

Review

Fungal endophthalmitis

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Endophthalmitis is a severe vision threatening intra-ocular infection. Endophthalmitis of fungal origin is mostly endogenous but may also occur following intra-ocular surgery, corneal ulceration or trauma. Clinically these conditions are difficult to diagnose and are often missed, without proper microbiological investigations. Hence, laboratory diagnosis is very important for the initiation of appropriate antifungal therapy. Whereas, laboratory diagnosis of fungal endophthalmitis is based mainly on conventional microbiological methods, these techniques are not sensitive enough for any aetiological conclusion to be established. The main reasons for this low sensitivity is minimal number of organisms in the eye, the small sample size, a tendency of these organisms to be loculated and inappropriate transport and storage of the samples, if collected during an emergency surgery when the laboratory is closed. Results of studies in India as well as abroad, indicate a low incidence of fungal endophthalmitis varying between 3-8%. Amongst the culture positive cases, *Aspergillus flavus* alone accounts for 36% of cases, followed by *Aspergillus fumigatus* in 20%, *A niger* in 10%, *Fusarium* in 21% and *Alternaria* in 13% cases. *Candida* and *Aspergillus* species, however, remain the predominant organisms isolated in endogenous endophthalmitis. *Histoplasma capsulatum var. capsulatum* and *Coccidioides immitis* are also reported to be the causative agents of metastatic endophthalmitis and should be considered in the clinical diagnoses of cases at places where these diseases are endemic as well as in patients who are immunosuppressed. In post-operative cases, *Aspergillus* species, *Fusarium* species, *Alternaria* species have been reported to be the common causative agents. *Aspergillus*, *Alternaria*, *Bipolaris*, *Acremonium*, *Fusarium* species mostly account for post-traumatic cases. A recent retrospective analysis of the laboratory records of all clinically diagnosed endophthalmitis cases during the period from 2001-2010 at our tertiary care eye centre revealed that fungi alone accounted for 11% all infectious endophthalmitis cases and 5 % of the cases had mixed etiology. Our data were in agreement with the findings of others in the USA who documented an overall positivity of fungal isolation amongst all cases of infectious endophthalmitis to be somewhere between 11 to 16 percent. Sensitive technique such as PCR of intra-ocular fluid has not only helped the clinician in timely administering appropriate antifungal antibiotics, but has also improved on the rapid laboratory identification and speciation of the fungal pathogens.

Keywords: Endophthalmitis, Clinical outcome, Fungal etiology, Post-operative endophthalmitis, Posttraumatic endophthalmitis, Metastatic endophthalmitis.

INTRODUCTION

Endophthalmitis is an inflammatory reaction of the intra-ocular fluid or tissues. Endophthalmitis can be infectious

or non-infectious. Infectious endophthalmitis is one of the most serious and vision threatening complications of ophthalmic surgery (Kresloff et al., 1998). Infectious endophthalmitis may be post-operative, post-traumatic or endogenous. As fungal endophthalmitis is always an emerging challenge before the ophthalmologist, it is very important to determine the prevalence and spectrum of

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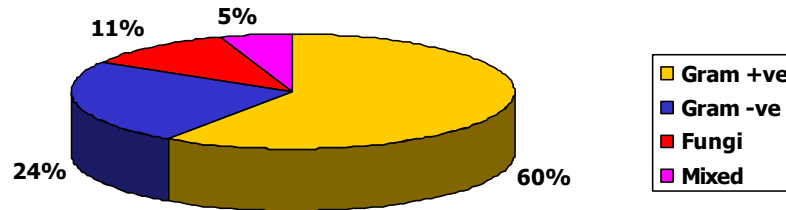


Figure1. Percentage isolation of fungi out of total culture positive isolates

the fungal agents causing endophthalmitis, to identify the risk factors involved and to assess the influence of fungal species on clinical presentation, therapeutic management and outcome of infection.

Spectrum of aetiological agents in fungal endophthalmitis

Reports from different sources account differently for the fungal aetiology of endophthalmitis. In post-operative cases, *Aspergillus* species, *Fusarium* species, *Alternaria* species have been reported to be the common causative agents (Kresloff et al., 1998; Kunimoto et al., 1999; Anand et al., 2000).

