Microbiology



MYCOLOGICAL PROFILE OF CORNEAL ULCER AT TERTIARY CARE CENTRE IN CENTRAL INDIA

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ABSTRACT Aim and objective: To study the Mycological profile of corneal ulcer in patients attending tertiary care hospital, from		

January 2019 to September 2019, IGGMC, Nagpur **Methods:** Corneal scrapings collected under slit-lamp examination with aseptic precautions were subjected to KOH mount, Gram stain, inoculated on Sabouraud dextrose agar and sent to Microbiology lab for further processing.

Results: Out of total of 56 patients with keratitis, 22 were positive for fungal growth. These included 43 (76.79%) men and 13(23.2%) women. Overall, 69.6% were rural inhabitants and 30.4% were urban. Most common affected age group was 31-40yrs (30.90%). Farmers were (50%) mostly affected occupational group. The commonest risk factor was trauma 68.17%. The incidence of fungal keratitis in our study was 39.3%. The most common fungal isolate was Aspergillus species (36.36%) and Candida (22.72%)

Conclusion: Mycotic keratitis is an avoidable disease. Early diagnosis and treatment can prevent the blindness.

KEYWORDS : Corneal ulcer, KOH mount, Sabouraud's Dextrose Agar

INTRODUCTION

Corneal ulceration results in 1.5–2 million new cases of corneal blindness annually, posing a major public health problem according to the World Health Organization (WHO) reports⁽¹⁾ Microorganisms can penetrate through a breach in the epithelium either, by trauma involving plant or vegetable matter sustained in agricultural farms, due to surgery often leading to infectious corneal ulceration and loss of vision⁽³⁾. Keratitis is the most frequently encountered fungal infections, although the orbit, lids, lacrimal apparatus, sclera, conjunctiva and intraocular structures may also be involved.⁽³⁾ Invasion of fungi into corneal layers followed by the subsequent tissue damage that follows is particularly devastating as it can disrupt the visual axis ⁽⁴⁾

Various risk factors have been implicated for increased incidence of fungal keratitis including widespread use of antibiotics and steroids, use of contact lenses, and postoperative infections⁽⁶⁾. The knowledge of epidemiological profile of mycotic agent will help in presumptive treatment where cultures are negative or facilities do not exist.

MATERIALS AND METHODS

Corneal scrapings from 56 patients with the clinical diagnosis of corneal ulcer with or without hypopyon attending ophthalmology department of IGGMC, Nagpur Hospital from January 2019 to September 2019 were included in the study.

Proper history was taken like age, sex, occupation, H/O trauma, surgery, antibiotic intake, steroid intake. Consent was taken for corneal scrapping. Corneal scrapping was taken in ophthalmology department under local anesthesia i.e. 4% paracaine eye drops without preservative.

Corneal scrapping was done from the leading edge and the base of the ulcer by using kimura spatula or 15 no sterile Bard Parker Surgical Blade with the help of slit lamp under aseptic conditions. Samples were Inoculated in SDA, Blood agar and slides were labeled and transported to microbiology laboratory, where SDA and Blood agar was incubated at 25°C and 37°C respectively in the incubator . Specimen was examined by Direct microscopic examination of the corneal scraping was performed with 10% potassium hydroxide (KOH) wet mount and by gram staining. The scraping were directly inoculated on two slants of Sabourand's dextrose agar incubated at 25°[°] . Identification was done by standard microbiological methods

for fungal diagnosis. Yeast were further prossed by gram staining , germ tube ,HiCHROM agar inoculation and dalmau plating culture . Filamentous fungi were processed by Lactophenol cotton blue prepration (after tease mounting) and slide culture .⁽⁶⁾.

RESULTS

Out of 56 patients of keratitis, 22 were positive for fungal growth. This included 43 (76.79%) men, 13(23.21%) women and 69.64% were rural and 30.36% were urban inhabitants.

The commonest age group to be affected was 31-40yrs (46.51%) followed by 41-50 years. Peak incidence of corneal ulceration was seen during the monsoon season (June to September) 77.27%. This was followed by a high incidence during the harvest season (Jan. to March) 86.36%. In our study, out of the 22 positive cases, 11(50%) were farmers by occupation, 7 (31.82%) were working as daily labourers, 2 (9.09%) were working indoors (office/home maker), and 2 (9.09%) were drivers. In our study we observed that the most common risk factor associated with corneal ulcer was trauma 68.17% (15/22) of which vegetative trauma accounted for 36.36% (8/22) cases, followed by dust particles 13.63% (3/22). The incidence of fungal keratitis in our study was 39.3%.

