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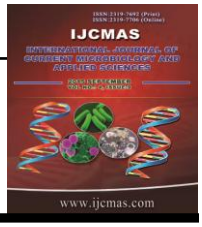

Original Research Article

Mycotic Keratitis in Patients Attending a Tertiary Care Hospital

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Keywords

Mycotic Keratitis, *Aspergillus flavus, Candida albicans,* Fungal culture

A B S T R A C T

Mycotic keratitis is a fungal infection of the cornea. Keratitis is defined as loss of corneal epithelial with underlying stromal infiltrate and suppuration associated with sings of inflammation with or without hypopyon. Fungal Keratitis is a suppurative, ulcerative and sight threatening infection of eye that sometimes leads to loss of eye. Worldwide reported incidence of mycotic keratitis is 17% to 36%. In India it is 44% to 47%. This infection is difficult to treat and it can lead to severe visual impairment or blindness. It is worldwide in distribution, but is more common in the tropics and subtropical regions. The incidence of fungal Keratitis has increased over the last few years. Trauma is the major predisposing factor. Objective of the study is to evaluate the incidence and most common species of the organism isolated from corneal ulcer patients and to identify the predisposing factors causing keratitis. The study was conducted at department of microbiology between Dec 2010 and May 2011, Corneal scrapings from 26 clinically diagnosed patients were subjected to direct examination by 10% KOH mount, Gram stain and fungal culture on Sabouraud's Dextrose agar. This study included 26 cases of corneal ulcers , based on clinical findings, out of which, 12 cases were diagnosed with mycotic keratitis (46.15%) in the laboratory. Among these 12 cases, culture showed fungal growth only in 8(30.76%) cases whereas the remaining 4 cases were positive only by 10% potassium hydroxide (KOH)(15.38%) preparation. Males were more commonly affected and were mostly in the age group of 31-45 years. *Aspergillus flavus* was the most common fungus isolated followed by candida albicans. Rapid diagnosis and early institution of antifungal therapy is necessary to prevent ocular morbidity and blindness. Although culture helps in definite diagnosis and identification, direct microscopic detection of fungal structures in corneal scrapes or biopsies permits a rapid presumptive diagnosis.The study highlights the importance of looking for fungi in infection of eye as appropriate use of antifungal drugs could result in saving the eyeball of patient.

Introduction

In India, there are about 12 million blind people.(9) The incidence of corneal blindness is 15.4%. (12) A recent report on the causes of blindness worldwide constituently lists corneal scarring second only to cataract as major etiology of blindness and visual disability in many of the developing nations in Asia.(1,13).

The term keratitis had been introduced by "James Wardrop" in 1869 in his essay on morbid anatomy of human eye.(10) Keratitis is the descriptive term used for any type of corneal inflammation. The loss of epithelium with inflammation in the surrounding cornea is called as corneal ulcer. It may be caused by bacteria, fungi, viruses or parasites.(2)

Keratitis constitutes the most common and serious ocular infection in developing countries mainly due to lack of medical awareness, inaccessibility to medical treatment in certain parts, having varied occupation like farming, queering and mining , labourers are uneducated and do not have access to safety equipments.(3a).

Infective keratitis rarely occurs in normal eyes without any predisposing factors. The predisposing factors such as trauma, contact lens use, dry eye, ocular surface disorders, immunosuppression may alter defense mechanism and permit pathogenic agent to invade the cornea.(17) Corneal ulcers pose a challenge to ophthalmologists as their etiology is varied. The origin and course of the disease is unpredictable. Similarly, for clinical microbiologist, it is challenging to identify the causative organisms.

As knowledge of pathogenic organisms is increasing and more and more laboratories are performing diagnostic work, many new fungi like Cylindrocarpon lichenicola, Exserohilum rostratum, Scedosporium apiospermum, Colletotrichum dematium etc. are being identified along with established fungi like *Aspergillus spp., Fusarium spp*. As species like Colletotrichum are susceptible to natamycin and azoles, the corneal ulcer due to these fungi if diagnosed early, can be treated successfully without any ocular morbidity.

