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Research Article

EPIDEMIOLOGICAL STUDY OF FUNGAL KERATITIS IN A TERTIARY HOSPITAL FROM SOUTH KARNATAKA

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A R T I C L E I N F O

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A B S T R A C T

Introduction: Corneal infections of fungal etiology are common in India (19-45%). *Aspergillus* (16–53%) and *Fusarium* (10–45%) species are the common pathogens. In agricultural country like India, where primary health care and referral systems are weak, minor eye injuries sustained in agricultural farms lead to corneal ulceration of fungal etiology and loss of vision **Purpose**: 1. To Identify and isolate the various fungi causing fungal keratitis. **2.** To know the various predisposing factors for developing fungal keratitis. **Materials and Methods**: Multiple corneal scrapings from suspected cases were collected on a set of SDA, Blood agar plates, for KOH wet mount and Grams stain. Molds were identified on the basis of colony morphology such as growth rate, colony texture, color, pigment, and aerial or vegetative hyphae. **Results**: Out of 68 study subjects, 18 (26.4%) were culture positive. 45 males and 23 were females. Mean Age was 40. *Fusarium sp* was predominant (44.4%) followed by *Aspergills sp* (38.9%). Majority 35 (51.4%) were farmer or from agricultural work, among which males 27(77.1%) were more than females 8(22.9 %). **Conclusion**: Among vegetative trauma, injury by sugarcane leaf itself contributed for 15(22 %) cases.

INTRODUCTION

Cornea is responsible for three quarters of dioptric power of the eye and hence any injury to it can cause considerable visual disturbances. Corneal ulcer is a major public health problem in the developing world causing prolonged ocular morbidity and visual impairment.[1] Corneal blindness is responsible for 1.5 to 2 million new cases of monocular blindness every year in which ocular trauma and corneal ulceration are significant contributors.[2]

In a vast agricultural country like India, particularly where primary health care and referral systems are weak, minor eye injuries sustained in agricultural farms often lead to corneal ulceration of fungal etiology and loss of vision.[3] The reported incidence of corneal ulceration in India is 1130 per million population.[4]

Corneal infections of fungal etiology are common in India (19-45%). *Aspergillus* (16–53%) and *Fusarium* (10–45%) are the common incriminating pathogens.[1] Ocular morbidity in fungal infections tends to be greater than that in bacterial keratitis, because the diagnosis is often delayed and available drugs are not very effective. History of injury with vegetable matter, dry looking ulcer, feathery edges, satellite lesions, endothelial plaque and thick hypopyon form a typical presentation. Hence this study is an attempt to study the etiology, clinical features, and diagnosis of fungal corneal ulcer and also to educate the patient for better follow up during the course of treatment.

EPIDEMIOLOGY OF FUNGAL CORNEAL ULCERS

Fungal infections occur all over the world but the incidence is highest in warm and humid climates and particularly in the rural environment. In a study conducted in 1997, 46.8% had pure fungal infections and 5.1% had mixed bacterial infections. [4]In India, a recent study suggests that approximately 1% of the population or~9 million people will acquire microbial keratitis in 10 years and 50% of the cases will be fungal. [5] In another study done in South India, fungal etiology was confirmed in 34.4% cases of corneal ulcers. [6]In Coastal Karnataka, higher incidence is reported in October, June and January. [7]In Hyderabad, higher incidence of fungal keratitis is reported during winter (October to January) and monsoon (June to September). [8]

The incidence of fungal keratitis was higher during the paddy harvesting and also during the time of the year when agricultural activity was greater. [6]The incidence of fungal keratitis was significantly higher in males, in individuals from rural areas and following corneal injury. It has been observed that younger people aged 21-50 years are more often affected by fungal keratitis compared to those above 50 years, who are affected by bacterial ulcers. [6]

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Fungal Etiology of fungal corneal ulcer: [9, 10, 11]

Septate Aspergillus sp., Cephalosporium sp., Fusarium sp., Penicillum sp., Cladosporium sp., Paeciolmyces sp., Curvularia sp., Alternaria sp.