Overall the most common isolate was Aspergillus species 8/22(36.39%) followed by Candida species 5/22 (22.72%) & then fusarium 3/22(13.64%). Among the Aspergillus species, Aspergillus fumigatus was the commenst 4(18.20%) and then followed by Aspergillus niger 3/22(13.64%) & Aspergillus flavus 1/22(4.55%).

The other fungi isolated were Mucor 2/22(9.09%), Acremoium1(4.55%), Cladosporium 1(4.55%), Curvuleria1 (4.55%) & Grapium 1(4.55%)

Table 1: Age & Sex distribution of patients with corneal ulcer

Age in years	Male (43) (76.79%)	Female (13) (23.21%)
0-10	0 (0%)	1 (7.7%)
11-20	1 (2.32%)	1 (7.7%)
21-30	5 (11.62%)	1 (7.7%)
31-40	20 (46.51%)	6 (46.2%)
41-50	13 (30.23%)	3 (23.0%)
51-60	3 (7.0%)	1 (7.7%)
61-70	1 (2.32%)	0 (0%)
Total	43 (100%)	13 (100%)

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 Table 2: Demographic profile of the positive patients with Corneal ulcer

 (n-22)

		(11-22)
Region :-	Rural	69.64%
_	Urban	30.36%
Month of Presentation :-	January-March	86.36%
	April-may	45.45%
	June-September	77.27%
	October- December	40.90%
Occupational Details :-		
Farmers	11	50%
Daily wages labourers	7	31.82%
Working indoors	2	9.09%
Drivers	2	9.09%
Total	22	100%

Table 3: Predisposing factors associated with positive corneal ulcer patients.

Predisposing factors	Number of cases	%(n=22)
Vegetative trauma	8	36.36%
Dust particles	3	13.63%
Others factors related with trauma	4	18.18%
Diabetes Mellitus	3	13.64%
Use of steroids	2	9.09%
Dust particles	1	4.55%
Contact lens usage	1	4.55%
Ocular abnormalities/ surgery	0	0
Total	22	100%

Figure 4: Distribution of fungal species in positive cases of Ocular mycosis





DISCUSSION:-

Corneal blindness is a major public health problem worldwide & Fungi are an important cause of ocular infections leading to the visual impairment. ^(7,8) Mycotic keratitis may occur at any age, but highest incidence coincides with the period of maximal activity. ⁽⁹⁾ In the present study, maximum number of cases were of the age group 31-40 yr (46.51%), followed by 41-50 yrs (30.23%). This is the most common actively working age group which was similar to the study by **Tapan Gogoi et al** ⁽¹⁰⁾ where most of the cases (33%) of keratitis were in the age group 31- 40 years. **G. satpathy et al** ⁽¹¹⁾ also reported maximum cases in the age group 31-40 yrs (30.8%) followed by 41-50 years (25.4%).

In our study gender distribution revealed that male preponderance (76.79%) is significantly high as compared to females (23.21%). Similar findings have been reported in **Parmjeet Kaur Gill et al**⁽¹²⁾, in which keratomycosis was found to be more common in men (79.97%) than women. The reason for this is that men in this age group have greater exposure to the fungal agents due to maximum outdoor activity which predispose them to occular trauma and other types of traumas with causative source contaminated with fungus⁽¹²⁾

We observed that fungal keratitis was more common in rural areas 39/56 (69.64%) than urban areas 17/56 (30.36%). **S Verma et al**, ⁽¹³⁾also observed higher prevalence in rural areas (85%). This may be because mycotic keratitis is an important cause of ocular morbidity in rural population of developing countries where agriculture is the main occupation. **Vasudha C.L et al** ⁽¹⁴⁾

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In this study, farmers were (50%) most commonly affected followed by daily wages workers. This is attributed to ubiquitous fungal spores and presence of these on senescent plant material(**AGupta et al**)⁽¹⁵⁾

Frequency of ocular trauma due to vegetative origin was predominant 8/22(36.36%) followed by Diabetes Mellitus (13.64%). **Srinivasan et al** ⁽¹⁶⁾ also showed farmers to be the most affected cases. In some other study done by **Mohod P.N et al**, also Vegetative trauma was the most common predisposing factor for the development of corneal ulcer representing 49% cases⁽¹⁷⁾.

