Evaluation of corneal scrapings smear examination method in the diagnosis of fungal keratitis

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Abstract

Aims & objective: To report the epidemiological features and laboratory diagnosis of 100 cases of fungal keratitis diagnosed at Regional Institute of Ophthalmology, Bangalore Medical College & Research Institute, Bangalore.

Methods: A total of 100 patients with suspected fungal corneal ulcer satisfying the inclusion criteria were studied at Regional Institute of Ophthalmology, Bangalore Medical College & Research Institute, Bangalore during the period December 2009 to February 2011. Socio-demographic data, risk factors, seasonal variation and laboratory findings were studied.

Results: Males (64) were affected significantly more than females (36). Of 100 patients, 78 were in the affected age group (20-50yrs). Ocular trauma was seen in 34 patients. There was a higher incidence of fungal keratitis during the monsoon and winter than summer. potassium hydra oxide (KOH), calcoflora white (CFW) and Gram stain revealed fungus in 46,51and17 patients respectively. Sensitivity of CFW was high (94.6%) followed by KOH (82.05%) and Gram stain (28.21%) respectively. Fusarium (42.1%), Aspergillus spp (28.93%) were predominantly isolated.

Conclusion: keratomycosis is predominant in young adults with trauma as the major predisposing factor. Direct microscopy with CFW was more sensitive in detecting fungal elements. A simple and rapid Calcofluor White smear examination of corneal scraping early during the disease result in significant progress of the disease in initiating early and specific therapy.

Keywords: Calcofluor white stain, Fusarium species, Potassium hydroxide mount, Mycotic keratitis.

Introduction

Fungal keratitis is a suppurative, ulcerative and sight - threatening infection of the cornea that sometimes leads to loss of the eye .Corneal infection of fungal etiology is very common and represents 30% - 40% cases of culture positive infectious keratitis in South India[1]. WHO estimates global blindness about 20-40 million. In India there are approximately 10 million blind persons and 3 million are corneally blind [2] .The majority of cases occur among agricultural workers following corneal trauma with vegetative matter contaminated by fungi. These are opportunistic organisms and colonize when natural defense of eye are abrogated by corneal trauma, use of topical steroids or any other predisposing factors [3]. Culture and direct microscopy are the two important investigations that are widely used. Culturing of microbial pathogens is considered to be the gold standard whereas direct microscopic evaluation of smears provides immediate information about the causative organisms for initiating treatment [4 ,5].

As the background knowledge of these factors accumulate, it is becoming important to identify the fungal corneal ulceration and to standardize the laboratory diagnostic methods so that an accurate early diagnosis can be made. Hence, this study aims to identify the most common causative organisms and also compares the efficacy of Calcofluor White staining with other methods like 10% KOH wet mount and Gram stain for early diagnosis of keratomycosis.

Objectives of the study

To evaluate the sensitivity, specificity and predictive value of Calcofluor White stain. To compare the efficacy of Calcofluor White stain with 10% KOH wet mount and Gram stain. To isolate and identify the fungal pathogens using 'c' streak method on Sabouraud's dextrose agar plate. 3. Patients and methods. This study included 100 patients with clinically suspected fungal corneal ulcers attending the department of ophthalmology, Regional institute of Ophthalmology, Bangalore and the duration of study period was from December 2009 to February 2011.

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Inclusion Criteria

1. Patients with corneal ulcer having loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon .
2. Patients who had received antimicrobial therapy and not responding to treatment were included in this study. Patients with suspected (or) confirmed viral keratitis, bacterial keratitis, interstitial keratitis and sterile neurotropic ulcers were excluded .

Sample preparation Multiple corneal scrapings were taken with the help of slit lamp using 26 gauge needle on a syringe. The scrapings from corneal ulcer bed and margins were taken under topical Anaesthesia using 4 % Lignocaine hydrochloride after removal of debris or discharge from the vicinity [6,7].