For diagnosis of microbial keratitis, culture is the gold standard test for isolation of organism and identifying it. But, even in small laboratories, organisms can be identified by microscopy which serves as a guide in the patient management. Because of the factor stated above, this study was taken up to evaluate the various risk factors, age and sex predilection, the type of microbes causing keratitis and the type of antibiotic to which organisms are sensitive so that a systematic approach may be adopted in treating and preventing this sight threatening disorder.

Materials and Methods

The present prospective study was carried out in the Department of Microbiology of S.R.T.R. Government Medical College and Hospital, Ambajogai, District Beed, Maharashtra. A total of 97 clinically suspected patients of keratitis attending Ophthalmology OPD of our hospital from December 2010 to December 2012 were studied. Ethical clearance from Institutional Ethical Committee was obtained.

A total of 97 samples of corneal scrapings collected and transported immediately to microbiology laboratory were processed. A thorough examination of the affected eye was done using slit lamp by the ophthalmologist and the ulcer details were noted. Detailed clinical history of patients such as, age, sex, occupation, duration of symptoms in the form of pain in the eye, lacrimation, photophobia, reddening, decreased vision, history of any trauma to the eye,
associated disease and other predisposing factors if any were recorded.

Collection of Sample

In the affected eye, the integrity of the

corneal epithelium was checked by using 2 % sodium fluorescein solution or 1% sodium fluorescein strips. It is kept in lower fornix for 2 seconds and then removed. The patient is asked to blink, the stain spreads and then the area is visualized. After a detailed clinical examination of the eye, the eye was cleaned with sterile normal saline to remove all necrotic exudates. The eye was then locally anaesthetized by using 2-3 drops of 4% lignocaine hydroxide or 4% xylocaine without preservative. Scrapings of the corneal ulcer were collected using a sterile Bard-Parker surgical blade no.15. The base of the ulcer as well as the edges was thoroughly scraped to obtain as much material as possible. Sample was immediately processed in the microbiology department of the tertiary care hospital, Ambajogai. Corneal scrapings of the patients were examined by direct microscopy by 10% KOH wet mount and Gram stain for demonstration of fungal elements.

Microscopy

KOH (Potassium hydroxide) wet mount: A small portion of scraped material was directly placed on a clean grease free slide and 1-2 drops of 10% KOH was added to the material. A clean cover slip is placed on the material. Slide is gently heated over a flame to hasten digestion of keratin. Care was taken to avoid overheating which causes crystallization of KOH. It was allowed to act for 5-20 minutes. The slide was then carefully observed under 10x and 40x objective to detect fungal elements.

Gram stain: The scraping is teased by a pair of teasing needles and smears were made using small portion of the material over clean grease free slides. The smear was then fixed by passing over a blue flame and was then stained by Jensen's modification of Gram stain method.

Cultures: The scraping was directly inoculated on two slants of Sabourand's Dextrose agar (Emmon's modification) with antibiotics (with Gentamicin and Chloramphenicol but without Cyclohexamide) and two slants without antibiotics. One set of slants (i.e with and without antibiotic) of the medium was incubated at 370C and the other set at room temperature. The cultures were examined for growth daily for first week and twice a week for the subsequent period of about three weeks. The cultures were retained for at least four weeks and then discarded as sterile if there was no growth.

The isolate was considered pathogenic if it grew on repeated cultures, if same isolate grew on more than one medium, if it grew at the point of inoculation and combined with the clinical findings corresponding to the culture findings. Further identification of the fungal isolates was done using the conventional parameters.

The growth rate; texture and morphology of colony on obverse and reverse side on SDA were noted for the fungal identification. Lactophenol Cotton blue (LPCB) preparation was done from the growth to observe the morphology of fungus. Confirmation of aetiological agent was done by putting slide culture on Potato Dextrose Agar (PDA). The yeast isolates were identified by standard tests like Germ tube test, growth on corn meal agar, urease production and sugar assimilation tests.

