A REPORT ON MICROBIOLOGICAL INVESTIGATION OF FUNGAL KERATITIS

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ABSTRACT

Purpose: To investigate the efficacy of various microbiological methods in the diagnosis of fungal keratitis and report the types of fungi involved. Methods: A retrospective review of microbiology records of fungal keratitis patients seen at L V Prasad Eye Institute, Bhubaneswar. All patients were investigated for detection of bacteria, fungi or parasitites as per a uniform institutional protocol which included examination of the corneal scrapings or tissue by direct microscopy and culture. The study period was between November 2006 and December 2009. Results: A total of 275 patients were diagnosed as microbiologically proven fungal keratitis during this period. The samples included either corneal scrapings (224) or corneal tissue (11) or both (33) and anterior chamber exudates or eviscerated contents (7). Detection of fungal filaments in 10% KOH/Calcoflour white (94.24%) was better in comparison to Gram stain (77.9%) and Giemsa stain (77.57%). Aspergillus species (28%) and Fusarium species (23.2%) were the major isolates. Concomitant bacterial infection was seen in 16.7% cases of mycotic keratitis and Staphylococcus species (34%) was the predominant bacterial pathogen. Conclusions: Potassium hydroxide with calcofluor white is highly efficient in demonstration of fungal elements in corneal scrapings. Aspergillus and Fusarium are the predominant genera of fungi involved. Simple laboratory techniques are useful for definite diagnosis of fungal keratitis.

Key words: Fungal keratitis, microscopy, culture, Aspergillus, Fusarium

Introduction

Enormous data is available regarding fungal keratitis from different parts of the world, some of which are population based studies and some deal with predisposing factors and causative agents.[1-6] The subtropical Indian climate is best suited for fungal keratitis and reports are published from all over the country, particularly large numbers from southern part of the country.[5-7] Although ophthalmologists at all levels of eye care come across cases of fungal keratitis, the tertiary eye care centres are likely to receive many of them because of referrals and are usually better equipped to investigate, analyze and present the local trends for the benefit of the ophthalmologists in a specific geographic area. A modest microbiology laboratory with basic facilities can help make definite diagnosis. Relatively simple techniques suffice to make a diagnosis of bacterial, fungal or parasitic keratitis. A common protocol is recommended for the purpose since there is considerable clinical overlap among these cases.[18]

At this point of time, reports on fungal keratitis from Eastern India are few. We present the data of first 275 patients of fungal keratitis who were referred to our four year old tertiary eye care centre from different parts of Orissa and neighboring states. Prior to commission of our institute, facilities of ocular microbiology service were not available or were sparsely available in Orissa.

Materials and Methods

A retrospective analysis of microbiology records was performed for all corneal scrapings received in the ocular microbiology service between November 2006 and December 2009. Corneal scrapings were collected by the treating ophthalmologist after a detailed ocular examination. Under aseptic conditions using sterile blade (No. 15) on Bard Parker handle,[18] the procedure was performed under magnification of slit lamp or operating microscope after instillation of 0.5 % proparacaine. Multiple scrapings were taken which were made in to smears on glass slides and inoculated on variety of culture

media (Table 1). Corneal tissue, when received was cut in to pieces to inoculate various media and no microscopic examination was done.

Table 1. Corneal scraping smear examination methods and culture media

Smear Examination Staining Methods	Culture Media	Expected organisms
l Gram l Giemsa l KOH+Calcofluor white	l Sheep blood agar (aerobic, anaerobic) l Sheep blood chocolate agar l Brain heart infusion broth l Sabouraud dextrose agar l Thioglycollate broth l Non-nutrient agar with E.coli	l Bacteria (aer-obic, anaerobic) l Fungi l Acanthamoeba l Microsporidia

All inoculated media were incubated aerobically. The Sabouraud dextrose agar (SDA) was incubated at 27°C, examined daily, and discarded at 2 weeks if no growth was seen. The inoculated blood agar, chocolate agar, thioglycollate broth, and brain heart infusion broth were incubated at 37°C, examined daily, and discarded at 14 days if no growth was seen. The inoculated non-nutrient agar plates were incubated at 37°C after overlaying with Escherichia coli suspension and were examined daily for the presence of Acanthamoeba trophozoites and cysts under the microscope and discarded at one week if there were no signs of growth. Microbial cultures were considered significant if growth of the same organism was demonstrated on more than one medium, and/or if there was confluent growth at the site of inoculation on one solid medium, and/or if growth of one medium was consistent with direct microscopy findings and/or if the same organism was grown from repeated scraping.[8]

Fungus grown in other media but not grown in SDA, was subcultured onto an SDA medium and incubated for a period of 15-21 days to facilitate sporulation. Following adequate growth of the fungal isolate on SDA, identification was done based on its macroscopic and microscopic features.