In endogenous endophthalmitis, *Aspergillus* species and *Candida* species have been incriminated as the commonest organisms (Kresloff et al., 1998; Samiy and D'Amico, 1996). *Aspergillus*, *Alternaria*, *Bipolaris*, *Acremonium*, *Fusarium* species mostly account for post-traumatic cases (Kresloff et al., 1998; Kunimoto et al., 1999). *Histoplasma capsulatum var. capsulatum* and *Coccidioides immitis* are also reported to be the causative agents of metastatic endophthalmitis and should be considered in the clinical diagnoses of cases at places where these diseases are endemic as well as in patients who are immunosuppressed (Gonzales et al., 2000; Culter et al., 1978).

A recent retrospective analysis of the laboratory records of all clinically diagnosed endophthalmitis cases during the period from 2001-2010 at our tertiary care eye centre revealed that fungi alone accounted for 11% all infectious endophthalmitis cases and 5% of the cases had mixed etiology (Figure 1). Our data were in agreement with the findings of others who documented an overall positivity of fungal isolation amongst all cases of infectious endophthalmitis to be somewhere between 11 to 16 percent (Kresloff et al., 1998; Katowitz, 1987; Boruchoff and Boruchoff, 1992).

In our series, *Aspergillus* species were the commonest agents in post-operative endophthalmitis and in endophthalmitis following keratitis, whereas *C. albicans* and *A. flavus* were mostly responsible for causing metastatic endophthalmitis. We further observed that *Fusarium* species and *A. niger* were found to be the commonest isolates in cases having mixed bacterial and

fungal infection. Dematiaceous fungi, however, were the most common agents obtained from cases of post trauma endophthalmitis (Table 1)

Relevant risk factors

The majority of the patients who develop endogenous endophthalmitis have a variety of conditions that may pre-dispose them to infection.¹ Most of the fungi require some form of host immunosuppression to cause endophthalmitis. Some of the relevant risk factors which predispose to endogenous endophthalmitis include diabetes mellitus, leukaemia, lymphoma, alcoholism, AIDS, prematurity, IV drug abuse, parenteral hyperalimentation and long term antibiotic therapy. Most important conditions which precipitate post-traumatic endophthalmitis include lens disruption, intra-ocular foreign body, plant and soil related injury, injury in a rural setting and penetration with an obviously contaminated device (Kresloff et al., 1998).

Post-operative endophthalmitis is however, governed by various conditions, which may be pre-operative, such as canaliculitis, dacryocystitis and contact lens use; intra-operative, like profound vitreous loss, prolonged surgery and inadequate eye lid/ conjunctival disinfection; and after the surgical procedure, in the form of wound leak or dehiscences, inadequately buried sutures following blebs and silicon lenses (Samiy and D'Amico, 1996).

Endophthalmitis sometimes may result from prolonged contact lens use (Rosenberg et al., 2006). There seems to be a higher correlation between fungal contamination of contact lens solution and development of fungal keratitis and endophthalmitis. Fungal endophthalmitis following penetrating keratoplasty is low (0.16%) (Benaoudia et al., 1999), but a higher correlation between fungal contamination of donor corneo-scleral rim and post-operative infection, mostly due to *Candida* species has been documented (Culter et al., 1978; Benaoudia et al., 1999).

Thus in most eye banks, cold storage at 4°C is practiced (Culter et al., 1978). Storage medium in most of the cases is tissue culture medium with antibiotics like gentamicin and streptomycin or amphotericin B, along

