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# Importance of accurate sampling techniques in microbiological diagnosis of endophthalmitis

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RESEARCH

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## Abstract

### Background

Endophthalmitis is an ocular emergency and bacteria are the commonest aetiological agents of infectious endophthalmitis. Any delay in treatment will result in serious complications like complete loss of vision. Therefore, obtaining the most appropriate sample is of paramount importance for a microbiologist to identify the aetiological agents that help the ophthalmologist in planning treatment.

### Objective

This study was undertaken to determine the intraocular specimen that is most likely to yield a positive culture on microbiological examination.

### Methods

From 60 cases, intraocular samples were collected in the operation theatre under anaesthesia. The samples obtained were aqueous humour and vitreous humour by vitreous tap, vitreous biopsy or pars plana vitrectomy. The specimens were processed within half an hour, first by inoculating onto culture media and then direct smear examination by Gram's Stain

### Results

Eighty samples were obtained from 60 cases of which the most were vitreous fluid (vitreous biopsy/tap + vitrectomy fluid), i.e., 75%. Culture was positive in 88% vitrectomy fluid as compared to 74% in vitreous tap/biopsy followed by 20% in aqueous fluid.

### Conclusions

Vitrectomy fluid appears to be the best sample for culture from clinically diagnosed endophthalmitis cases.

Key words: Endophthalmitis, vitreous fluid, sampling techniques.

## Background

Endophthalmitis is the most challenging complication seen in ophthalmic practice.[1] This clinical entity without early and timely intervention and appropriate therapy leads to rapid loss of vision and blindness. Despite various research and the best therapeutic efforts, even today the prognosis remains extremely variable.

As endophthalmitis is not a single entity, but a heterogeneous group of infections with diverse aetiological agents, it is of foremost importance to establish a rapid, accurate diagnosis. Inability to diagnose and treat endophthalmitis promptly amounts to neglect of the standard of care and this is grievous. [2]

Endophthalmitis is an inflammatory process that involves the ocular cavity and adjacent structures including the central cavity of the eye which is filled with vitreous fluid and surrounding tissues like the choroid and retina that are responsible for vision.[3]Bacteria are the commonest aetiological agents and clinical as well as experimental

studies have firmly established that delay in therapy will result in poor visual outcome, especially in severe cases. [4]

Culture of intraocular specimens like aqueous humour, vitreous humour and vitrectomy fluid, is still considered the gold standard in the diagnosis of endophthalimitis. Nevertheless, even under the most appropriate care traditional microbiological methods yield positive results in only 25–60% of the clinically diagnosed typical cases.[5] Accurate, appropriate and adequate sampling is the key to precise microbiological diagnosis and its importance cannot be overemphasized especially in the case of endophthalmitis where management is tailored by the laboratory report and confirmation.

Hence this study was undertaken to determine the intraocular specimen that is most likely to yield a positive culture on microbiological examination.

## Method

A prospective study of three years was conducted between February 2004 and February 2007 in the Department of Microbiology. A total of 60 clinically diagnosed cases of endophthalimitis who presented to a regional institute of ophthalmology were included in the study. Informed consent was taken for diagnostic procedures and management. Ethical clearance has been obtained from the Institutional Ethical Clearance Committee.

The samples were collected by the ophthalmologists in the operating theatre within six hours of presentation in the case of adults and up to one day in the case of children. For adults, ocular fluids were collected under local retrobulbar or peribulbar anaesthesia and for children; it was collected under general anaesthesia.

The following specimens were collected:

I. Aqueous humour

Aqueous tap was obtained by a paracentesis using a 26 or 27 gauge half an inch needle mounted on a tuberculin syringe. About 0.1 ml of fluid was withdrawn.

II. Vitreous humour

This was collected either by vitreous tap or biopsy or pars plana vitrectomy.

Vitreous tap

This was done by insertion of a 22 to 27 gauge needle attached to a 1ml tuberculin syringe through the limbus or parsplana region into the cavity and aspiration of 0.1 to 0.3ml of undiluted vitreous humour.

