td>6 / 60</td>
</tr>
<tr>
<td>2</td>
<td>Vitreous humor</td>
<td>Negative</td>
<td>Negative</td>
<td>negative</td>
<td>few fungal filaments</td>
<td>PI Pr</td>
<td>Hm Cf</td>
</tr>
<tr>
<td>3</td>
<td>Vitreous &amp; Tissue</td>
<td>Negative</td>
<td>Negative</td>
<td>negative</td>
<td></td>
<td>PI Pr</td>
<td>no Pl</td>
</tr>
<tr>
<td>4</td>
<td>Vitreous tap</td>
<td>Gram Positive Bacteria</td>
<td>Negative</td>
<td>negative</td>
<td>Negative</td>
<td>Hm</td>
<td>6./9</td>
</tr>
<tr>
<td>5</td>
<td>Vitreous asp</td>
<td>Gram Positive Bacteria Streptococcus/ Enterococcus</td>
<td>enterococci</td>
<td>Negative</td>
<td></td>
<td>PI Pr</td>
<td>no Pl</td>
</tr>
<tr>
<td>6</td>
<td>Vitreous asp</td>
<td>Negative</td>
<td>negative</td>
<td>negative</td>
<td>Negative</td>
<td>Cf 1 mt</td>
<td>Cf 3mt</td>
</tr>
<tr>
<td>7</td>
<td>Vitreous Tap</td>
<td>Gram Positive Bacteria</td>
<td>Negative</td>
<td>negative</td>
<td>Negative</td>
<td>Missing data</td>
<td>Missing data</td>
</tr>
<tr>
<td>8</td>
<td>Vitreous tap</td>
<td>Negative</td>
<td>Negative</td>
<td>negative</td>
<td>Negative</td>
<td>PI Pr</td>
<td>Hm Cf</td>
</tr>
<tr>
<td>9</td>
<td>Vitreous tap</td>
<td>Negative</td>
<td>Negative</td>
<td>negative</td>
<td></td>
<td>6./60</td>
<td>6./24</td>
</tr>
<tr>
<td>10</td>
<td>Capsular bag</td>
<td>CONS, Candida, E. aerogenes, P. aeruginosa*</td>
<td>Candida</td>
<td>negative</td>
<td></td>
<td>6./24</td>
<td>6./24</td>
</tr>
<tr>
<td>11</td>
<td>Vitreous tap</td>
<td>Negative</td>
<td>Negative</td>
<td>negative</td>
<td></td>
<td>6./60</td>
<td>6./6</td>
</tr>
<tr>
<td>12</td>
<td>Vitreous tap</td>
<td>S. aureus positive</td>
<td>Negative</td>
<td>negative</td>
<td>Negative</td>
<td>Cf 2 M</td>
<td>CF 2 M</td>
</tr>
<tr>
<td>13</td>
<td>Vitreous tap</td>
<td>Varicella Zoster Virus</td>
<td>Negative</td>
<td>negative</td>
<td></td>
<td>Cf 1 mt (6 weeks post op)</td>
<td>Cf 1 mt (6 weeks post op)</td>
</tr>
</tbody>
</table>

Sensitivity 53.85% 15.38% 0% 7.69%

*CONS: coagulate-negative staphylococci; E. aerogenes; Enterobacter aerogenes; P. aeruginosa; Pseudomonas aeruginosa. Abbreviations: PI - perception of light; Pr – projection of rays; Hm – hand motion in front of face; Cf – counting of fingers

Patients were reassessed three months after discharge. Institutional review board (IRB) approval was obtained for this analysis.

RESULT

The study sample included 61.5% men and 38.5% women, with a mean age of 60.62 years (range: 28 years -78 years). Three of these patients had been diagnosed with diabetes while two of them had hypertension. One patient had both diabetes and hypertension. The results of the diagnostic evaluation have been given in table 1.

As can be seen from the table 1, 7 out of 13 cases were positive for bacteria or fungi or virus when tested on SES. Out of Seven positive two were also culture positive and concordant. Three out of these seven positives did not improve in visual acuity while two cases improved and in two cases there was deterioration in visual acuity noted. Six out of the 13 cases SES did not detect any organisms. It can also be seen that 5 out of six negatives improved in visual acuity with treatment.

DISCUSSION

Along with conventional culture techniques, molecular biology techniques have now become an important complementary diagnostic method in the diagnosis of endophthalmitis.

or difficult to grow owing to their intrinsic properties, presence in a small inoculum, sequestration on prosthetic materials, or inactivation by prior antibiotic therapy[8].

While the syndrome specific hybridization made possible with the SES method allows for higher sensitivity, re-naturation of amplified signature gene to its chemically identified complementary gene sequence on the SES ensures higher specificity[7]. However the organisms detected in SES- Endophthalmitis cover most of the pathogens known to cause postoperative endophthalmitis and endogenous endophthalmitis

In the current analysis, the SES assisted detection rate was 3.5 times higher than that of culture (sensitivity with SES and culture was 53.85% and 15.38%, respectively) while the concordance with culture was 100% (SES was able to detect enterococci and candida as noted with culture results). Additionally, SES was able to detect the following pathogens in comparison to culture testing:

i) Enterococci and Gram positive bacteria Streptococcus

ii) E. aerogenes, P. aeruginosa and Coagulase-Negative Staphylococcus

In a similar retrospective review of consecutive cases with infective endogenous endophthalmitis in Hong
Kong, the overall culture positive rate with intraocular specimen was 24% and was considered to be relatively low by the authors. They also noted that such a low rate would make it difficult to arrive at a microbiological diagnosis in endogenous endophthalmitis[6].

Discordance was noted with 1 case of calcofluor white staining showing few fungal filaments. However, the sample was culture negative, as well as SES negative. Viral detection was possible in one case with SES wherein the presence of varicella zoster virus (as suspected) was identified in the vitreous sample.

The recruitment of cases discussed in this report occurred after an initial trial of intra-ocular antibiotic. Only the cases with no clinical improvement were taken in to this study and thus the improvements seen due to SES guided therapy are not dramatic. Molecular diagnostics like SES, if used early enough during the episode of endophthalmitis, may therefore prove to be crucial for better patient outcomes. The treatment decisions were taken based on the results obtained following SES. Accurate diagnosis followed by early therapy improves prognosis, especially in rare conditions such as endogenous endophthalmitis[9]. Molecular diagnostic techniques promise lesser diagnostic times with more accurate diagnosis than other modalities. Such methods would enable initiation of targeted therapies at a much earlier stage of the disease to ensure better prognosis[2].

CONCLUSION
In conclusion, SES is an accurate, quick and reliable diagnostic method in endophthalmitis whose sensitivity is much higher than culture and Gram’s staining. This diagnostic technique can help in administering targeted therapies at a much earlier stage and hence improve the overall outcome of the endophthalmitis cases. Although this study involved a small number of patients, a larger study with more number of patients is required to accurately assess the impact of SES in patient outcomes.

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COMPETING INTERESTS
None declared.

REFERENCES

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