Table 1. Spectrum of etiological agents

Clinical diagnosis	Total no. of cases	Bacterial Isolates (%)	Fungal isolates (%)	Fungi as mixed isolates (%)
Post-operative	200	82 (41%)	16 (8%) A fumigatus 4 A flavus 4 Fusarium 4 Alternaria 4	2 (1%) Fusarium
Post-traumatic			106	38 (35.8%)
Metastatic	34	14 (41.1%)	6 (17.6%) C albicans 4 A flavus 2	-
Following keratitis	78	28 (35.8%)	4 (5.12%) A fumigatus 2 A flavus 2	2 (2.56%) A niger
Miscellaneous	652	218 (33.4%)	22 (3.37%) Fusarium 8 Alternaria 2 A flavus 4 A niger 4 A fumigatus 4	16 (2.45%) A flavus 4 A niger 4 Curvularia 4 Alternaria 2 Fusarium 2
Total	1070	380	50	24

with penicillin and streptomycin (Merchant et al., 2001; Mass-Reijs et al., 1997). During storage, a screening for any microbiological contamination of donor corneal medium is always employed. If contamination is detected, those are discarded. Viable non-contaminated donor corneas are further incubated for 24 hrs. and are used for surgery if no turbidity is noted. It is debatable if a prolonged period of warming of the donor button in storage medium could reduce the incidence of fungal contamination.

The method of disinfection of donor globes may also affect the rate of contamination of donor corneas. It is advocated that use of 1% solution of povidone-iodine to decontaminate the globe might result in significant reduction in microbial growth, especially that due to *Candida* species (Ainley and Smith, 1965).

Clinical features

Most of the symptoms of fungal endophthalmitis are similar to those seen in bacterial endophthalmitis. These include, blurring of vision, pain, photophobia and redness (Michaelson et al., 1971; Griffin et al., 1973). In addition, patient may present with some important external signs of inflammation which include ciliary congestion, chemosis, lid oedema, raised intra-ocular pressure, restriction of external ocular movement and proptosis. Other severe signs and symptoms include diminished visual acuity, altered pupillary defect, increased pain and redness, hypopyon, corneal oedema, corneal infiltrate,

retinitis, severe vitreous inflammation with persistent iritis alongwith visible puff balls and strands. However, there are certain features which are unique to some fungi. In *Candida* endophthalmitis, for example, there is a creamy, white, well circumscribed lesion involving the choroid and retina (Samiy and D'Amico, 1996). These lesions may be multiple, are most often located in the posterior pole and have associated retinal hemorrhage and perivascular sheathing. The vitreous may contain yellowish white opacities in the form of 'string of pearls' or puff balls (Kresloff et al., 1998; Samiy and D'Amico, 1996). It is also true that, severe vitreous inflammation with persistent iritis along with whitish puff balls and strands, in association with hypopyon and optic nerve edema are quite characteristic of endophthalmitis due to mycelial fungi (Kresloff et al., 1998).

Pathology

In contrast to bacterial infections, fungal infections of the vitreous progress slowly and remain localized for a longer duration and tend to form multifocal microabscesses. Invariably the inflammatory response is granulomatous, characterized by a predominant mononuclear cell infiltration, although at times *Candida* and *Aspergillus* may give rise to suppurative type of lesions. The inflammatory process gets exacerbated once these infiltrates reach vascular structures such as iris and ciliary body. Progressive inflammation of the ciliary body and vitreous may potentiate further damage due to

proliferation of the ciliary epithelium accompanied by capillary and fibrous tissue proliferation. Such fibrovascular proliferative disorder exerts traction on the ciliary body, resulting in ciliary and choroidal detachment. Inflammatory exudates in the vitreous may cause structural changes leading to liquefaction, opacification, posterior vitreous detachment and eventually abscess formation.

Laboratory diagnosis

Prompt and rapid laboratory diagnosis is very important in endophthalmitis, because this condition is invariably vision threatening. Delay in the management may lead to dreaded complications with the compulsion to eviscerate the eye ball. Therefore, when the patient presents with signs and symptoms suggestive of infectious endophthalmitis, the best approach is to obtain intra-ocular sample for microbiological investigation. Secondly, any post-operative inflammation, which is more severe than is normally expected after intra-ocular surgery and is unresponsive to a course of intensive topical corticosteroids is always suspicious of infective origin and requires immediate culture of intra-ocular fluid. Thirdly, vitreous opacities with strands and the presence of hypopyon after intra-ocular surgery are strong indications for vitreous biopsy and culture of the intra-ocular sample. Hence in all the above clinical settings, samples should be collected without delay so that prompt laboratory diagnosis can be made.