A. Non *septate Mucor sp., Rhizopus sp., Absidia sp.*
B. *Yeasts Candida sp., Cryptococcus sp., Rhodotorula sp.*
C. Dimorphic fungi Blastomyces, Coccidiodes Histoplasma, Sporothrix

Table 1 Frequency of various fungi responsible for keratomycoses worldwide [12]

Fungi	Hyderabad	New Delhi	Singapore	Ghana	Florida
Fusarium	38%	11%	52%	42%	52%
Aspergillus	30%	40%	17%	17%	4%
Penicillium	-	7%	3%	0	2%
Curvularia	4%	7%	3%	1%	9%
Other *dematiaceae*	16%	17%	0	6%	10%
Candida	0	1%	10%	1%	8%
Total no of cases	557	211	29	109	240

Table 2 Contribution of different predisposing factors and traumatic agents according to a study in South India[13]

Predisposing factors and traumatic agents	Percentage
Corneal trauma	92.15
Co-existing ocular diseases	6.67
Spheroidal degeneration	2.37
Post-operative(suture infiltrates)	1.46
Pre-existing viral keratitis	1.29
Lid abnormalities	0.64
Lagophthalmos	0.73
Adherent leucoma	0.18%
Use of topical steroids	1.19%
Systemic diseases like diabetes mellitus, leprosy and tuberculosis	16.07%

In a study to assess whether the presence of characteristic clinical features can be used as a diagnostic aid, it was found that serrated margins, raised slough, dry texture, satellite lesions and discolourations other than yellow occurred more frequently in cases of filamentous fungal ulcers than bacteria. The probability of fungal infection if one clinical feature was present was 63% and 83% if all three were present. [14]

MATERIALS AND METHODS

Source of Data

Study design: Prospective study.

Stud period: 1 year from June 2013 to May 2014.

Sample size: Consented subjects, who are attending ophthalmology OPD and clinically suspected to have fungal keratitis .

Sampling method: Purposive sampling

Inclusion criteria: All patients who presented to Ophthalmology OPD of Mandya Institute of Medical Sciences, and suspected with clinical diagnosis of fungal keratitis and agreed to participate in the study were included.

Exclusion criteria

- Patients who refuse to give consent.
- Patients who were clinically suspected to have bacterial, viral, protozoal and non infective etiology were excluded.

Method of collection of data: Written informed Consent was obtained. Clinical history was noted and detailed examination with slit lamp biomicroscope by an Ophthalmologist was done. After removal of debris or discharge in the vicinity, corneal scrapings were collected using sterile Bard-Parker blade no. 15.[15]

Multiple scraping of the affected part of cornea, ulcer margins and bed was done under aseptic precaution with topical anaesthesia (4% lignocaine hydrochloride) with the aid of a slit-lamp, used in a sequence first to inoculate on a set of SDA with Chloramphenicol plates and a set of Blood agar plates, then scrapings were placed on 2 different clean and labelled glass slides for KOH wet mount, Grams stain respectively.

A. Potassium Hydroxide (KOH) Wet Preparation:[15,16,17,18]

Corneal scraping sample was placed on a first labelled glass slide and a drop of 10% KOH is put on it. Cover slip is placed and the preparation warmed for better digestion of tissue material. Presence of branching refractile fungal filaments was observed and findings were noted.

B. Gram Stain: [17,19,20] :Scrapings on the second labeled glass slide were spread to make a smear and fixed with 95% methanol. Gram stain was done, Gram positive fungal filaments, septate or aseptate, branching or non branching were noted.

Gram positive budding yeast cells with pseudohyphae if was found it was noted.

Culture:[17,19,20,21]

Specimens were inoculated on the surface of culture media, making multiple "C" shaped marks without cutting the agar.

Corneal scrapings were inoculated onto a set of SDA with Chloramphenicol and on a set of Blood agar plates, under strict aseptic precautions.

One each set was incubated at 37^0C and another at room temperature. Culture media were inspected daily, and the growth was noted and standard procedures for description of colony morphology followed for identification of filamentous fungi.

Molds were identified on the basis of standard description of colony morphology such as growth rate, color, colony texture, and aerial or vegetative hyphae with diffusible pigment.