The material obtained from scraping was inoculated directly onto the surface of solid media such as Blood agar, Chocolate agar, Non-nutrient agar in a rows of C-shaped streaks so that a differentiation between

contaminations can be made and also into the depth of liquid media like Brain-Heart infusion broth. The inoculated media like Blood agar incubated aerobically at 370C and Chocolate agar in candle jar in presence of 5 to 10% CO2, were evaluated at 24 hours and 48 hours and then discarded if there was no growth. BHI broth was incubated for 7 days and examined daily for the presence of growth or turbidity and was considered negative at the end of 7 days of incubation.

Results and Discussion

Keratitis is emerging as a major cause of preventable blindness throughout the world. The incidence of keratitis varies from country to country and region to region. This variation can be due to seasonal and climatic differences and also due to occupation involved in different parts of the world. With this knowledge, the present study was undertaken to know the clinical pattern of keratitis and its correlation with mycological investigation along with various predisposing factors.

This was a kind of first study in our area. This study included 97 cases of corneal ulcers, based on clinical findings, out of which, 31 cases were diagnosed with mycotic keratitis (32%) in the laboratory. All age groups and both the sex were included in this study. The incidence was maximum in the age group 41-50 years (31%), followed by 31-40 years (27.83%) and above 60 years (27.83%) [Table No. 1].

The incidence of fungal keratitis was more in males (72.16%) than in females (27.83%). Male to female ratio was 2.59:1. [Table No. 2] Corneal infection among males could be attributed to their greater involvement in outdoor activities, thus being prone to corneal injury with external agents. Younger age groups in the both the sexes are more physically active and are at risk for corneal injury (4).

It is evident, that keratitis is predominantly seen in people engaged in occupation of farming (58.76%) followed by labourers (14.43%). The region of the proposed study being a rural region, majority of the patients attending the ophthalmology OPD were farmers engaged in outdoor physical activity.

On a global level, predisposing risk factors for mycotic keratitis vary tremendously between developing and developed countries. Corneal trauma is the leading cause for microbial keratitis in South India. (4) The most important predisposing factor in the present study leading to keratitis was trauma (88.65%) followed by topical antibiotic use (69.07%). [Table No. 3] It was the predominant predisposing factor for the fungal keratitis, i.e. 28 (39.43%) cases, trauma with vegetative matter was the cause of keratitis in 71(82.5%) cases. Among them, injury with sugarcane leaf was found in 37(43.02%) cases and with jowar was found in 26 (30.23%) cases with predominance.[Table No. 4] These two crops are the principal crops of this region and the agricultural community is engaged in cultivation of these crops.

In the present study, fungi were the most prevalent (32%), followed by bacteria (23.8%) and mixed infection in 2(2.06) cases.[Table No. 5] Present study showed that, out of 97 cases of corneal ulceration 23 (23.8%) showed the growth of fungus, whereas in 31(32%) fungal element were seen in KOH preparation. Various comparative study were shown in [Table no. 8].

Among the various fungal isolates

Aspergillus spp. was the most prevalent 23(74.19%), among which Aspergillus flavus 8(25.8%) was most common, followed by Aspergillus fumigatus 5(16.12%), then Fusarium spp. 3(9.67%) and Mucor, Candida albicans, Aspergillus niger 2(6.45%) each. 3.22% of the isolates remain unidentified that was the hyaline fungi.[Table No.7] Filamentous fungi accounted for 90.32% of total 31 cases of fungal keratits. The majority of filamentous fungi associated with corneal ulceration in the tropics are saprophytic thermophilic moulds, which are found widely in this environment. The Aspergillius are ubiquitous and have been found almost elsewhere on every conservable type of substrate, including soil and decaying organic debris.

