Original Research Paper

Volume - 13 | Issue - 10 | October - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Clinical Microbiology

MYCOTIC KERATITIS AT A TERTIARY CARE HOSPITAL IN NORTHERN INDIA, BIHAR

Md Shabbir Azad	Tutor, Department of Microbiology, DMCH Laheriasarai, Darbhanga, Bihar India
Saroj Kumari*	Tutor, Department of Microbiology, DMCH Laheriasarai, Darbhanga, Bihar India *Corresponding Author
Murtaza Ali	Senior Resident, Department of Ophthalmology, DMCH Laheriasarai, Darbhanga, Bihar India
Ramshanker Prasad	Associate Professor and Head of Depatment, Department of Microbiology, DMCH Laheriasarai, Darbhanga, Bihar India.

ABSTRACT Fungal keratitis or keratomycosis refers to an infective process of the cornea caused by any of the multiple pathogenic fungi capable of invading the ocular surface. In developing countries like India, Mycotic or fungal keratitis (FK) is a sight-threatening disease, caused by infection of the cornea by filamentous fungi or yeasts. Filamentous fungi, in particular Fusarium spp., the aspergillus and dematiaceous fungi, are responsible for the greatest burden of disease. The predominant risk factor for filamentous fungal keratitis is trauma particularly during crop harvest in countries like India, typically with organic, plant-based material. This study was conducted to know the mycolological profile of fungal isolates from patients with a corneal ulcers. A total of 297 patients who were clinically diagnosed with cases of infective keratitis in the Department of Ophthalmology were included in the study. The sample collected by an ophthalmologist was received in the Microbiology department. Fungal identification was done using the conventional method. Out of 297 patients, 69% were male and 31% % were females. The Majority of cases were from the age group 20-60 years. Of 297 cases, 76 (25.58%) showed fungal etiology in culture. The most common isolated fungus was Fusarium species detected in 43 (56.57%) cases followed by Aspergillus species in 25 (32.89%) cases. Keratomycosisis most typically a slow, relentless disease that must be differentiated from other types of corneal conditions with similar presentation; especially its bacterial counterpart, which accounts for the majority of the microbial corneal infections in order to effectively treat corneal ulcers and prevent further complications that can lead to blindness.

KEYWORDS : Mycotic Keratitis, Corneal ulcer, Aspergillus, Fusarium, Keratomycosis

INTRODUCTION

Mycotic or fungal keratitis (FK) is a severe and potentially blinding infection of the cornea that if not properly treated can lead to corneal destruction and endophthalmitis with severe loss of vision.[1]

The corneal infection of fungal etiology is very common and comprising at least 50% of all culture positive cases in India.[2]

According to The National Blindness and Visual Impairment Survey (2015-2019), corneal related diseases are still among the major causes of blindness in India.[3]

Fungal keratitis is caused by yeasts and filamentous fungi but the pattern of infection varies globally with respect to aetiology and predisposing risk factors relating to geographical location and occupational exposure. Infections due to Candida spp. And other yeasts are typically associated with steroid use, ocular surface disorders, previous ocular surgery, contact lens wear and underlying illness resulting in immuno-incompetency [4], mostly occurring in temperate climes.

However, the main burden of disease globally is attributable to the filamentous fungi and these infections predominantly affect the poorest patients in warm, humid, tropical climatic regions.[5]

Although viral pathogens like Herpes Simplex Virus type 1 are more commonly associated with the disease, the other common infectious agents leading to infectious keratitis are bacteria, including Streptococcus pneumonia, Staphylococcus aureus, coagulase-negative Staphylococci, Pseudomonas aeruginosa; fungus including Candida albicans, Aspergillusflavus, Fusarium species, Penicillium species and Aspergillusfumigatus as well as parasites like Acanthamoeba. [6]

This study was conducted to know the various fungal agent implicated in patients with corneal ulcers.

MATERIALS AND METHODS

The study was planned 297 clinically suspected cases of infectious keratitis as a prospective observational collaborative study in the Department of Microbiology with the department of Ophthalmology at DMCH Laheriasarai, Darbhanga, Bihar India. The study was conducted for one year from 1st April 2022 to 31st March 2023. The ethical clearance was taken from the Institutional ethical committee.

Inclusion criteria

Patients of both gender and 20-60 years age group, who were clinically diagnosed as cases of infectious corneal ulcer after thorough clinical evaluation by an ophthalmologist were included in this study.

Exclusion criteria

Patients who were already on topical antimicrobial medications were excluded from the study.

Sample collection

Informed consent of the patient was taken before sample collection. The patient was explained in detail about the procedure before sample collection. After making patient comfortable, the ophthalmologist performed a slit lamp examination. Before collection of the sample, a local anesthetic agent (topical 4% xylocaine) was instilled into the affected eye. (Figure 1)

With aid of slit lamp, corneal scrapings were collected from the edge of the cornea using a kimura spatula. Scrapings were taken in sufficient amounts for both staining and culture purposes. Part of the sample was collected on two clean slides for Gram Staining and Potassium Hydroxide (KOH) mount.

