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Prevalence of Scedosporium keratitis in a rural tertiary care hospital

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Abstract:
Aim: To study the prevalence of Scedosporium in fungal keratitis infection from a rural tertiary care hospital.
Method: Corneal scraping samples from October 2018 to October 2019 had come to Microbiology central laboratory at Narayana nethralaya hospital,Bommasandra Bangalore. 94 samples were collected using a surgical scalpel blade no. 15 and it was subjected to microscopic Gram stain examination and 10%Pottasium hydroxide (KOH) mount and culture techniques. 16 samples on Sabouraud dextrose agar (SDA) started growing after 72 h and these were initially floccose, with a white hairy growth with greyish surface and yellow reverse. No growth was seen on blood agar and chocolate agar after 1 week of incubation. Lacto phenol cotton blue (LPCB) staining was performed after seven days of incubation by tease mount method and observed by binocular compound microscope with low power x10 and high power ×45, which shows septate hyphae, ovoid conidia with truncated bases suggestive of Scedosporium species.
Results: 16 samples on Sabouraud dextrose agar (SDA) started growing after 72 h and these were initially floccose, with a white hairy growth with greyish surface and yellow reverse, suggestive of Scedosporium species.10 samples showed white, floccose, cottony myceleal growth on SDA and LPCB mount shows slightly curved micro and macro conidia accumulating around tips of phialides, suggestive of fusarium species.
10 samples revealed phaeoid, brown fungus with hairy growth on SDA and LPCB mount showed septate, slightly tapering curved conidia suggestive of Curvularia species.
Conclusion: majority of Scedosporium growth was observed in our study(44%), compared to Fusarium(27%) and Curvularia(27%) species. In our retrospective study we found that males(35%) are more infected with fungal keratitis than females(8.3%), age group 50 to 60 years were affected more with Scedosporium keratitis infection.

Key words: KOH, LPCB, SDA, Scedosporium, Keratitis.

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1. INTRODUCTION

Fungal infections of the eye are recognized as an important cause of morbidity and blindness worldwide. Fungal keratitis is the most common encountered clinical entity. These infections are one of the most difficult form of fungal eye infection to diagnose and treat successfully. It is difficult for correct diagnosis and topical antifungal preparations[1]. Fungal keratitis remains as a challenge to ophthalmologists through out the world. The incidence of fungal keratitis is particularly higher in agricultural and developing countries.

The most common pathogens reported are *Fusarium* species and *Aspergillus* species. *Scedosporium* species commonly found in soil or decaying plant are previously thought to be opportunistic infection in immunocompromised individual only. Recently, *Scedosporium apiospermum* and its sexual form, *Pseudallescheria boydii* have been identified as emerging opportunistic pathogen responsible for mould infection in immunocompromised and occasionally immunocompetent patients.[2]

The purpose of this study was to reveal rare fungus Scedosporium causing microbial keratitis investigated at this hospital from past one year. Cornea evaluation was carried out by a cornea specialist using a slit-lamp biomicroscope, documentation of the ulcer was done and recorded on a daily basis[3].

2. MATERIALS AND METHODS:

Corneal scrapings samples of the 94 patient were collected using a surgical scalpel blade no. 15 and inoculated in the form of letter C and Y on Sabouraud dextrose agar (SDA) plates, blood agar and chocolate agar plates, SDA incubated in ambient air at 28 °C in a BOD (Biological Oxygen Demand) incubator, blood agar and chocolate agar plates were incubated at 37 °C, a piece of scraping was smeared onto clean glass slides for Gram stain and potassium hydroxide (KOH) mount. 37 samples revealed septate fungal filaments on 10 % potassium hydroxide (KOH) mount. No bacteria were seen on Gram staining. 57 samples showed no fungal filaments and no bacteria in KOH and Gram stain respectively. 16 samples on SDA started growing after 72 h and these were initially floccose, with a white hairy growth with greyish surface and yellow reverse. No growth was seen on blood agar and chocolate agar after 1 week of incubation. Lacto phenol cotton blue (LPCB) staining was performed after seven days of incubation by tease mount method and observed by binocular compound microscope with low power x10 and high power ×45, which shows septate hyphae, ovoid conidia with truncated bases suggestive of *Scedosporium species.*

10 samples showed white, floccose, cottony myceleal growth on SDA and LPCB mount shows slightly curved micro and macro conidia accumulating around tips of phialides, suggestive of fusarium species.

10 samples revealed phaeoid, brown fungus with hairy growth on SDA and LPCB mount showed septate, slightly tapering curved conidia suggestive of Curvularia species.