Table.1 Age wise distribution of cases of keratitis

Completed age group in years	Number of cases	Percentage
below 20 years	5	5.15 %
21-30 years	6	6.18%
31-40 years	27	27.83%
41-50 years	30	31%
51-60 years	7	7.22%
above 60 years	27	27.83%
Total	97	100%

Table.2 Sex wise distribution of cases of keratitis

Sex	No. of cases	Percentage
Male	70	72.16%
Female	27	27.83%
Total	97	100%

Table.3 Showing distribution of predisposing factor of keratitis

Predisposing factor	Number of cases (n=97)	
	Total number of cases studied (%)	Number of positive cases (%)
History of corneal trauma	86(88.65)	44 (45.36%)
Topical antibiotic	67(69.07)	28 (28.86%)
Surgery (cataract)	1 (1.03%)	0
Use of contact lens	0	0
Use of herbal medicine	11 (11.34%)	10 (10.30%)
Other local / systemic conditions	5 (5.15%)	4 (4.12%)
No significant history	4 (4.12%)	3 (3.09%)

Table No.4 Distribution of cases according to type of trauma

	Nature of trauma	No. of cases	Percentage (%)
Vegetative matter	Sugarcane leaf	37	43.02
	Jowar	26	30.23
	Groundnut	06	6.97
	Thorn prick	02	2.32
Foreign body	Stone/Dust	09	10.46
	Wood chip	02	2.32
	Iron piece	01	1.16
	Buffalo tail	03	3.48
Total		86	100

Table No.5 Etiological agents in cases of keratitis

Isolated organisms	No. of cases	Percentage
Bacterial isolates	23	23.8
Fungal isolate	31	32
Mixed	2	2.06
Total	52	53.60

Table No.6 Microscopy and culture positivity of fungal isolate.

	No. of samples	Percentage (%)
Total KOH +ve	31	32
Total culture +ve	23	23.8
KOH +ve Culture -ve	08	8.25

Table No.7 Showing Fungal pathogen isolated in cases of keratitis

Fungal isolates	No. of cases	Percentage(%)
Aspergillus spp.	23	74.19%
Aspergillus Flavus	8	25.8%
Aspergillus fumigatus	5	16.12%
Aspergillus niger	2	6.45%
Fusarium spp.	3	9.67%
Mucor	2	6.45%
Unidentified Hyaline fungus	1	3.22%
Candida albicans	2	6.45%
Total	31	100%

Table no.8 Percentage of fungi isolated from various studies

Studies	Place	% Positivity
Saha *et al*[14]	Delhi	22.25
Chowdhary *et al*[7]	New Delhi	39
Gugnani *et al*[8]	Nigeria	80.76
Srinivasan *et al*[13]	Madurai	46.8
Pichare *et al*[11]	Aurangabad	20
Ragini *et al*[15]	Varanasi	45.55
Baradkar *et al*[6]	Mumbai	13.07
Williams G et al [16]	Bagladesh	21.21

Photo No 1: Keratitis with Hypopyon

Photo No 3: Keratitis with ulcer having feathery appearance

Photo No 2: Clinical presentation of Corneal ulcer with ring abcess.

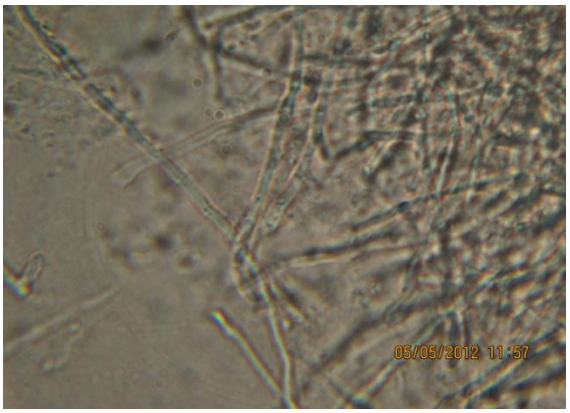

Photo No 4: KOH mount showing Fungal element.