Results:

During the study period a total of 1490 clinical samples were received in ocular microbiology laboratory, out of which 318(21.3%) were positive for fungal culture. From the 318 positive samples 275 (86.4%) were from keratitis patients. Of the 275 of keratitis patients 193 (70.1%) were clinically diagnosed as fungal, 28 (10.1%) as bacterial, 4 (1.4%) as viral, 1 (0.3%) as Acanthamoeba, and 53(19.2%) as non- specific microbial keratitis. The samples included either corneal scrapings (224) or corneal tissue (11) or both (33) and anterior chamber exudates or eviscerated contents (7). Detection of fungal filaments in 10% KOH/Calcoflour white (94.24%) was better in comparison to Gram stain (77.9%) and Giemsa stain (77.57%). Figure 1a shows fungal filaments in the corneal scraping of a patient with fungal keratitis. Corresponding chocolate agar (Fig.1b) and blood agar (Fig.1c) show fungal as well as bacterial colonies that were identified as A. flavus and Staphylococcus sp. SDA (Fig.1d) from the same patient shows confluent typical colonies of A. flavus.

Put together, microscopy and culture for fungus was positive in 275 patients, however, culture alone was positive in 228 (82.9%) cases. Table 2 shows the types of fungi that were isolated from the samples. Aspergillus species (28%) and Fusarium species (23.2%) were major isolates. Among the Aspergillus, A.flavus (36) was most common and among the Fusarium, F.solani (24) was most common. Concomitant bacterial infection was seen in 47(16.7%) cases of mycotic keratitis. and Staphylococcus species 16 (34%) was the predominant bacterial pathogen.

Table 2. Distribution of various fungal species in patients with mycotic keratitis (n=228)

Sl. No.	Fungal isolates	Number	Percentage (%)
1.	a. A. flavus	36	15.78
	b. A. fumigatus	14	6.14
	c. Other Aspergillus species	14	6.14
2.	a. F. solani	24	10.53
	b. Other Fusarium species	29	12.72
3.	Acremonium species	10	4.38
4.	Colletotrichum species	3	1.32
5.	Trichosporon species	2	0.87
6.	Penicillium species	2	0.87
7.	Paecilomyces species	1	0.44
8.	Rhodotorula glutinis	1	0.44

9.	Unidentified hyaline fungus	23	10.09
10.	Curvularia species	2	0.87
11.	Curvularia lunata	6	2.63
12.	Scedosporium apiospermum	6	2.63
13.	Cladosporium species	3	1.32
14.	Lasiodiplodia theobromae	2	0.87
15.	Nigrospora species	2	0.87
16.	Bipolaris spicifera	2	0.87
16.	Exophiala species	2	0.87
17.	Alternaria alternata	1	0.44
18.	Aureobasidium pullulans	1	0.44
19.	Cladophialophora bantiana	1	0.44
20.	Phialophora verrucosa	1	0.44
21.	Sepedonium species	1	0.44
22.	Unidentified dematiaceous fungus	37	16.23
23.	Candida species	2	0.87

Discussion:

The present study shows that fungal keratitis is common in Orissa and the neighbouring states as it is in rest of India. The efficacy of direct micrscopy of corneal scraping by staining with KOH+CFW and examining under fluorescence microscope is one of the most rewarding methods for diagnosis of fungal keratitis. In this study 94.24% of the corneal scrapings showed fungal elements by this method. Efficacy of this technique has been appreciated by several studies in the past and this method continues to rule over all others.[7] In contrast Gram stain was positive in 77.9% of cases and Giemsa stain in 77.57% in demonstration of fungal filaments in corneal scraping. One important factor that has been highlighted before and we would like to reiterate is that a