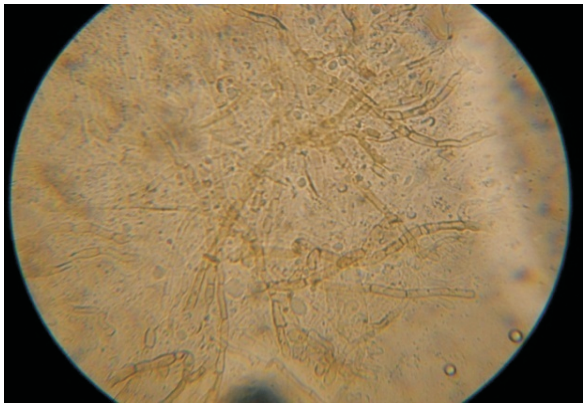

Figure . 1 Fungal elements in KOH wet mount

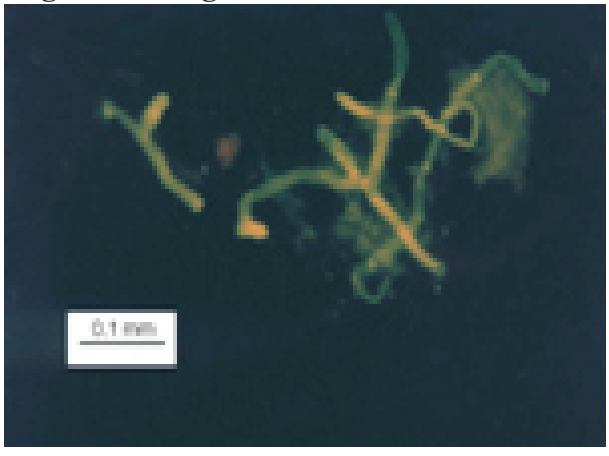

Figure 2. Fungal elements in CFW mount

Direct microscopic examination

The materials from the corneal scrapings were examined under 10% KOH, Calcofluor white stain and Gram stain. a. KOH preparation: wet mount preparation in 10% KOH was done to detect the presence of fungal elements. [Figure 1] b. Calcofluor white stain: one drop of calcofluor white stain (comprising 1g/L calcofluor white M2R and Evans blue Sigma-Aldrich, catalog No 18909 100 ml) and one drop of 10% KOH was added to the slide. The slide was then left to stand for 10 mts and was examined under fluorescence microscopy using blue light excitation (300-400 nm for the emission wavelength with excitation at around 355 nm)[11]. [Figure 2] c. Gram stain: was done to look for hyphae, budding yeast cells and pseudohyphae.

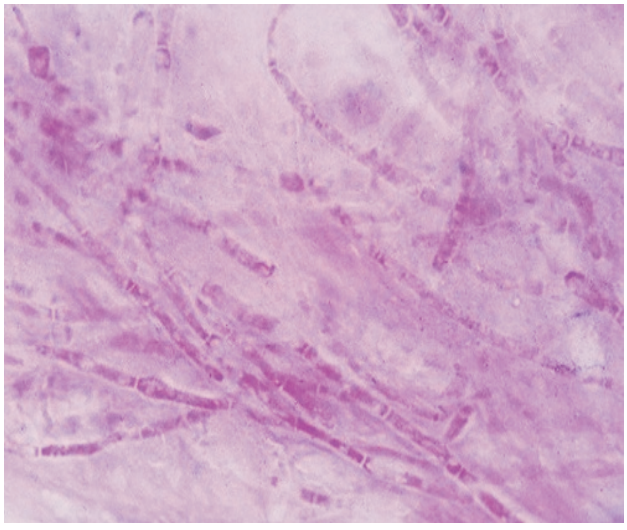

Figure 3. Fungal elements in Gram's stain

Culture of specimens

The corneal scrapings obtained were inoculated directly onto Sabouraud's dextrose agar with antibiotics but without actidione. The material was directly inoculated onto the surface of Sabouraud's dextrose agar media in a row of c-shaped streaks and incubated aerobically at 25 ℃ and 37°C . The plates were examined daily during the first week and twice weekly during the next two weeks.[4,5] The isolates were identified by standard laboratory procedures [4]. The mycelial isolates were identified by their Colony characteristic on SDA microscopic morphology on LPCB wet mount and slide culture. The yeast isolates were identified by standard tests, Germ tube test Chlamydospore production on cornmeal agar and growth on CHROMagar media [4]. No growth was observed even after 3 weeks of incubation the culture was considered as sterile and the plates were discarded [4, 8,9].