Collection and transport of specimens

Both anterior chamber (AC) and vitreous aspirates are ideal samples for the laboratory diagnosis of this condition. However, AC aspirate is not that helpful as vitreous sample. About 0.2 to 0.3ml of the intra-ocular fluid is collected with the help of sterile tuberculin syringe and 22 gauge needle. The AC is tapped via the limbus. The needle should be removed before the specimen is submitted in order to avoid the chances of danger to laboratory personnel. However, the nozzle of the syringe should be sealed with a sterile rubber bung and the whole set should be transported to the microbiology laboratory, soon, for processing. Vitreous fluid is collected either by vitreous needle tap or by vitreous biopsy. Vitreous needle tap is best collected by a sterile tuberculin syringe and a 22 gauge needle by approaching the anterior portion of vitreous cavity through pars plana region. About 0.1 to 0.3ml of fluid is collected by manual aspiration.

Occasionally, it is not possible to collect vitreous aspirate by simple needle tap because of severe vitreous inflammation. Hence, vitreous biopsy is collected, with a vitrectomy cutting/aspirating probe, which is attached to a

tuberculin syringe and needle. Vitreous cavity is reached through pars plana approach. Nearly 0.2 to 0.3ml material is obtained by manual aspiration into the syringe during the activation of the cutting mechanism. The specimen is sent to the laboratory, preferably undiluted to increase the yield.

Aqueous and/or vitreous specimens thus collected are usually sent to the laboratory for further processing without any delay. In case of delay, one should take care that the sample is not refrigerated. It is always advisable to preserve the sample in a 25°C BOD incubator, till it is processed. Either the aqueous or vitreous or both can be cultured as recommended (Allansmith et al., 1970; Koul et al., 1990). However, culture yield is more with vitreous than with aqueous (Koul et al., 1990). Often therapeutic vitrectomy has proved an alternative modality for obtaining vitreous for microbiological investigations.

Processing of the sample and identification of the fungal pathogen

The procedure of microscopic examination and culture is the same as used routinely for any other ocular specimen described elsewhere (Das et al., 1995) with minor variations, which are described below. Smear examination is, no doubt, a rapid method for the diagnosis (Das et al., 1995), but it is very less sensitive; more so for fluid specimens like aqueous and vitreous, in which the organisms are as such diluted. The commonly used Gram's and Giemsa's staining techniques have 60% and 41% sensitivities respectively (Sharma et al., 1996). Calcofluor white fluorescent staining has a better sensitivity as compared to Gram staining procedure. However this needs expertise and facilities for a fluorescent microscopy.

The conventional culture does not require any selective media as aqueous and vitreous are normally sterile body fluids. However, concentrating the specimen by passing it through a membrane filter system is advocated for better yield of the micro-organism (Das et al., 1995). The vitrectomy specimen is first processed by passing through a 0.2µm membrane filter. The filter is then removed aseptically and cut into segments for direct inoculation onto the culture media. Processing of both vitreous biopsy and vitrectomy cassette fluid by this technique provides greater yield (Sharma et al., 1996).

Limitations of fungal culture in endophthalmitis

There are certain limitations to culture in establishing an etiological diagnosis of fungal endophthalmitis. First of all the sample size is very small, and organisms being in a fluid sample are diluted and small in number. So a little delay in processing may result in loss of viability of the organisms. Secondly, fungi are ubiquitous and chances