3. RESULTS

On microscopic examination of Gram stain and KOH mount reveals numerous branching septate hyphae(Fig.a). On SDA plates, rapidly growing isolate produced initially white hairy colonies and the colonies turned yellow with a cottony texture after further incubation for one week (Fig.c). Microscopically on LPCB mount hyphae were septate and hyaline, conidia were

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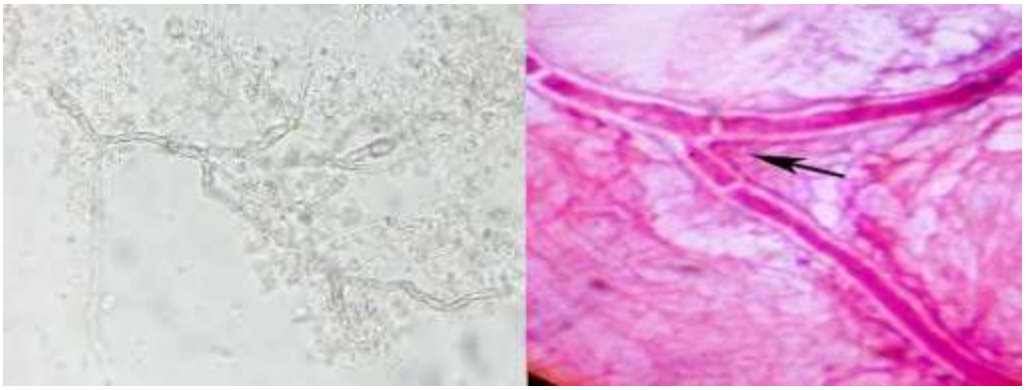
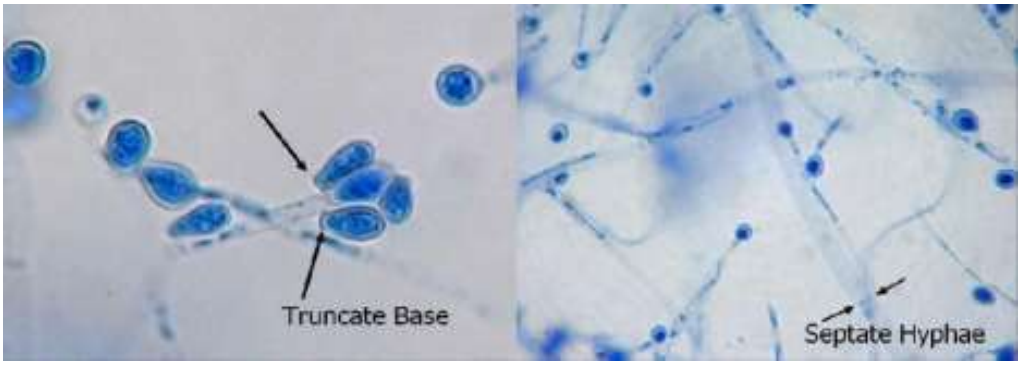
single celled, ovoid with a truncate base with single or branched conidiophores(Fig.b), the majority of Scedosporium growth was observed in our study(44%), compared to Fusarium(27%) and Curvularia(27%) species. In our retrospective study we found that males(35%) are more infected with fungal keratitis than females(8.3%).



a. KOH mount and Gram stain showing branching septate hyphae



b. LPCB mount showing Truncate base and septate hyphae of Scedosporium

c. Growth of Scedosporium on SDA plate

d. Table 1: Percentage of isolates obtained

Organisms	**Percentage of isolates(%)**
Scedosporium species	44
Fusarium species	27

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Curvularia species	27

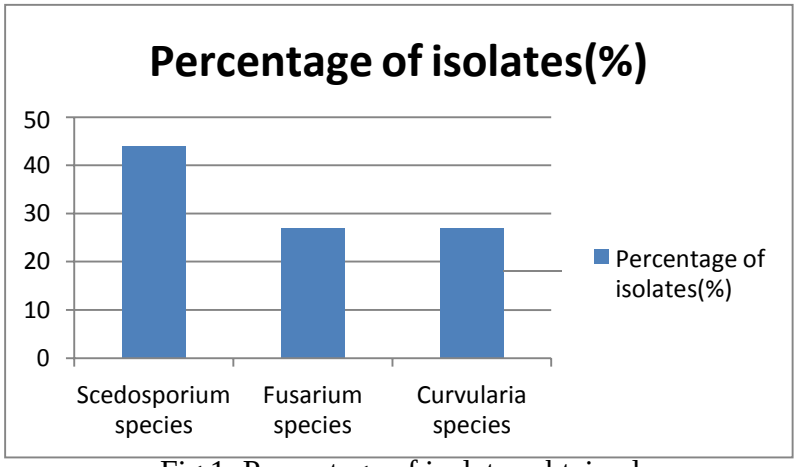

Fig 1: Percentage of isolates obtained

Table 2: Agewise distribution of Scedosporium species

Sex	Age group 1-20	Age group 21-40	Age group 41-60	Age group >60
Male	1	2	5	4
Female	0	2	1	0