This may be due to an Injury with vegetative origin like sugarcane leaf which reaches the average level of the human eye & fungi are known soil saprophytes and plant pathogens. Furthermore Indian climate favors the growth of these fungi. This is in accordance with study done by **Kanishtha Sharma et al**, in which Ocular trauma due to Vegetative origin was the the predominant cause (81.48%) followed by Diabetes Mellitus (50%)⁽¹⁸⁾. In contrast, A study from Philadelphia, showed chronic ocular surface disease, contact lens usage and use of topical corticosteroids as commonest risk factors.⁽¹⁹⁾

The incidence of fungal keratitis was higher during the paddy harvest season (Jan. to March)- 86.36% and the monsoon season (June – September) 77.27%, when agricultural activity was greater. The peak incidence correlates with windy and dry weather during the month of June through September and during hot, humid, windy climate which makes fungal keratitis more frequent in tropical zones⁽²⁰⁾. **Our study correlates with Bharathi M. J et al**⁽²¹⁾.

The incidence of fungal keratitis in our study was 39.3%. which is similar to study done by **Bharathi M J et al**⁽²¹⁾. The incidence of fungal keratitis in this study was 34.4%. Incidence reported in other regions of India are 7.3% in North India⁽²²⁾, 32% in East India⁽²³⁾, 38.9% in West India⁽²⁴⁾ and 39.8% in South India. ⁽²⁵⁾ **Mohod P.N et al** reported a very high incidence of 59.09% ⁽¹⁷⁾. This regional variation could be because fungal keratitis is expected to be more common in the tropical and subtropical regions than in the temperate regions.

Overall the most common isolate in this study was Aspergillus species 8/22(36.39%) followed by Candida 5/22 (22.72%) & fusarium 3/22(13.64%). **Parmjeet Kaur Gil et al**, also got most common fungal isolates as Aspergillus species (50%), Candida (20%) and Fusarium (15%)⁽¹²⁾ **Rumpa Saha et al** ⁽⁹⁾ got similiar result. As has been reported worldwide as well as from India, Aspergillus species is the most common isolate in fungal keratitis⁽²⁶⁾.

In contrast to our Study, Mohod et al ⁽¹⁷⁾ and Shrinivasan et al ⁽¹⁶⁾ (31)showed Fusarium to be the commonest isolate followed by Aspergillus spp. The other fungi isolated in this study were Mucor 2/22(9.09%),Acremoium1(4.55%),

cladosporium 1(4.55%),Curvuleria1 (4.55%) & graphium1(4.55%). **Bharathi M J et al** reported other fungal isolates as Mucor 0.54%, Cladosporium 6.64%, Curvularia 2.64% $^{(21)}$

CONCLUSION-

To conclude, Mycotic keratitis is an avoidable vision-threatening disease that still represents a considerable proportion of daily cases which leads to severe ocular morbidity.

Though bacteria, fungi and parasites are responsible for keratitis, fungal agents deserve special mention due to their opportunistic behavior. Early, meticulous examination of corneal scrapings by microbiological diagnostic methods and timely institution of antifungal therapy may limit ocular morbidity and its disastrous sequelae. Proper understanding of microbiological and clinical characteristics of keratomycosis will decrease the indiscriminate use of drugs.

REFERENCES

- Insan NG, Mane V, Chaudhary BL, Danu MS, Yadav A, Srivastava V. A review of fungal keratitis: Etiology and laboratory diagnosis. Int J Curr Microbiol App Sci 2013;2:307 314.
- Robert C. Arff, Grayson's Diseases of the Cornea, 4th edition, Mosby Publications, p257-269.
 Srinivasan R, Kanungo R, Goyal JL. Spectrum of Oculomycosis in South India. Acta
- (3) Srinivasan R, Kanungo R, Goyal JL. Spectrum of Oculomycosis in South India. Acta Ophthalmol 1991; 69:744-9.
 (4) Ansari, D. Miller, A. Galor Current thoughts in fungal keratitis: diagnosis and treatment
- (4) Ansari, D. Minet, A. Galor Current inoughts in tungat keratuts, utagnosis and treatment Curr Fungal Infect Rep, 7 (3) (2013), pp. 209-218 CrossRef View Record in Scopus Google Sc.
- (5) Bakšhi R, Rajagopal R, Sitalakshmi G, Sudhi R, Madhavan H, Bagayalakshmi R. Clinical and Microbiological Profile of Fungal Keratitis: A 7 Year Study at a Tertiary Hospital in South India. Cornea Session III; AIOC 2008 Proceedings: 207 209.