Statistical methods

Chi-square /Fisher exact test has been used to find the significance of study. Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Table 1. Age and gender distribution

Age group (years)	Male		Female		Total	
	No.	%	No	%	No	%
1-10	2	3.12	1	2.77	3	3
11-20	2	3.12	2	5.55	4	4
21-30	10	15.62	5	13.88	15	15
31-40	11	17.18	8	22.22	22	22
41-50	14	21.87	9	25.00	20	20
51-60	16	25.00	5	13.88	21	21
>60	9	14.06	6	16.66	15	15
Total	64	100	36	100	100	100%

Table 2. Occupational distribution

Occupation	No. of patients (%)
Farmers	44
Housewives	16
Industrial workers	15
Manual labourers	14
Business class	5
Unemployed	4
Students	2
Total	100

Results

Of the 100 cases studied, 64 (64%) were males and 36 (36%) were females. Male to female ratio was 1.76:1. The age distribution showed the incidence of fungal keratitis predominantly between the 40- 60 yrs i e .the working period of life. The mean age was 40.85 years [Table-1]. Fungal keratitis was predominantly seen in people engaged in farming (44%) followed by housewives (16%), industrial workers (15%) and labourers (14%) respectively . Agriculture is the most common occupation predisposing to fungal corneal ulcers [Table - 2]. Out of the 100 patients, 68 belonged to low socio economic strata while 32 patients were from middle socio economic status. The majority of our patients were involved in agriculture and daily labourers . These persons were therefore exposed to a high risk of corneal trauma with vegetative matter while working in the fields. Table 3 describes the occupational distribution of patients. In our study as many as 34 (62.96%) had corneal trauma as predisposing factor .The other risk factors were use of topical steroids (14), chronic dacryocystitis (1), HIV (1), Diabetes mellitus (1) and contact lens usage (2). As seen from the table 4 corneal trauma with vegetative matter seen in 20 (34.48%). Other causes of trauma were foreign body ,stone ,dust. Corneal trauma with vegetative matter was the most important risk factor for fungal keratitis .Table 5 describes 31 (57.4%) patients had some sort of medication before presenting to the department . 15(27.77%) of them had just antibiotics /steroids eye drops , while 6 (11.1%) of them had received antifungal treatment without any significant improvement. 10 patients had treatment but they did not know the details of medications .

Table 3. Predisposing factors

Predisposing factor	No of cases	Percentage (%)
Trauma	34	62.96
Topical steroids	14	25.92
Diabetes mellitus	1	1.85
Chronic dacryocystitis	1	1.85
Contact lenses	2	3.70
HIV	1	1.85
None	1	1.85
Total	54	100

Out of 100 cases studied ,KOH was positive in 46 patients, CFW was positive in 51 patients and Gram stain positive in 17 patients. In the present study ,positive fungal culture was taken as the gold standard showed the sensitivity of CFW (94.6)

higher in detection of fungal filaments compared to KOH and Gram stain (Table 6,7, 8). shows the fungal culture and direct microscopy methods and sensitivity & specificity of staining methods relative to fungal culture results .

Table 4. Nature of trauma

Nature of trauma	No. of cases
Vegetative matter	20
Paddy	12
Thorn prick	05
Wooden sticks	03
Foreign body	14
a. Lime injury	4
b. Stone	3
c. Dust	5
d. Finger nail injury	2
Total	34

Table 5. Prior treament received

No treatment	23
Antifungal	6
Antibiotics/ steroids	15
Not known	10

Table 6. Direct demonstration of organisms

Method	Positive	Percentage	Negative	Percentage
KOH	46	46%	54	54%
CFW	51	51%	49	49%
Gram stain	17	17%	83	83%

Of the 38 cultures positive on SDA ,Fusarium spp (42.1%) was the most common isolate followed by Aspergillus spp (28.93%) among which Aspergillus fumigatus was the predominant isolate .The other common isolates were Curvularia (5.26%), Penicillium spp .(7.89%) ,Candida albicans (7.89%), Acremonium spp, (5.26%) and Cladosporium spp (2.63%) .The total percentage of culture positivity was 38% .Curvularia spp. (3.44%) was most common among the pigmented fungi followed by Cladosporium spp (1.72%) [9].

Table 7. Correlation of KOH ,CFW ,Gram with Culture

Methods	Direct microscopy positive Culture Positive	Direct microscopy positive Culture negative	Direct microscopy negative Culture positive	Direct microscopy negative Culture negative	Total
KOH	32	14	7	47	100
CFW	36	13	5	46	100
GRAM	11	6	28	55	100

Table 8. Comparison of sensitivity ,specificity , positive predictive value and negative predictive values.