Slide cultures was done on Potato Dextrose Agar for sporulation pattern

For identification of Yeasts: [17,19,20,21]

Growth pattern on SDA with Chloramphenicol and BA plate was observed and further speciation done by Germ tube test, pattern of sporulation on Cornmeal agar, and Urease production test.

An isolate is more likely to be considered significant if,

1. Confluent growth at the site of inoculation on any one medium.
2. Correlation of Smear results with growth on one medium.
3. The same organism, was grown on more than one media.

Bacterial contamination of Saboraud's dextrose agar was avoided by incorporating chloramphenicol in a concentration of 50ug/ml. The majority of fungi causing keratitis were detected on SDA within 72 hours.

Aspergillus and *Fusarium* species showed growth on SDA within 48 hours. Culture media were observed for at least 2 weeks before reporting them as negative.[22]

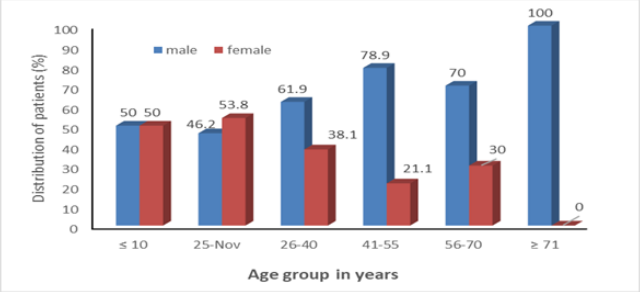
Plan for data analysis

The data was entered in Microsoft Excel sheet and analysed using SPSS software version 15.

Data was analysed using the descriptive statistics, 'chi-square' test and other statistical tests as required.

RESULTS

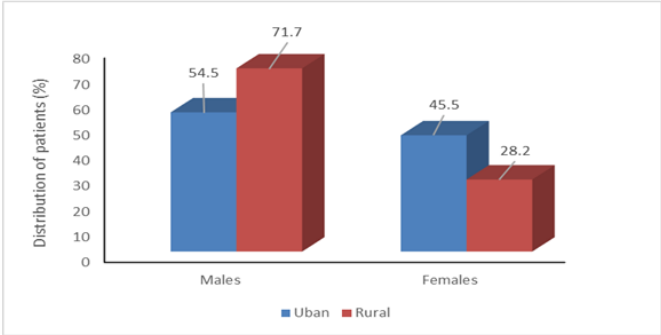
Sixty Eight patients attending Ophthalmology OPD who were suspected clinically of fungal keratitis were studied in the Department of Microbiology in Mandya Institute of Medical Sciences, Mandya. Observations were made as follows:



Graph.1 Age wise distribution of patients

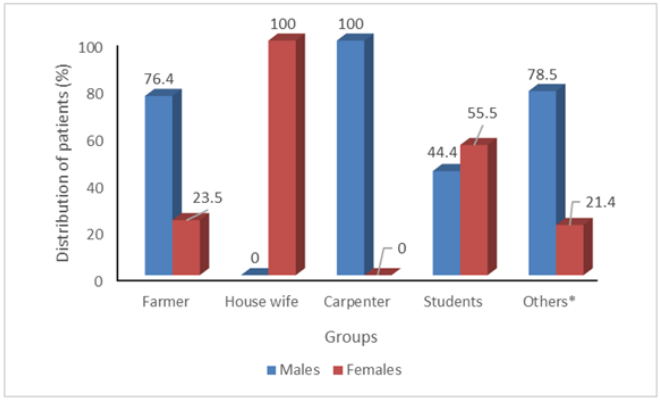
Age Distribution in the present study subjects ranged from 3 to 75 years. Maximum numbers were seen in 26 to 40 years age group. Mean Age was 40.42. Out of 68 study subjects 45 (66.1 %) were males and 23 (33.9 %) were females.

Male to female ratio was 1.95:1. Among males majority 15 (78.9%) were 41-55 age group followed by 13(61.9%) in 26-40 age group. Among females majority 8 (38.1%) were from age group 26-40 years.



Graph 2 Area Wise Distributions of Patients

Majority 45 (66.1%) of the patients were from rural area. Among the men it was 71.7 %.