- Textbook of Medical Mycology, Jagdish Chander MD DNB MAMS Professor and Head, Department of Microbiology, Government Medical College and Hospital, (6)Chandigarh, Haryana, India, edition -illustrated, publisher JP medical Ltd ,2017, ISBN 9386261839, 978938626183
- Chander J. 2017. Text Book of Medical Mycology. 4th Edn. Mehta Publishers, New (7) Delhi.India
- (8) Nath R, Baruah S, Saikia L, Devi B, Borthakur AK, Mahanta J. Mycotic corneal ulcers in upper Assam. Indian J Ophthalmol 2011;59:367 71 Rumpa Saha & Shukla Das, Mycological profile of infectious Keratitis from Delhi,
- (9) Indian J Med Res 123, February 2006, pp 159-164 (10) Dr Tapan Gogoi , Dr. Sikha Deori , et al,Current Trend of Corneal Ulcer In Patients
- Attending a Tertiary Healthcare Centre, International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064 Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391
- Gita Satpathy, Nishat H. Ahmed, Niranjan Nayak et al, Spectrum of mycotic keratitis in (11)
- (12)
- Gita Satpathy, Nishat H. Ahmed, Niranjan Nayak et al, Spectrum of mycotic keratitis in north India: Sixteen years study from a tertiary care ophthalmic centre, Journal of Infection and Public Health Volume 12, Issue 3, May–June 2019, Pages 367-371 Parmjeet Kaur Gill, Pushpa Dev, Keratomycosis A retrospective study from a North Indian tertiary care institute, JIACM 2011; 12(4): 271-3 S Verma, V Sharma, A Kanga, R Sharma, A Angrup, K Mokta, A Garg, Current spectrum of oculomycosis in North India: A 5 year retrospective evaluation of clinical and microbiological profile, Indian Journal of Medical Microbiology, (2016) 34(1): 72-75 Vasudha CL, Anuradha B, Krishna BN. A study on mycological profile of corneal ulcers in a tertiary care hospital Indian I Microbiol Res (2019;6(1): 15
- (14)in a tertiary care hospital. Indian J Microbiol Res 2019;6(1):1-5. Gupta A, Capoor M R, Gupta S, Kochhar S, Tomer A, Gupta V. Clinico-demographical (15)
- profile of keratomycosis in Delhi, North Indi Microbiol 2014;32:310-4 SrinivasanM, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, et al.
- (16)Epidemiology and actiological diagnosis of corneal ulceration in Madurai, South India. Br J Ophthalmol 1997;81:965 71.
- Mohod PN, Nikose AS, et a l, Incidence of various causes of infectious keratitis in the (17)part of rural central India and its visual morbidity: A prospective hospital-based observational study. J Clin Ophthalmol Res 2019;7:31-4.
- Kanishtha Sharma, Dipender Kaur Najotra and Varsha A. Singh. 2017. Clinico-Epidemological Profile of Corneal Ulcer Cases from Rural Hospital of Haryana, India. (18)Int.J.Curr.Microbiol.App.Sci. 6(9): 2410-2416. doi: https:// doi. org/ 10. 20546/ijemas.2017.609.296
- Tanure MA, Cohen EJ et al. Spectrum of keratitis at Wills Eye Hospital. Philadelphia, (19) Pennsylvania. Cornea 2000; 19: 307-12. Gopinathan U, Garg P, Fernandes M, Sharma S, Athmanathan S, Rao GN. The
- (20)epidemiological features and laboratory results of fungal keratitis: A 10-year review at a referral eye care center in south India. Cornea 2002;21:555-59.
- M Jayahar Bharathi,; R Ramakrishnan, MS; Samala Vasu, DNB; R Meenakshi, DNB; R (21)Palaniappan, MSc, PhD, Epidemiological Characteristics and Laboratory Diagnosis of Fungal Keratitis. A Three-year Study, Indian J Ophthalmol 2003;51:315-21
- (22) т T. . Chander J, Sharma A. Prevalence of fungal corneal ulcers in Northern India. Infection 1994;22:207-9.
- U Dutta LC, Dutta D, Mohanty P, Sharma J. Study of fungal Keratitis. Indian J Ophthalmol 1981;29:407-40. (23)
- V Varenkar MP, Shubhangi B, Pinto MJM, Naik PA. Study of mycotic keratitis in Goa. (24)Indian J Med Microbiol 1998;16:58-60.
- W. Gopinathan U, Garg P, Fernandes M, Sharma S, Athmanathan S, Rao GN. The (25) epidemiological features and laboratory results of fungal keratitis: A 10-year review at a referral eye care center in south India. Cornea 2002;21:555-59.
- Upadhyay MP, Karmacharya PCD, Koirala S, Tuladhar NK, Bryan le, Smolin G. Epidemiologic characteristics, predisposing factors and etiological diagnosis of corneal (26)ulceration in Nepal. Am J Ophthalmol 1991; 111: 92-9.

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