Methods	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
KOH	82.0	77.05	69.57	87.04	79.00	<0.001**
CFW	94.06	75.41	69.38	90.20	80.00	<0.001**
GRAM	28.21	90.16	64.71	66.27	66.00	0.017*

Table 9. Distribution of isolates in fungal keratitis

Species	No of isolates	%
Fusarium species	16	42.10
Aspergillus fumigatus	06	15.78
Aspergillus flavus	03	7.89
Aspergillus niger	02	5.26
Acremonium species	02	5.26
Curvularia	02	5.26
Cladosporium	01	2.63
Penicillium species	03	7.89
Candida albicans	03	7.89
Total	38	100%

Discussion

Keratomycosis is an important ophthalmic problem in all parts of the world, as it leads to corneal blindness and sometimes loss of the vision. Its incidence is reported to vary from 7%- 40% in various parts of India [9,10]. Early and rapid identification of the causative organism is the key to ensuring successful medical therapy for corneal infections and is particularly true for fungal keratitis. Thus, microscopical evaluation of corneal scrapings from patients with suspected fungal keratitis is of the utmost importance because early and rapid detection of fungal structures permits early initiation of antifungal therapy[11].

The mean age in the present study was noted to be 40.85yrs (20yrs-70yrs). This is comparable with the study of Samar K Basak et al [5]. The age distribution showed the incidence of fungal keratitis predominantly between the 4th to 6th decades, reflecting the active working period of life and hence the increased vulnerability to injury during outdoor activities. The male predominance of fungal keratitis (64%) noted in the present study correlates with the studies of Bharathi et al, Srinivasan et al and Chowdhary et al [6,12,13]. Trauma was the major risk factor in 34% of the affected eyes. The sensitivity of KOH used in the present study for diagnosing mycotic keratitis was determined to be (82.05%) and is comparable to the studies by Jagdish Chander et al and Tahereh Shokohi et al[14,15,16]. Calcoflour white was found to have a better sensitivity (94.6%) as compared to KOH and similar findings have been reported by Savithri Sharma et al, Tahereh Shokohi et al, Zhang et al and Usha Gopinathan et al [17,18, 19]. Specificity of Gram stain, KOH and CFW stain were 90.16, 77.05 and 75.41% respectively. Chander et al [18] in their study stated that CFW on direct microscopy detected fungi in 20(95.2%) patients in comparision with 15(71.4%) patients by both KOH wet mount and culture. They confirmed the superiority of KOH+CFW in comparision of with KOH alone and culture. Sharma et al [19] also confirmed the superiority of KOH+CFW stain in detection of fungal elements in corneal scraping. It is not only highly specific and sensitive but also has a high NPV and PPV for both early and advanced keratitis. Since they had seen some false positive results in advanced keratitis, they recommended resorting to antifungal therapy whenever a KOH+CFW stain smear is positive for fungus. Shokohi et al[10] in their study revealed a sensitivity of 71.4% for KOH and 42.9% for Gram stain. They concluded that KOH with CFW as the single most important screening test for rapid diagnosis of fungal corneal ulcer. Zhang et al[11] found out sensitivity of KOH+CFW was 96.6%. This combined staining method, enhancing the rate of correct diagnosis.

In the present study, 38 of the 100 corneal ulcer cases (38%) yielded fungal isolates on culture. Of these, Fusarium spp (42.1%) was the most common isolate followed by Aspergillus spp (28.93%). Aspergillus fumigatus was most frequently isolated among Aspergillus spp the other common isolate were Penicillium spp (7.89%), Candida albicans (7.89%), Curvularia spp (5.26%), Cladosporium spp (2.63%). These observations were comparable with studies by Gopinath et al, M J Bharathi et al [9,20]. Aspergillus spp was the predominant isolate as compared to Fusarium spp in the various studies by Samar K Basak et al (59.8%), Jagdish Chander et al (41.1%), Jagdish Chander et al (35.9%), Javadi et al (34.8%), Shokohi et al (33.3%) [3,16,20,23,10]. Gopinath U et al stated that reports from South India, Florida, and Ghana have identified species of Fusarium and Aspergillus as common corneal pathogens [22]. Curvularia spp were foremost among the pigmented fungi. Vajpayee et al noted that cultures were positive in 51.46% cases, although the fungus was demonstrated by KOH in 94.3% of the culture proven cases [23]. In our study yeasts play a minor role because Candida infection predominates in colder climates and is preceded by corneal disease, local immunosuppression or systemic diseases like diabetes mellitus[12].

In the present study, 16 samples remained sterile on culture despite positive direct microscopic examination. The reasons for cultures to be sterile could be patients already using topical steroids or antifungal agents before corneal scrapings were performed and also sometimes insufficient material was obtained for inoculation.

Conclusion

Central corneal ulceration leading most often to uniocular blindness continues to be a common ophthalmologic problem. Keratomycosis is the leading fungal elements than fungal culture. Early and rapid identification of the pathological organism is the key to ensuring successful medical therapy for corneal infections and is particularly true for fungal keratitis.

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