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Original Research Article

Isolation and identification of microorganisms causing Keratitis with special reference to Mycotic agents in a tertiary care hospital of Odisha

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Abstract

Infective keratitis is a common, potential sight threatening ocular infection of the cornea by infective organisms like bacteria, fungi, virus or parasite. India and specifically Odisha being an agricultural land with hot humid climate, our study has been designed to specifically focus on Mycotic Keratitis. Corneal scraping was collected from the affected eye and subjected to Gram Stain and 10% KOH mount. The samples were inoculated into blood agar, McConkey Agar or bacterial culture and Sabouraud Dextrose Agar (SDA) for fungal culture. The culture plates were incubated at 37 °C and 27 °C for bacterial and fungal culture respectively. The bacterial pathogens were identified by colony morphology, Gram Staining and standard biochemical Tests. The fungal pathogens were identified by growth, texture of the colony and pigmentation on obverse and reverse in SDA media. Microscopic examination was done by lactophenol cotton blue mount. Yeasts were identified by Gram Staining (Germ tube test) and morphology on Corn meal agar. Odisha being a tropical agricultural based area, most corneal ulcers are mycotic in origin. *Aspergillus* spp being the commonest pathogen followed by *Penicillium* spp (24.32%), *Candida* spp (13.51%), *Aspergillus niger* (10.81), *Fusarium* spp. (8.1%). males outnumbered females with maximum preponderance in 31-45 years age group.

Keywords: Mycotic keratitis, *Aspergillus*

Introduction

Corneal disease is the second most common cause of blindness in tropical countries after cataract [1]. Infective keratitis is a common, potential sight threatening ocular infection of the cornea by infective organisms like bacteria, fungi, virus or parasite. In India, previous studies have highlighted that mycotic keratitis(MK) occurs more frequently in men, those in an agricultural occupation, and is more likely following trauma to the eye [2, 3]. Specifically, injury with vegetative matter or soil can predispose to mycotic keratitis. It occurs in areas with warm climate and incidence increases with seasonal increase in temperature and humidity [4]. Hence, India and specifically Odisha being an agricultural land with hot humid climate, our study has been designed to specifically focus on Mycotic Keratitis.

Materials and methods

This study was conducted in the PG Department of Microbiology, SCB Medical College, Cuttack from May 2017 to April 2018. 96 clinically diagnosed cases of Keratitis were included in the study. Age, Sex, occupation, socioeconomics status, risk factors like history of trauma, use of topical steroids, use of contact lenses were specifically noted. Corneal scraping was collected from the affected eye and subjected to Gram Stain and 10% KOH mount. The samples were inoculated into blood agar, McConkey Agar or bacterial culture and Sabouraud Dextrose Agar (SDA) for fungal culture. The culture plates were incubated at 37 °C and 27 °C for bacterial and fungal culture respectively. The bacterial pathogens were identified by colony morphology, Gram Staining and standard biochemical Tests. The fungal pathogens were identified by growth, texture of the colony and pigmentation on obverse and reverse in SDA media.

Microscopic examination was done by lactophenol cotton blue mount. Yeasts were identified by Gram Staining (Germ tube test) and morphology on Corn meal agar.

Statistical analysis

The data were analyzed using SPSS (version 21.0). The qualitative data were analysed using Chi square test. A P value < 0.05 was considered statistically significant.

Result

Table 1: Bacterial Pathogens Isolated

| Bacterial isolates | Number (%) |
|-------------------------|------------|
| <i>S. aureus</i> | 13 (46.42) |
| <i>Pseudomonas</i> spp. | 09 (32.14) |
| CoNS | 05 (17.85) |
| <i>Klebsiella</i> spp. | 01 (3.57) |
| Total | 28 |

Table 1: Fungal Pathogens Isolated

| Fungal Isolates | Number (%) |
|---------------------------|------------|
| <i>Aspergillus flavus</i> | 16 (43.24) |
| <i>Aspergillus niger</i> | 04 (10.81) |
| <i>Penicillium</i> spp | 09 (24.32) |
| <i>Candida</i> spp | 05 (13.51) |
| <i>Fusarium</i> spp | 03 (8.10) |
| Total | 37 |

Table 3: Age and Gender distribution of Mycotic Keratitis patients

| Age Group | Male | Female | Total |
|-----------|------|--------|-------|
| 0-15 | 02 | 01 | 03 |
| 16-30 | 04 | 03 | 07 |
| 31-45 | 12 | 06 | 18 |
| 46-60 | 06 | 01 | 07 |
| >60 | 01 | 01 | 02 |
| Total | 25 | 12 | 37 |

Chi square: 1.6885, P=0.7928

Out of 96 cases, bacterial pathogens were isolated from 28(29.17%) cases and fungal pathogens from 37(38.54%) cases. Rest 32 were culture negative. Among the bacterial pathogens, as seen in Table I, *S. aureus* was the commonest 46.42%, followed by *Pseudomonas* 32.14%. Table II shows, *Aspergillus flavus* (43.24%) was the most common fungal pathogen followed by *Penicillium* spp (24.32%), *Candida* spp (13.51%), *Aspergillus niger* (10.81), *Fusarium* spp. (8.1%). Out of the 37 laboratory confirmed mycotic keratitis cases, 19, 12 and 6 belonged to low, middle and high socioeconomic status respectively. 18 out of the 37 subjects were agricultural workers. History of trauma was present in 17 cases, 10 patients had h/o topical steroids use, 3 were contact lens users, Rest 7 elicited no pertinent history. Table III shows males outnumbered females with maximum preponderance in 31-45 years age group.

Discussion

Odisha lies in the tropics with a climate and humidity that best suits fungal growth. Majority of population survives on agriculture [4]. They are more prone to trauma with vegetative matters. This justifies the magnitude of mycotic keratitis observed in our study and similar studies [5]. The male preponderance found in our study, though statistically insignificant, is similar to other studies in different parts of India [6, 7, 8]. In our study most affected

cases were of low socioeconomic status, may be due to unhealthy living conditions, lack of awareness and less access to proper medical care. Commonest etiological agent of MK in this region was *Aspergillus* spp. Similar to other reports from North India [9]. In contrast to other studies from South India [10], our study reports less cases of *Fusarium* spp.

Summary and conclusion

Odisha being a tropical agricultural based area, most corneal ulcers are mycotic in origin. *Aspergillus* spp being the commonest pathogen with male preponderance. The present study highlights the importance of identification of fungal pathogens in corneal ulcer, so that early diagnosis with adequate and appropriate treatment can be done so that a major preventable cause of vision loss can be prevented.

Conflict of Interest: None

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