Vitreous biopsy

This consists of removal of 0.2–0.3 ml of vitreous humour with a vitrectomy aspiration cutter inserted through a pars plana incision.

Vitrectomy fluid

Here, vitreous infusion fluid is collected by parsplana vitrectomy. As this fluid is highly diluted, the vitrectomy sample was concentrated by centrifuging at 2000rpm for 15min, the supernatant carefully removed and the sediment used for further processing.[6,7]

Transport of the specimen

After the collection of the specimen, a sterile disposable needle was immediately fixed to the syringe containing the sample. The needle was then capped with a sterile rubber bung and placed in a sterile test tube container and was sent to the laboratory, preferably within half an hour of collection.[6]

The specimens were processed immediately first by inoculating onto culture media and then direct smear examination by Gram's Stain to avoid contamination.

## Results

Table 1 shows that all the 60 cases underwent diagnostic aspirations and in 33% (20 cases) both aqueous and vitreous samples were sent for microbiological work-up, in 23 cases (38%) only vitreous fluid was sent and in 17 cases (29%) vitrectomy fluid was sent. A total of 80 samples were sent of which 20 samples (25%) were aqueous fluids, vitreous fluid in 43 cases (54%) and 17 cases (21%) samples were vitrectomy fluid.

Table 1: Distribution of samples in the study

| Sample | Sample | Percentage |
|---|---|---|
| Aqueous + vitreous tap | 20 | 33% |
| Vitreous tap | 23 | 38% |
| Vitrectomy fluid | 17 | 29% |
| Total | 60 | 100% |

Table 2 shows that a total of 80 samples were collected and processed from 60 patients. In post-traumatic cases 12 (60%) aqueous fluid, 30 (70%) vitreous fluid and 8 (47%) vitrectomy fluids were sent for examination. In post-operative cases, 7 (35%) aqueous fluids, 11 (26%) vitreous fluid and 9 (53%) vitrectomy fluid were sent to the laboratory. In endogenous type, 1 (05%) aqueous fluid and 2 (04%) vitreous fluids were sent to the laboratory for microbiological work-up.

Table 2: Samples collected from different clinical types of endophthalmitis

| Type of endophthalmitis | Aqueous fluid | % of total | Vitreous fluid | % of total | Vitrectomy fluid | % of total |
|---|---|---|---|---|---|---|
| Post-traumatic type | 12 | 60 | 30 | 70 | 8 | 47 |
| Post-operative type | 07 | 35 | 11 | 26 | 09 | 53 |
| Endogenous type | 01 | 05 | 02 | 04 | – | 00 |
| Total | 20 | 100 | 43 | 100 | 17 | 100 |

Our study showed that the direct microscopy results correlated 100% with the culture results. Table 3 shows that the culture was positive in 88% of vitrectomy fluid followed by vitreous tap/biopsy fluid 74%. Aqueous fluid yielded growth in only 20% of the samples.

Table 3: Comparison of culture and gram stain on endophthalmitis

| Sample | Total | Gram stain Positive | Culture Positive | Gram stain negative | Culture negative |
|---|---|---|---|---|---|
| Aqueous Fluid | 20 | 4 (20%) | 04 (20%) | 16(80%) | 16(80%) |
| Vitreous Fluid | 43 | 32 (74%) | 32 (74%) | 11 (26%) | 11 (26%) |
| Vitrectomy | 17 | 15(88%) | 15(88%) | 02(12%) | 02(12%) |

## Discussion

Rapid diagnosis and accurate treatment are very essential for successful management of endophthalmitis, therefore appropriate sample collection is important to isolate the causative micro-organisms. Although initial management of endophthalimitis with antibiotics may not be dependent on microbiological results, subsequent modification and tailoring of treatment requires identification and the susceptibility pattern of the infecting organisms so that suitable treatment is given.