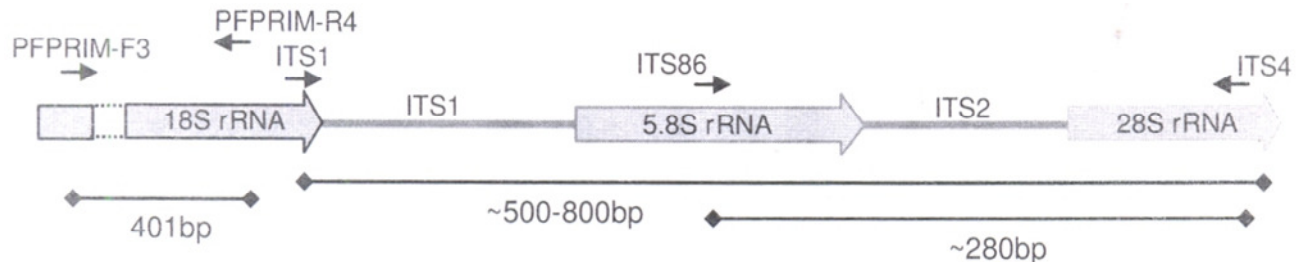


Figure 2. Schematic representation of the fungal genome

of laboratory contamination can be a possibility. So one should always inoculate more than one fungal culture media for the same sample. Unlike in case of fungal keratitis, repeat sampling is not possible in this case for confirmation of the etiological agent. Thirdly, review of the major reports in the literature shows that only 64% of vitreous specimens obtained from eyes with clinical diagnosis of endophthalmitis are culture positive²⁰. Fourthly prior use of antibiotics may yield negative results in culture and lastly it should always be remembered that fungi are slow growing organisms and so there is always a time lag between processing of the sample and getting a positive culture.

Molecular methods for the diagnosis of endophthalmitis

Owing to the major disadvantages of the conventional culture method as mentioned above, molecular assays such as Polymerase Chain Reaction (PCR) in the rapid diagnosis of fungal endophthalmitis seems quite promising. In most of the works conducted earlier, the fungal ribosomal DNA gene cluster (Odds, 2003) has been chosen as the target for amplification in the PCR assay. The ribosomal DNA (rDNA) gene is a tandem array of at least 50-100 copies in the haploid genome of all fungi. It comprises the subunits; r DNA (18S) gene, the 5.8 S gene and the large subunit r DNA (28S) gene (Chen et al., 2002). Separating the 18S and 5.8S, is ITS1 region and separating the 5.8S and 28S subunits, is the ITS2 region. These two are called internal transcribed spacer regions. The intergenic spacer region (IGS), however, occupies the site between each of these set of transcripts. It has been documented that rDNA genes (coding regions) are highly conserved, the ITS regions are moderately variable and the IGS region is highly variable between different fungi (Reiss et al., 1998) (Figure 2). This has led the researchers in the designing of universal primers based on the conserved regions, which will amplify the rDNA gene cluster from a large number of fungal species, and of species specific primers/probes based upon the variable regions, that can be used to identify and differentiate the various fungal species. At

least 16 *Candida* and 5 *Aspergillus* species specific probes have been designed based upon this principle (Klotz et al., 2000; Elie et al., 1998).

A recent study from India (Anand et al., 2001a) reported that in intra-ocular specimens, PCR detected fungi in more number of samples, which were negative by the conventional method. Average time required for culture was 10 days, whereas PCR needed only 4 hours. The same group of workers (Anand et al., 2001b) in another study observed that PCR was more sensitive and rapid as compared to the conventional microbiologic methods for diagnosing fungal endophthalmitis. In yet another study (Hidalgo et al., 2000), the observers noted that PCR was positive in all the four patients of suspected endophthalmitis, by using species-specific PCR for *Candida* in the vitreous samples, while the culture of vitreous was negative in two specimens.

Therefore, apart from being a highly sensitive and rapid technique, PCR is, no doubt, a very useful laboratory tool, especially in the tropical countries, where incidence of fungal eye infections is quite high. Due to its rapidity, PCR not only helps the Ophthalmologist in planning the effective and quick therapeutic measures, but also aids in accurately diagnosing the cases with ultimate impact on the prognosis of the patients. This is especially true for post-operative endophthalmitis following cataract surgery, which is the commonest form of fungal endophthalmitis, where early diagnosis is very important for effective management of the cases in order to avoid severe and vision threatening ocular morbidity. Investigating on the entity of post-operative endophthalmitis, researchers (Tarai et al., 2006) from India recently observed that PCR, for the detection of fungal DNA, was found to be a rapid and a more sensitive method compared to the conventional culture methods for the early diagnosis of this condition. Over and above, use of species specific primers. (Reiss et al., 1998; Jaeger et al., 2000; Ferrer et al., 2003; Mancini et al., 2005) as discussed above, has added further to the accuracy and effectiveness of this molecular tool for detecting various fungal pathogens up to the species level in the intraocular samples. Both the ophthalmologist and the microbiologist will surely benefit from the outcome of using this method so far as better prognoses of their patients are concerned.

