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Clinico-Epidemiological Study of Mycotic Keratitis at a Tertiary Care Hospital

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Abstract

Aims: To study the various clinical presentations of mycotic keratitis based upon the type of fungi and predisposing factor. To study, the epidemiological features of confirmed culture proven cases of mycotic keratitis and to identify the etiological agents. *Settings & Design:* A prospective hospital based cross sectional study at a tertiary care hospital for a period of 3 years from October 2013 to September 2016. *Material & Methods:* 800 culture confirmed cases of mycotic keratitis were enrolled in the study and followed regularly. The demographic, epidemiological characters and clinical examination findings by slit lamp examination were noted. Corneal scrapings and swabs were sent to laboratory for cultivation and identification of fungi. *Statistical Analysis:* The collected data was entered in Microsoft excel spread sheet, verified and entered in MEDCALC software and analyzed for statistical significance. *Results:* Males were more common in the study (66.25%), 21-30 years age group were more common(29.4%) and the incidence of mycotic keratitis was 38.28%. 78% were of rural background and 65.5% of cases were agricultural workers/farmers. Trauma was the common predisposing factor (78.75%) and traumatic agent was vegetative matter like husk, straw etc. Anterior chamber reaction was the commonest finding in slit lamp examination (91%).Fusarium sp was the common fungi (34.2%) followed by Aspergillus sp (32.5%). Candida sp accounted for 18.2% in the study. Pseudomonas aeruginosa was the commonest bacterial pathogen isolated (62.6%). *Conclusion:* our study highlights the demographic particulars of mycotic keratitis and epidemiological characters and clinical presentation in our geographic region. Early diagnosis of the condition is imperative for management either surgical or medical management. KOH wet mount is a simple microscopic diagnostic choice with more specificity which can be used in diagnosis of fungal keratitis.

Keywords: Mycotic Keratitis; Fusarium; Aspergillus SP; Pseudomonas Aeruginosa; KOH Wet Mount.

Introduction

Keratitis is one of the common preventable causes of blindness. This is more commonly seen in the tropical climates and rare in temperate areas. Based on the geographic location, its incidence is between 6% - 20% as reported by various studies [1]. Several studies have shown that etiological agents of this condition may be bacterial, viral, fungal and parasitic. The clinical features produced by these agents are protean and overlapping, hence diagnosis based upon clinical features alone is difficult task for the Ophthalmologist. Corneal infection of fungal etiology is very common in tropical countries like India and accounts to 30-40% of culture positive infectious keratitis [2]. Keratomycosis is caused by around 60 different species of Fungi. Clinical features produced by these fungi itself are variable and accurate identification requires the cultivation of

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Fungus. These fungi are placed under two categories, filamentous fungi and Non-filamentous fungi [3]. The frequency and spectrum of fungi involved in causation of mycotic keratitis varies from place to place, as it is influenced by multiple factors like climate, age, sex, socioeconomic and geographical conditions. While tropical climates show a preponderance of filamentous fungi, temperate climates show higher percentage of yeast infections. The predisposing factors in development of mycotic keratitis are also variable, trauma being a common factor in developing countries where cultivation is a major source of income. In developing countries, increased usage of contact lens is a significant risk factor for mycotic keratitis [4]. The spectrum of fungal pathogens is constantly changing based upon the changes in the climatic factors, geographical conditions and increased usage of contact lenses. Hence a thorough knowledge of the spectrum is essential in identification, prevention and management of cases of mycotic keratitis.

The present study was undertaken to study the various clinical presentations of mycotic keratitis based upon the type of fungi and predisposing factor. To study, the epidemiological features of confirmed culture proven cases of mycotic keratitis and to identify the etiological agents. Our study also compares the direct microscopy with LOH wet mount examination, gram staining and culture on Sabourad's Dextrose agar in cultivation of the fungi.

Materials & Methods

A hospital based prospective cross sectional study was conducted at Narayana medical college and General Hospital by Department of Ophthalmology for a period of 3 years from October 2013 to September 2016. All the patients attending the Outpatient department of Ophthalmology and Emergency department were selected in the study based on the inclusion criteria. Patients clinically suspected of Bacterial and fungal corneal ulcers were included in the study. Ulceration is defined as a loss of corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without Hypopyon. Corneal ulcers with features suggestive of viral keratitis, healing ulcers and ulcers associated with autoimmune conditions were excluded from the study. The study was approved by the institutional ethical committee and all the procedures were performed as per the ethical guidelines of the institution. The patient's demographic data, occupational history, duration of symptoms, History of trauma and systemic illness were interviewed and entered in a predesigned questionnaire form. The study was explained to the cases in the study and written consent was obtained from the cases.