Graph 3 Sex distributions in different risk groups

Majority 35 (51.4%) were farmer or from agricultural work, among which males 27(77.1%) were more than females 8(22.9 %).

Among the predisposing factors 44 (64.6%) had history of vegetative trauma, followed by foreign body 26(38.2%).

Among vegetative trauma, injury by sugarcane leaf itself contributed for 15(22 %) cases. The study group mainly comprises sugarcane growers. In 18 (26.4 %) cases gave no history of any predisposing factor.

Among 68 cases, Gram stain was positive for fungal elements in 17 cases, out of which 16 (94.1 %) were confirmed by culture. Gram stain was negative in 51 cases, among which 2 showed positive fungal culture. Sensitivity of the Gram stain was found to be 88.9 %. Specificity Gram stain was found to be 98%.

Out of 68 cases, KOH wet mount was positive for fungal elements in 14 cases, all showed growth. Out of 54 cases negative by KOH wet mount 4 cases showed fungal growth.

Table 3 Distribution of predisposing factors

Predisposing Factors	Males (%)	Females (%)	Total (%)
Injury by sugarcane leaf	9 (60)	6 (30)	15 (22.0)
Other Vegetative Trauma	19 (65.5)	10 (34.5)	29 (42.6)
Foreign Body	16 (61.5)	10 (38.5)	26 (38.25)
Diabetes Mellitus	9 (90)	1 (10)	10 (14.75)
Local steroids	4 (66.6)	2 (33.3)	6 (8.8)
Chronic Dacryocystitis	3 (60)	2 (40)	5(7.3)
Surgery Same Eye	1 (100)	0	1 (1.4)
Unknown	13 (72.2)	5 (27.8)	18 (26.4)

Table 4 Comparison of Gram staining and fungal culture

Gram staining	Number(68)	Culture Positive (%)	Culture negative
Positive for fungal filaments	17	16 (88.88)	1
Negative for fungal filaments	51	2 (11.11)	49

Table 5 Comparison of KOH mount and fungal culture

KOH wet mount	Number (68)	Culture Positive (%)	Culture negative
Positive for fungal filaments	14	14 (77.77)	0
Negative for fungal filaments	54	4 (22.22)	50

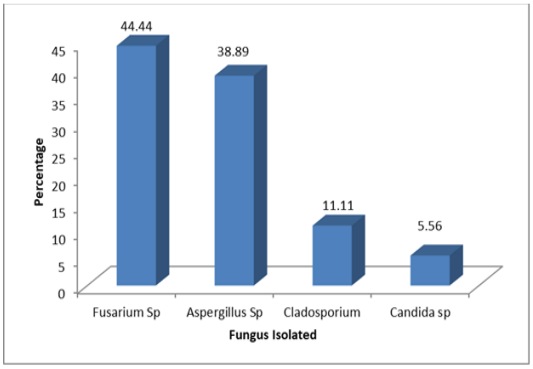
Table 6 Population Wise Distribution of Fungi

Fungal isolate	Urban (%)	Rural (%)
Aspergillus	0	7 (43.7)
Candida	0	1 (6.2)
Cladosporium	0	2 (12.55)
Fusarium	2	6 (37.5)
Total	2 (11.11)	16 (88.9)

Sensitivity of KOH was found to be 77.8 %. Specificity of KOH was found to be 100 %. Among 68 cases studied 18 (26.4%) were culture positive, out of which 16 showed single growth and 2 cases showed mixed growth with bacteria. Bacteria were isolated from 21(30.9%) cases, there was no growth in 31 (45.5%) cases. Among the bacterial flora Gram positive cocci were isolated from 18 cases, out of which predominant flora was CONS and 3 were Pseudomonas. Fungal growth was found in 16 cases singly and 2 with mixed bacterial growth.

In this study among the fungi isolated, Fusarium sp was predominant (44.4) followed by Aspergills sp (38.9). Cladosporium was isolated from 11% of cases and Candida was isolated from 5.6% cases only.

Aspergillus sp was found to be more common in rural area and followed by Fusarium sp. 2 cases from urban population were Fusarim sp.