Photo No 5: Growth on SDA of *Aspergillus flavus*

Photo No 8: Growth on SDA of *Fusarium* spp

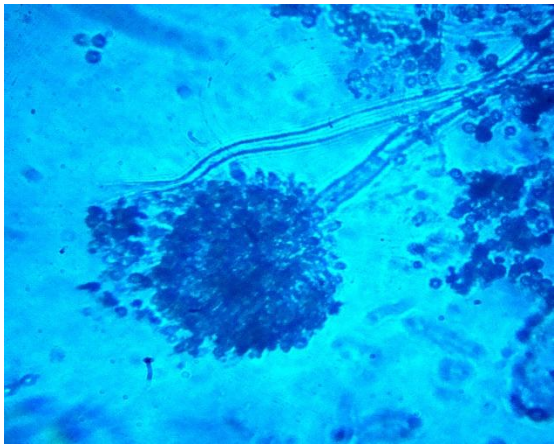

Photo No 6: LPCB mount of *Aspergillus flavus.*

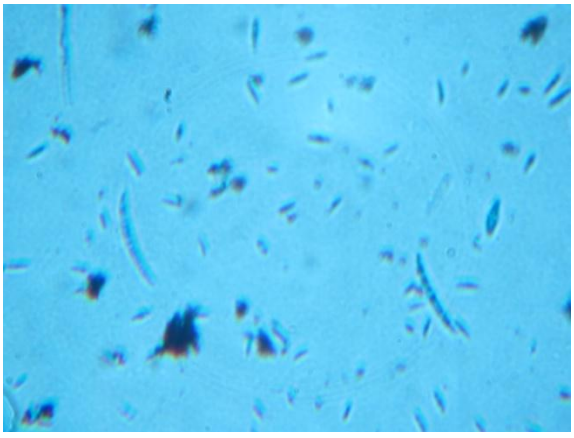

Photo No 9 : LPCB of *Fusarium* spp

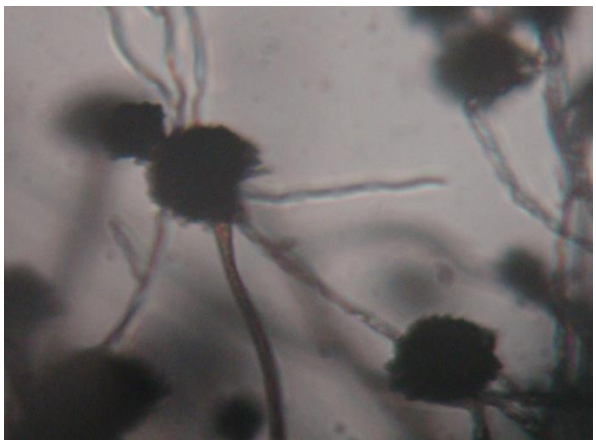

Photo No 7: Slide culture of *Aspergillus* spp

In this study, positivity of KOH mount was more than positivity of culture for fungal keratitis. [Table No. 6] The reason for this observation could be that viable fungus may not be present in all areas of the ulcer crater and therefore every scraping from a corneal ulcer might not have contained viable fungus. (15)

Globally, bacterial keratitis remains the leading cause of microbial keratitis. In developed countries, fungal keratitis is rare, however fungal keratitis occurs with higher frequency in developing countries like India, as fungal keratitis is expected to be more common in tropical & sub-tropical regions than in the temperate regions. In this study, 53.6% cases were microscopy and culture positive, and 46.39% cases had no definite laboratory diagnosis. Possible reasons for this could be some patients were already on topical medication when they arrived, defective scraping techniques by ophthalmologist on call, and/ or problems in microbiology transport and handling.

Among 34.48% of fungal keratitis cases in this study, improved with prompt treatment with antibiotics, antifungal therapy respectively. 19.09% of bacterial, 37.93% of fungal and 50% of mixed keratits deteriorated in spite of treatment. Keratits irrespective of aetiology (bacterial, viral, fungal, parasitic or iatrogenic), if not treated and managed properly may lead to serious complications like glaucoma, anterior staphyloma, corneal perforation, cataract, descemetocele, corneal scarring, endophthalmitis, etc.

In conclusion, Mycotic keratitis continues to be an important cause of ocular morbidity, mostly in the person inhabiting rural areas, involved in outdoor agricultural activity. Young male adults affected in this circumstances are often the bread earners of their family and blindness in them is of much grave economic consequences. Therefore we conclude that early institution of antifungal therapy following meticulous examination of corneal scraping by direct microscopy at rural centers may limit ocular morbidity and disastrous sequelae among these patients.

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