Eighty samples were obtained from 60 cases of which the maximum were vitreous fluid (vitreous biopsy (54%) + vitrectomy fluid (21%) that is 75%. The fewer number of aqueous taps sent when compared to vitreous could be because, in post-operative cases, it is risky and difficult to insert a needle in an inflamed recently operated eye, and it is also equally difficult to collect anterior chamber specimens following non-surgical trauma.[8]

Of the 80 samples, aqueous fluid showed smear positivity in 20% cases, vitreous fluid in 74% cases and vitrectomy fluid in 88% of the cases. Okumoto et al.[9] have reported that 80% of patients from whom a positive culture was obtained were also smear positive and hence performing a smear is worthwhile, since a smear can be stained and read in a matter of minutes, can be performed as a bedside investigation and a presumptive diagnosis can be obtained many hours before culture results are available. [9]

In our study, the culture positivity rate is 78% which is similar to studies done by Barza et al. [10] at 80% and Rowsey et al. [11] at 77%. In the endophthalmitis vitrectomy study (EVS) in 1996, culture positive cases were seen in 69.3% of cases which is less than compared to this study.[8]

*Contribution of each chamber to determination of infection*

The present study showed that vitreous fluid gave a culture positivity of 74% (32 of 43 samples); vitrectomy fluid gave a maximum culture positivity of 88% (15 of 17 samples). The aqueous fluid was culture positive in only 20% of cases (4 out of 20 samples) and yielded the same organisms as the vitreous sample. Maylath and Leopold in 1955 gave the first understanding of the pathophysiology of microbial endophthalmitis when they demonstrated that in a rabbit model, the aqueous and iris had the ability to eliminate infection but not the vitreous, indicating the need for a more conclusive culture positive diagnosis of endophthalmitis.[12]

Allensmith et al. in 1970 recommended that culture of the aqueous from an anterior chamber paracentesis would be a valuable tool in the diagnosis of the aetiology of postoperative endophthalmitis.[13] In 1972, Tucker and Forster confirmed the potential value of anterior chamber paracentesis in the diagnosis of endophthalmitis.[9]

The aqueous fluid did not contribute much towards diagnosis in the present study. The aqueous fluid was not culture positive in any of the cases when vitreous was negative which is similar to reports by Mandelbaum et al.[14] (26%), Weber et al. [15] (22%) and Mamalis et al. [16] (14%).

The anterior chamber appears to clear itself of organisms better than vitreous and also aqueous humour has been demonstrated to possess immunoglobulin and complement components that contribute to antimicrobial properties. This may explain why anterior chamber cultures are negative. [17]

Forster was the first to do diagnostic aspiration of vitreous, recognising the importance of the vitreous in the infection process. Further studies confirmed that in some cases positive vitreous taps and negative aqueous cultures were seen, hence it has been recommended that both aqueous and vitreous fluids be sampled to provide the maximum yield of positive culture in patients with endophthalmitis. [18]

Later on Maylath and Leopald in their experimental studies

using animal models demonstrated that the anterior chamber has a greater ability to resist an infecting agent than the vitreous does and hence the vitreous is an excellent culture medium.[19]

We found that undiluted vitreous culture was positive in 74% of cases which is similar to the study of Jain et al.[20] who had 88% positivity and the vitrectomy fluid was positive in 88% cases (15 of 17 samples) that is significantly higher when compared to Donahue et al.[21] who have reported 76% culture positivity. According to the study done by Madhavan et al.[6], the vitreous specimen is much more likely than the aqueous to offer an etiological diagnosis.

Vitrectomy fluid provides the maximum amount of specimen for diagnostic purposes and is most appropriate as it yields higher positivity, this could be because vitrectomy fluid provides a sufficient quantity of specimen for diagnostic purposes.

Hence, the present study revealed that the processing of both samples undiluted tap/biopsy and vitrectomy cassette fluid provided greater sensitivity.

## Conclusion

This study showed that in all cases of suspected endophthalmitis, the vitreous should be sampled either by vitreous aspiration or diagnostic vitrectomy as it is possible that bacteria are more protected in the vitreous humour than aqueous. Vitrectomy fluid appears to be the best sample for culture from clinically diagnosed endophthalmitis cases.

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PEER REVIEW

CONFLICTS OF INTEREST

None

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