Graph 4 Percentage distributions of fungal isolates

DISCUSSION

In present study an attempt was made to study 68 cases suspected of fungal corneal ulcer .The results are compared with other studies and are discussed as follows:

The commonest age group affected was between 26-40 years, it correlates well with the study by Parvez Ahmed *et al*[23], Chowdhary et al[24], and Kamlesh et al[25]. In all studies Maximum number of cases were seen between 21-50 years. The second common affected group was 41-55 years, it correlates with Chander J *et al*.[26] Mean age of patients in the present study was 40.42 , The mean age reported by different workers varied from 40.6 years to 69.3 years. The age range involved reflects the most active period of life and increased vulnerability to injury during outdoor activities. This has a considerable socioeconomic impact because this age group people are bread winners of the family.

Sex:

Author	Male (%)	Female (%)
Srinivasan et al(1997)[4]	61.3	38.7
Bharathi *et al*(2003)[6]	65	35
Chowdhary *et al*(2005)[24]	68	32
Chander J *et al*(2008)[26]	80.2	19.8
Gopinathan *et al*(2009)[8]	69.3	30.7
Pervez*et al*(2013)[23]	69	31
Present study	66.1	33.9

In the present study male preponderance was seen with male to female ratio of 1.95:1, and it is comparable to study by Srinivasan et al[4] 1.6:1. Kotigadde *et al*found female preponderance with ratio 1.14 .[7] The study correlates well with Bharathi et al[6], Chowdhary et al[24] and Gopinathan et al[8]. This is because predominant outdoor work is done by males and their increased vulnerability to trauma as their activity involves more risk.

Area wise distribution

In present study 46 (67.6%) of the patients were from rural area which is comparable to that of 74% in Kamlesh et al[25] series, whereas the rest of the 22(32.4%) were from urban areas. This data coincided well because patients involved in agricultural activity were more commonly from rural area.

Distribution of the patient- risk groups

Study series	Farmer	House wife	labourer	student	Unknown / others
Bharathi *et al*(2003)[6]	64.7	5.1	14.5	8.1	7.5
Basak *et al*(2005)[27]	57.5	11.3	13.1	2.6	6.5
Gopinathan *et al*(2009)[8]	-	-	20.3	-	22
Present study (2013)	51.4	10.2	16.2	9	13

Results of the present study are in agreement with that of Samar K Basak et al[27] and Bharathi et al[6] . This occupational preponderance was justified because trauma with vegetative matter was an important risk factor for the occurrence of fungal keratitis

Comparison of predisposing factors

In the present study trauma was the commonest risk factor, which is correlating with Gopinathan et al. Second common being diabetes mellitus in our study which is also correlating with their study.

Among the vegetative trauma, injury by sugarcane leaf contributed to 22% cases, as majority of our study group were sugarcane growers and Mandya is called Sugar city. Local steroids application was found in 8.8 % cases correlating well with Samar K Basak [27], Bharathi et al[6] and Jagdish Chander et al[26]. Presence of Chronic dacryocystitis was 7.3 % which was comparable with Kamlesh et al[25] ,bur parvez et al[23] found it to be 16 %.

Our study showed Diabetes mellitus in 14.75 % cases which is in agreement. In the present study no predisposing factor was established in 26.4% cases.

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Study series	Vegatative Trauma	Contact lens	Local corticosteroids	Chronic dacryocystitis	Foreign Body	endophthalmitis	Surgery same eye	Diabetes Mellitus	Entropion/ Ectropion	Others/ unknown
Kamlesh et al (1983)[25]	46.4	_	_	5.8	_	_	_	_	2.8	45
Bharathi et al (2003)[6]	54	-	7.8	-	6.89	-	-	-	-	1.89
Samar K Basak et al (2005)[27]	56	-	10	-	7.6	-	-	-	-	-
Jagdish Chander et al (2008)[26]	53.4		9.78	-	-	-	-	-	-	-
Gopinathan et al (2009)[8]	61.8	--	-	-	-		-	-	-	1.8
Pervez et al (2013)[23]	44	-	-	16	-	-	-	-	-	14
Present study (2013)	64.6	-	8.8	7.3	-	-	1.4	14.75	-	26.4

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