Clinical Examination

All the cases were examined by a slit lamp microscope and features of the ulcer, presence or absence of hypopyon, pigmentation, margins of the ulcer were noted.

Collection of Corneal Scrapings

Scrapings from the edges of the ulcer were collected by Ophthalmologist under aseptic conditions using sterile Bird-Parker blade (No 15). The collection was done by using operating microscope after installation of 4% lignocaine. The scrapings were transferred immediately to the laboratory for further processing. Swabs were also collected from the deep base and leading edges of the ulcer.

Laboratory Procedures

The scrapings received were transferred immediately onto sterile sheep Blood agar, Chocolate agar and two sets of Sabourad's Dextrose agar with and without actidione for growth of the fungi. The scrapings were inoculated in a pattern of "C" shaped streaks deeply into the solid media. The swabs were spread thinly on a new clean glass slides for wet mount examination with 10% KOH solution and Gram's staining. The inoculated media were incubated aerobically at 25^0c and 37^0c for four weeks and examined daily for 1st week and twice a week thereafter.

Identification of Isolates

Bacterial isolates were identified by standard biochemical tests and grams staining. Fungal isolate was considered significant if it was consistent with clinical signs, growth occurred on the C streaks and same organism was grown for second time also. Fungi were identified by colony morphology; features on LCB mount and slide culture as per standard mycological guidelines. Candida species were identified by germ tube formation, chlamydospore formation on corn meal agar and sugar assimilation tests.

Statistical Analysis

All the data was entered in Microsoft excel spread sheet and checked. Diagnostic parameters like

sensitivity, specificity and predictive values were calculated using MEDCALC software.

Results

In our study, a total of 2090 cases with clinical features suggestive of corneal ulceration were examined and evaluated. In 2090 cases, 800 of cases were fungal growth positive (38.28%), 684 cases were bacteriological culture positive (32.73%), 74 cases were both bacterial and fungal growth positive (3.54%) and 532 cases were sterile with no growth (25.45%). The incidence of mycotic keratitis in the study was 38.28%. 812 fungal isolates were recovered from 800 cases of fungal keratitis, pure growth was seen in 788 cases and mixture with two fungi and one bacterial were seen in 12 cases of the study. 684 bacterial isolates were isolated in the study.

800 confirmed cases of mycotic keratitis were included in the study and studied for various factors. In total 800 cases males were predominant (66.25%) almost equal to twice the females (33.75%) in the study. The male to female ratio in the study was 1.96:1 and mean age was 41.32 years. The common age group in the study was 21-30 years (29.4%) followed in order by 31-40 years (25.3%), 41-50 years (23.5%) and 12.1% in >50 years age group. 77.5% of cases were from rural locality and 65.5% were agricultural workers/ farmers. 155 were labourers, 5 were students and 5.75% professionals. Trauma was the commonest predisposing factor for mycotic keratitis (78.75%) followed by ocular conditions like surgical conditions, lagophthalmos, lid injury etc. Diabetes mellitus and topical steroid use was also observed in few cases in our study. 68.9% of cases had injury with vegetative matter like (paddy husk etc), other less common causes were insects, fingers, contact lens injuries etc. Some of the cases in the study were associated with systemic diseases like diabetes mellitus (3%) and topical corticosteroid usage (2.25%). Statistically significant association was found with male sex, trauma and vegetative matter as a causative agent (p valve<0.001) [Table 1].

Table 1: Demographic data of cases in the study.

Gender	No	%
Male	530	66.25
Female	270	33.75
Total	**800**	100
Age in years		
<21 years	78	9.8
21-30 years	235	29.4
31-40 years	202	25.3
41-50 Years	188	23.5
>50 years	97	12.1
Residence		
Rural	620	77.5
Urban	180	22.5
Occupation		
Agricultural worker	524	65.5
Labourer	120	15
Student	56	7
Household	32	4
Salaried	46	5.75
Others	22	2.75
Predisposing factors		
Trauma	630	78.75
Occular conditions:	128	16
Topical steroids	18	2.25
Diabetes mellitus	24	3
Traumatic agents		
Vegetative matter	434	68.9
Soil/Dirt/sand	98	15.6
insects	30	4.8
fingers	24	3.8
Miscellaneous	18	2.9
Conatct lens	26	4.1
Total	**630**	100

On slit lamp examination, features observed are presented in Table 2. Anterior chamber reaction was seen in 91% of cases; Conjunctival injection in 81.8%, Hypopyon in 86.3%, Suppuration in 40.4% and raised corneal surface in 33.4% of cases. Less commonly observed findings were satellite lesions (10.5%), feathery margins (15.8%), immune rings (5.8%) and perforation (3%) of cases.