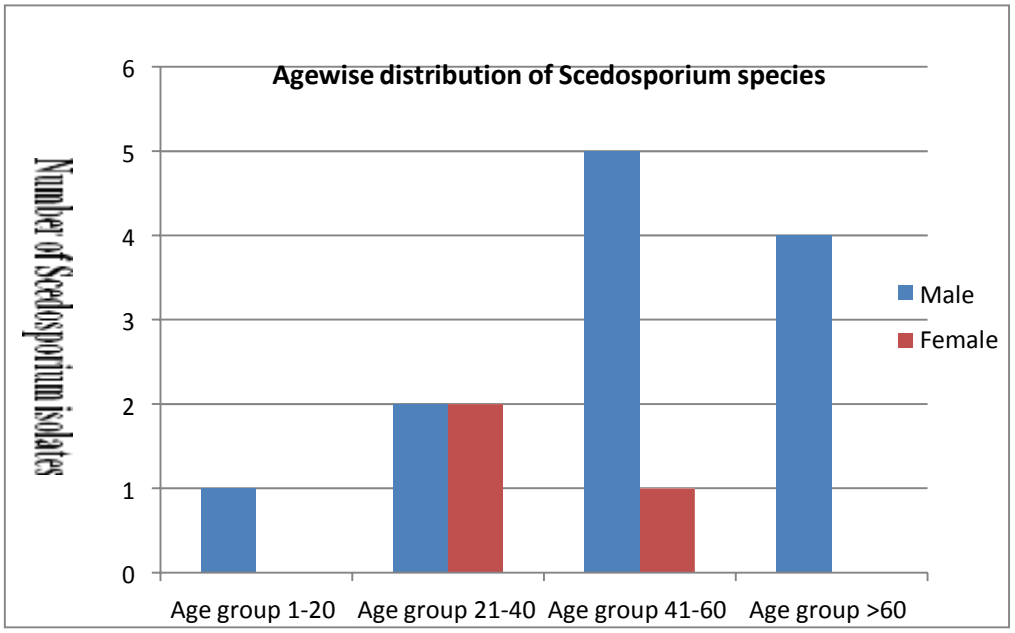

Fig 2: Agewise distribution of Scedosporium species

4. DISCUSSION:

Fungal infections of the cornea (fungal keratitis or keratomycosis) may constitute 6 to 53% of all cases of ulcerative keratitis, depending upon the country of origin of the study. Mycotic

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keratitis occurs in conjunction with trauma to the cornea brought about by fungus contaminated plant material (leaves, grain, branches or wood) or occasionally metal. Filamentous fungi may constitute up to one to two-thirds of all cases of traumatic infectious keratitis . As a group, the dematiaceous fungi have gained importance as the agents causing keratitis in most incidence reports of corneal ulcer (second to hyaline hyphomycetes such as Fusarium and Aspergillus species)[4].

Majority of Scedosporium growth was observed in our study(44%), compared to Fusarium(27%) and Curvularia(27%) species (as shown in Table:1,Fig.1). Being a rare filamentary fungus to affect the eye, reports of *Scedosporium* ocular infections in literature are usually solitary and uncommon. The literature has attributed the low incidence of *Scedosporium* keratitis to difficulty in distinguishing *Scedosporium* from *Aspergillus*, *Fusarium*, and other hyaline hyphomycetes due to common histopathological and clinical features. However, sporulation in culture followed by staining with lactophenol cotton blue, a stain commonly used to identify fungi, helps in identifying scedosporium[5] .

Scedosporium is known to be resistant to many antifungals and S. prolificans is generally more resistant than *S. apiospermum*[6]. Fungi of the yeast variety are more frequently opportunistic than filamentous fungi and are seen in chronic corneal disease, contact lens wearers, and immunocompromised patients. Filamentous fungal keratitis is often preceded by corneal trauma and occurs mostly in previously healthy individuals[7].

In our study we found that male predominance of keratitis infection with Scedosporium in the age group of 41-60 and above 60 years, elderly age group are more affected (as shown in Table:2,Fig.2).

Possible risk factors of fungal keratitis, one of the major causes of fungal ocular infection, include ocular injury, long-term therapy with topical or systemic steroids, immunosuppressive agents, and underlying diseases such as pre-existing corneal surface abnormality and diabetes mellitus, and wearing contact lenses[8].

Scedosporium colonization of the airways in patients with Bronchial colonization may lead to chronic inflammation or even to life-threatening invasive disease in cases of severe immunosuppression, such as lung transplant or hematological malignancies[9]. Other infections caused by S. apiospermum and S. prolificans are sinusitis, meningitis, arthritis and osteomyelitis, endocarditis, cutaneous and subcutaneous infection (nonmycetoma), keratitis, endophthalmitis, and disseminated disease. In most cases, inoculation of spores in skin or soft tissue is due to penetrating trauma or surgery[10].

5. CONCLUSION

From our retrospective study we would like to conclude that Scedosporium is a rare septate fungal infection causing keratitis infection when compared to aspergillus, fusarium, curvularia species and its more predominance in male elderly age group.

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