Another part of the sample was directly inoculated by the ophthalmologist on the blood agar, chocolate agar, MacConkey agar and two Sabouraud dextrose agar (SDA) in multiple C shaped streaks with help of a surgical blade in OPD. Without any delay, the slides and culture media were sent to the Microbiology department.

Staining

Two Slides were received from the ophthalmology department, one slide was heat fixed and stained with Gram staining to observe the presence of bacteria.[7]

KOH mount

From other slide with corneal scraping a KOH mount was prepared by adding 10% KOH on the slide, which was covered with coverslip and observed under microscope for presence of any fungal elements.[8] (Figure 2)

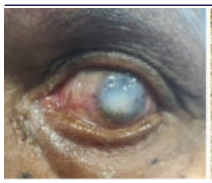
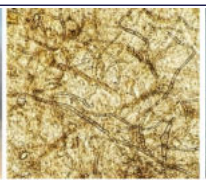
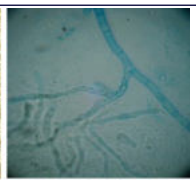

Figure: 1 **Figure: 2** **Figure: 3**

Fungal Culture

The culture media that was inoculated in the ophthalmology department were received in the mycology section of the Microbiology laboratory.

The inoculated media for fungal culture (two inoculated Sabouraud Dextrose Agar containing 0.05mg/mL of chloramphenicol) was incubated at 37°C and 22°C for 14 days and were checked at regular intervals (3rd day, 7th day & 14th day) for any evidence of any fungal growth. When there was fungal growth, identification was done based on colony characteristics and microscopically by observing the fungal morphology in lactophenol cotton blue (LPCB) mount (Figure 3).[9,10] Diagnosis of fungal keratitis was made only when the KOH mount and fungal culture were both positive or when the same growth was observed in both (Sabouraud Dextrose Agar media).

RESULTS

In patients presenting with corneal ulcers,fungal etiology was seen in 77(25.93% incidence) out of 297 suspected cases. So the incidence of keratomycosiswas 25.93% among suspected corneal ulcer patient.

Most of the patients belonged to the low socioeconomic group. Most of the patients gave history of trauma, mostly while working in farms, gardens and outdoor. Male were affected five times more than female (5:1).

The age incidence of keratomycosis in the current study shows (Table 1) that the age range of 51 to 60 years has the highest incidence (31), followed by 41 to 50 years (24), 31 to 40 years (09) and 20-30 years (03)

Table 1

Age group	No. of cases (N=77)	Percentage
20-30	03	3.9%
31-40	09	11.7%
41-50	27	35%
51-60	38	49.4%

The predominant fungal species was Aspergillus species (38=49.35%) followed by Fusarium species(25=32.47%). In a relatively smaller incidence Curvularia species (07=9.09%), Bipolaris spp. (05=6.49%) and Alternaria species (02=2.6%) were also isolated. (Table 2)

Table 2

Fungal isolates	No. of isolates (n=77)	Percentage
Aspergillus spp.	38	49.35%
Fusarium spp.	25	32.47%
Curvulariaspp.	07	9.09%
Bipolaris spp.	05	6.49%
Alternaria spp.	02	2.6%

DISCUSSION

Infective keratitis is still one of the leading causes of blindness especially in developing countries like India. Although both bacteria and Mycotic keratitis frequently mimics as bacterial or parasitic keratitis, and it is very difficult to differentiate clinically. For delayed presentation and difficulty in diagnosis large number of patients do not get proper medication in time andpresent with delayed presentation. To estimate the load of fungal keratitis and evaluate the spectrum of causative organisms, we have conducted this study in our institute over 1 year from 1st April 2022 to 31st March 2023.

Our study revealed that 25.93% of microbial keratitis is due to fungal by origin. This high incidence of fungal keratitis can also be seen in neighbouring area of same climatic region, like Bangladesh (39.9%) and Orissa (32%). [11,12] Whereas incidence of mycotic keratitis in South India is higher, which is 44%, because of hotter and more humid climate than Northern India, which favours the growth of fungus.

Aspergillus (49.35%) and Fusarium (32.47%) were predominant causative organisms for mycotic keratitis in our study. Whereas Fusarium is predominant in South India and Aspergillus is predominant in rest of the India. Candida and other yeasts had been isolated mainly from the North Bengal region and Sub Himalayan part of West Bengal, Sikkim. The climate of this region matches with European countries where yeasts isolated predominantly. [13] Following Aspergillus and Fusarium, Curvularia was isolated in 9.09% of cases in our study. Curvularia is found mostly in hot and humid area. It is reported thatit is predominant along the US gulf coast of Mexico and in India it is predominant in North India which is in agreement with our study. [14]

Males in the age group 40-60years were found to be most vulnerable probably due to their out-door and farm work activities, susceptibility to trauma particularly injury with vegetative matter and poor immunity due to low socioeconomic conditions.[15] Variation in prevalence of mycotic keratitis in males and females depending on local socioeconomic conditions has been recorded. [16,17] In our study, males were found to suffer five times more than females.