The sensitivity of KOH examination in detection of fungal elements was higher (95.42%) than gram stained smear (93.145%). The specificity was better by KOH examination (99.185%) and gram staining (99.18%) than Slit lamp examination (92.43%) PPV was almost equal in KOH wet mount and gram staining than slit lamp examination, whereas the negative predictive value is less in gram stain examination than by slit lamp and KOH wet mount examination [Table 3].

Table 2: Slit lamp examination findings in Cases of Mycotic keratitis.

Sign	No of Patients	%
Conjunctival injection	654	81.8
Anterior chamber reaction	728	91.0
Suppuration	323	40.4
Hypopyon	690	86.3
Raised corneal surface	267	33.4
Satellite lesions	84	10.5
Stromal infiltrtae with feathery margins	126	15.8
immune rings	46	5.8
Perforartion	24	3.0

Table 3: Correlation between KOH and gram stained examination with slit lamp examination and culture growth of cases in the study

S. No	Name of the Investigation	Results	Number	Presence of Fungal growth		Sensitivity	Specificity	PPV	NPV
				Positive	Negative				
1	*10% KOH mount*	Positive	844	834 (TP)	10 (FP)	95.42%	99.18%	98.82%	96.79%
		Negative	1246	40 (FN)	1206 (TN)				
		Total	2090	874	1216				
2	*Detection of fungal elements in gram stain*	Positive	824	814 (TP)	10 (FP)	93.14%	99.18%	98.79%	95.26%
		Negative	1266	60 (FN)	1206 (TN)				
		Total	2090	874	1216				
3	*clinical examination by slit lamp*	Positive	924	832 (TP)	92 (FP)	95.19%	92.43%	90.04%	96.40%
		Negative	1166	42 (FN)	1124(TN)				
		Total	2090	874	1216				

Table 4: Bacterial and Fungal isolates from the cases in the study

Fungal isolates	No	%
Aspergillus Sp	264	32.5
A.niger	168	20.7
A.fumigatus	43	5.3
A.flavus	28	3.4
Other species of Aspergillus	25	3.1
Candida Sp	148	18.2
C.albicans	108	13.3
C.tropicalis	24	3.0
C.parapsiliosis	11	1.4
C.glabarata	5	0.6
Curvalaria Sp	43	5.3
Fusarium Sp	278	34.2
Pencillium sp	16	2.0
Alternaria Sp	11	1.4
Mucor Sp	18	2.2
Rhizopus	24	3.0
unidentified fungii	10	1.2
Total	812	100
Bacterial isolates		
Pseudomonas aeruginosa	428	62.6
Staphylococcus aureus	148	21.6
CONS	70	10.2
Streptococcus pneumoniae	38	5.6
Total	**684**	**100**

The predominant fungal species identified was Fusarium (34.2%), followed in order by Aspergillus sp (32.5%) and candida sp (18.2%). Less commonly observed fungi were Curvalaria (5.3%), Rhizopus (3%), Mucor (2.2%), Pencillium sp (2%), Alternaria (1.4%). 1.2% of fungi were unable to be identified. Aspergillus niger (20.7%) and candida albicans (13.3%) were commonest among the species. Among the bacterial isolates in the study Pseudomonas aeruginosa was the commonest (62.6%) followed by staphylococcus aureus (21.6%). Other isolates were CONS (10.2%) and streptococcus pneumoniae (5.6%). [Table 4].

Discussion

Presence of fungi is ubiquitous and their distribution widely across is responsible for different types of infections. Among many fungal diseases, mycotic keratitis is one of the several diseases, which is responsible for blindness if not timely diagnosed and managed. Fungi causing keratitis can be either yeasts or moulds or filamentous fungi. Fungi reproduce sexually or asexually and are grown in asexual phase when cultivated from the scrapings of the cornea.

The incidence of mycotic keratitis differs from place to place, country and country based upon geographical factors, climatic conditions and nature of occupation. The incidence of mycotic keratitis in our study was 38.28%, which is almost similar to findings in reports of various studies. As reported by Gopinathan U et al, Srinivasan M et al, the incidence from south India is 32-38%[1,2]. The incidence of mycotic keratitis was more in males, in the age group of 21-30 years. These findings suggest that males are more actively engaged in work with outdoor activities, and age group is most active period of work in the life making them vulnerable to injuries of the eye. Trauma was the most common predisposing factor and was most commonly observed in rural population in our study. In our study area, paddy cultivation is the most common source of economy and vegetative matter (paddy, Husk, straw etc) were the most common agents of injury for the cornea. Findings of our study were on par with the reports of Parmjeet Kaur Gill et al, Agrwal J et al and many other studies globally [5,6]. Higher incidence of fungal keratitis is observed in males of age group 21-40 years and rural areas and more common in farmers and Agricultural labourers as per the reports of many studies which coincides with the findings of our study [7].