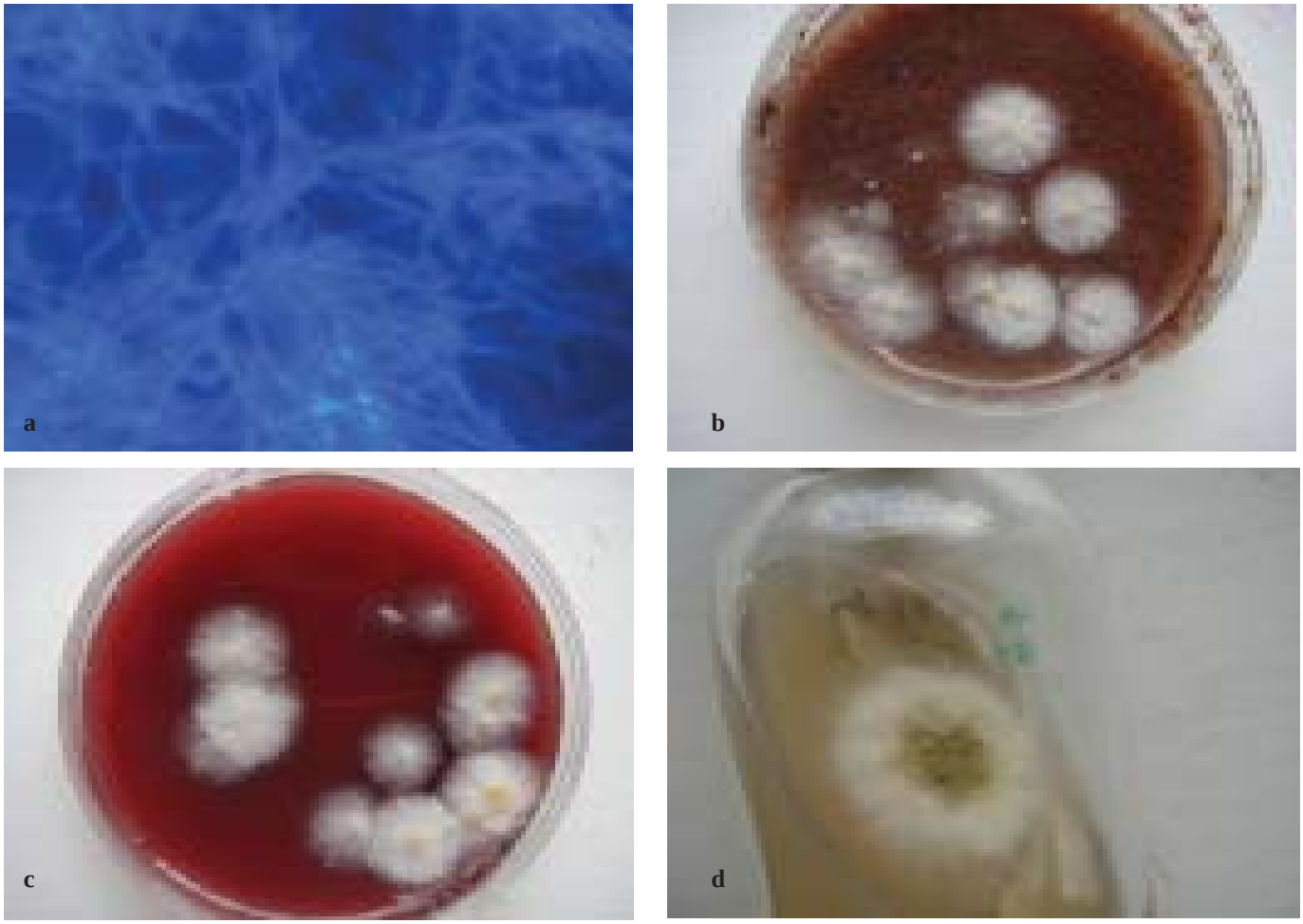

Figure 1: Corneal scraping from one of the patients shows septate fungal filaments in (a) KOH+CFW (x400), mixed growth of Staphylococcus sp. and A. flavus on chocolate (b) and blood agar (c) and A. flavus on SDA (d). Colonies of A. flavus show characteristic features of white, fluffy periphery with yellowish green granular centre.

wet smear like KOH+CFW allows the spread of the scraped material uniformly and the fungal elements stand out strikingly bright in a darker background. In fact, with time the fungal elements retain their brightness while the background becomes dull and darker. It is important to remember to place the scraped material at one place without smearing on the slide for KOH+CFW preparation. This advantage of clear visualization is missing in Gram and Giemsa stain where the scraped material must be smeared in a thin layer on the slide failing which heaps of tissue may hide the fungal elements.

In the present study Aspergillus species 64 (28%) was predominant isolate followed by Fusarium species 53 (23.2%). This is similar to reports from other studies in India.9,10 but in contrast to report by Bharathi et al 6 where Fusarium species was the predominant species. Similar to other studies from India Candida species remains an uncommon cause of fungal keratitis.

We conclude that fungal infection of the cornea is common in this part of the country and a large number of different species cause the disease. However, Aspergillus and Fusarium are the predominant genera involved and simple laboratory techniques are useful for definite diagnosis and specific treatment of fungal keratitis.

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