Table 2. Amphotericin B (Amp B) for intraocular injection (7.5 μ gm/0.1 ml)

a.	Inject 10ml water into the bottle with 50 mg dry AmB. Shake until transparent (50 mg Am B)
b.	Take 1 ml of this into a 10-ml syringe (5 mg Am B)
c.	Dilute with water to 10 ml, shake well
d.	1 ml of the above(500 μ gm Am B) is diluted with water to 6.7 ml and must be shaken (74.64 μ g/ml Am B)
e.	Take 0.1 ml of this with an insulin syringe (7.4 μ g Am B).

Recommended therapy

Intravitreal injection

Historically, amphotericin B has been the preferred antifungal agent in fungal endophthalmitis. Systemically delivered amphotericin B does not achieve the required therapeutic concentrations in the eye, even in the presence of intraocular inflammation. However, intravitreal injection of the drug achieves therapeutic concentrations and limits systemic toxicity. The recommended dosage of intravitreal amphotericin B is 7.5 μ g in 0.1 ml (prepared from a 50 mg vial by serial dilutions). Table 2 illustrates the details of the steps for preparation of intravitreal formulation of amphotericin B.

Systemic therapy

Ketoconazole 200mg t.i.d. is also advocated. However, intravenous amphotericin B, despite its toxicity is the most effective. This is administered by I.V. infusion in 5 % dextrose solution, starting with 0.25 mg/kg body weight on the 1st day, gradually increasing by 0.25 mg/kg body weight/day till a total dosage of 0.6 mg/kg is achieved.

Recently newer antifungal agents have expanded the treatment armamentarium. Voriconazole, a new triazole compound possesses an excellent oral bioavailability and intraocular penetration. Recent reports have suggested that voriconazole may have a broader spectrum of antifungal activity compared with amphotericin B (Ozbek et al., 2006; Prakash et al., 2008). Even other newer antifungal agents like posaconazole, another triazole with excellent oral bioavailability and intraocular penetration; caspofungin, an echinocandin derivative administered parenterally have been used successfully in managing intraocular fungal infections (Charles et al., 2008).

Influence of fungal species on clinical presentation, therapeutic management and outcome of infection

The therapeutic implications of this vision threatening condition is often influenced by the various fungal species causing the infection. Endophthalmitis due to *Candida*

species is well documented, both as a consequence of fungemia and as a result of dissemination from endogenous source in an immunocompromised individual. The situation is even more alarming in cases of endophthalmitis caused by species of *Candida* other than *C. albicans*. These non-*C. albicans* species are reportedly showing in vitro resistance to fluconazole. In addition, *C. tropicalis* is intrinsically resistant to many azole compounds. Thus newer azoles such as voriconazole and posaconazole as discussed above, seem to be quite promising for the sake of management of such deep and recalcitrant cases of fungal infections.

Intra-ocular signs and symptoms such as diminished visual acuity, severe vitreous inflammation with persistent iritis, whitish puff balls and strands are more commonly seen in endophthalmitis due to *Candida* and *Aspergilli*. In addition, infection due to *C. albicans* may result in choroidal neovascularisation, which is a potential cause of late visual loss in patients who have had sepsis and endogenous chorioretinitis due to this organism.

CONCLUSION

Owing to modern therapeutic management facilities and increasing number of immunocompromised patients, fungal infections in general and ocular fungal infections in particular are emerging as major threats to the clinicians. Out of all oculomycoses, endophthalmitis is the most vision threatening. Thus our research needs to focus entirely on improvement in the diagnostic techniques, development of new antifungal agents and standardization of their sensitivity testing techniques. and a better understanding of the pathogenesis of this condition. In all tertiary care hospital settings, laboratory control of antifungal sensitivity testing is very important because of the therapeutic implications involved in some cases of endophthalmitis caused by certain classes of fungi.

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