Trauma was the most common predisposing factor of fungal keratitis in our study (78.75%) which is most common in labourers and agricultural workers, farmers. Similar findings were reported by Leck AK et al who reported the incidence of trauma as 76% in his study as predisposing factor for fungal keratitis [8]. Schafer et al have reported associate ocular co morbidities like lagophthalmos, lid injuries also have an important role as predisposing factor, in our study the incidence of ocular conditions was 16% (Surgical conditions etc) which is little less than the findings reported from developed countries. The factors associated with mycotic keratitis are variable from developing to developed countries. Contact lens as a traumatic agent has gained importance due to wide spread usage among developed countries, it is reported as important traumatic agent in studies of UK and Florida, but in our study its role as causative agent was restricted to only 4.1% whereas studies from developed countries have reported its as an important agent which ranges from 10-12% [9]. This represents a significant lower incidence of contact lens usage among our patient population or higher incidence of other risk factors in our study. Some of the studies have reported diabetes mellitus, topical corticosteroid usage as significant risk factors in development of fungal keratitis; however in our study the rate of association of DM and steroid usage was 3% and 2.5% which is lower than some of the studies. This is however explained by the fact that our study population mostly was agricultural farmers and laborers who are actively engaged in regular physical exertion and age group in our study was mostly between 21-40 years where the incidence of DM is usually low.

Clinical manifestations of fungal keratitis are protean and are multifactorial dependent. These are mainly dependent upon age of the case, type of fungus, severity of the pathogen etc. In our study, by slit lamp examination anterior chamber reaction was the most common entity followed by hypopyon and Conjunctival injection [10,11]. Other associated lesions were Suppuration (40.4%), raised corneal surface (33.4%), stromal infiltrate with feathery margins (15.8%) and immune rings (5.8%). These findings are always variable and sometimes may be misleading in diagnosis [12]. The sensitivity and specificity of slit lamp examination was 95.19% and 92.43% in our study and Positive predictive value was 90.04% and Negative predictive value was 96.4%.

In our study, good old traditional method of KOH wet mount examination of corneal scrapings has sensitivity of 95.42% and specificity was 99.18%

which was higher when compared with Grams staining with sensitivity of 93.14%. These findings were on par with the findings of Sharma S et al who reported similar findings in their study [13]. Hence grams' staining alone lacks good sensitivity in identification of cases and results in underreporting of cases of Keratomycosis. Isolation of fungal pathogen by cultivation on SDA still remains as the gold standard in the diagnosis, bur delay in the growth and errors in identification of fungi leads to underreporting in cases of fungal keratitis. Absence of growth in KOH positive cases could be due to administration of antifungal topical preparations.

The type of the fungal pathogen implicated in mycotic keratitis is variable from place to place and is dependable upon the type of occupation involved. In our study, Fusarium sp was the most common pathogen (34.2%) with very little variation with Aspergillus sp which accounted for 32.5% of cases in our study. As described in many studies, filamentous fungi were more common than the yeasts in our study also. The difference in the isolation rates of the pathogens could be due to difference in the traumatic agent and predisposing risk factors of the cases in the study. Higher incidence of fusarium in the study than Aspergillus could be due to the fact, fusarium is a plant pathogen and most of the cases in the study were farmers and agricultural labourers [14]. Aspergillus niger was the commonest species among Asergillus sp (20.7%) and Candida albicans was the commonest among Candida sp. (13.3%). Similar findings were reported by Saha et al from New Delhi [15].

Mixed infections with bacterial and fungal were reported in 74 cases (3.54%) in our study. Similar incidence was reported by Hagan M et al and Capriotti JA et al in their studies as 4% and 13% in their studies [16,17]. Pseudomonas aeruginosa was the most common bacterial pathogen (62.6%) in the study followed by Staphylococcus aureus. Other bacterial pathogens were Coagulase negative staphylococci and Streptococcus pneumoniae.

To conclude, our study highlights the demographic particulars of mycotic keratitis and epidemiological characters and clinical presentation in our geographic region. Fungal keratitis is still a concern for ophthalmologist. Early diagnosis of the condition is imperative for management either surgical or medical management. KOH wet mount is a simple microscopic diagnostic choice with more specificity which can be used in diagnosis of fungal keratitis. Trauma with a vegetative material appears to be most common cause of fungal keratitis in our study. Aspergillus sp and Fusarium sp were common causative fungi implicated in the condition.

Acknowledgements

Nil.

Conflict of Interest

Nil.

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