CONCLUSION

Mycotic keratitis is a fungal infection of the cornea. 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. Trauma is the major predisposing factor, followed by ocular and systemic defects, prior application of corticosteroids, and prolonged use of antibiotic eye-drops. 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.

Conflicts of Interest

The authors declare that there are no conflicts of interestregarding the publication of this paper.

Acknowledgments

The author would like to thankDepartment of Ophthalmology, DMCH Laheriasarai, fortheir support during patient screening andsample transportation.

Abbreviations: KOH – Potassium hydroxide; LPCB- lactophenol cotton blue

REFERENCES

1. Castano G, Elnahry AG, Mada PK. Fungal Keratitis. 2022 Aug 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 29630244.
2. Khurana A, Kumar A, Chauhan L. Clinical profile and treatment outcomes of Fusarium keratitis. Indian J Ophthalmol. 2022 Mar;70(3):852-859. Doi: 10.4103/ijo.IJO_999_21. PMID: 35225530; PMCID: PMC9114586.
3. Dr.Rajendra Prasad Centre for Ophthalmic Sciences, AIIMS, New Delhi. National Blindness and Visual Impairment Survey 2015-2019. "https://npcbvi.Mohfw.gov.in/writeReadData/mainlinkFile/File341.Pdf". Accessed on 30th September, 2022.
4. Qiao G.L., Ling J., Wong T., Yeung S.N., Iovieno A. Candida Keratitis: Epidemiology, Management, and Clinical Outcomes. Cornea. 2020;39:801–805. doi: 10.1097/ICO.0000000000002306. Qiao G.L., Ling J., Wong T., Yeung S.N., Iovieno A. Candida Keratitis: Epidemiology, Management, and Clinical Outcomes. Cornea. 2020;39:801–805. doi: 10.1097/ICO.0000000000002306.
5. Hoffman JJ, Yadav R, Sanyam SD, Chaudhary P, Roshan A, Singh SK, Arunga S, Hu VH, Macleod D, Leck A, Burton MJ. Diagnosis of Fungal Keratitis in Low-Income Countries: Evaluation of Smear Microscopy, Culture, and In Vivo Confocal Microscopy in Nepal. J Fungi (Basel). 2022 Sep 13;8(9):955. Doi: 10.3390/jof8090955. PMID: 36135680; PMCID: PMC9502267.
6. Singh P, Gupta A, Tripathy K. Keratitis. [Updated 2023 Feb 22]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559014/
7. Mackie, McCartney. Practical Medical Microbiology. Collee JG, Fraser AG, Marmion BP, Simmons A. 14thEdition. New York: Churchill and Livingstone; 1996.
8. Rathi VM, Thakur M, Sharma S, Khanna R, Garg P. KOH mount as an aid in the management of infectious keratitis at secondary eye care centre. Br J Ophthalmol. 2017 Nov;101(11):1447-1450. Doi: 10.1136/bjophthalmol-2017-310241. Epub 2017 Jul 24. PMID: 28739646.
9. Walsh, Thomas & Hayden, Randall & Larone, Davise. (2018). Larone's Medically Important Fungi: A Guide to Identification, 6th Edition. 10.1128/9781555819880.
10. Chauhan J, Kaur N, Kumar H, Bala R, Chauhan S. Mycological profile of acute invasive fungalRhinosinusitis during COVID-19 pandemic at a tertiary Care hospital. Med J Babylon 2022;19(4):595-600. Doi:10.4103/MJBL.MJBL_193_22
11. Dunlop AA, Wright ED, Howlader SA, et al (1994) Suppurative corneal ulceration in Bangladesh. A study of 142 casesexamining the microbiological diagnosis, clinical and epidemiological features of bacterial andfungal keratitis. Aust NZ J Ophthalmol. 22:105–110
12. Paty BP, Dash P, Mohapatra D, Chayani N (2018) Epidemiological profile of mycotic keratitis in a tertiary care centerof eastern Odisha. J NTR Univ Health Sci 7:23-25
13. Rondeau N, Bourcier T, Chaumeil C, et al. (2002) Fungal keratitis at the Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts: retrospective study of 19 cases. J Fr Ophtalmol. 25:890–896
14. Wilhelmus KR, Jones DB (2002) Curvularia keratitis. Trans Am OphthalmolSoc 99:111-132
15. Lin Y, Zhang J, Han X, Hu J. A retrospective study of the spectrum of fungal keratitis in

southeastern China. Ann Palliat Med. 2021 Sep;10(9):9480-9487. doi: 10.21037/apm-21-1949. PMID: 34628873.
16. Satpathy G, Ahmed NH, Nayak N, Tandon R, Sharma N, Agarwal T, Vanathi M, Titiyal JS. Spectrum of mycotic keratitis in north India: Sixteen years study from a tertiary care ophthalmic centre. J Infect Public Health. 2019 May-Jun;12(3):367-371. doi: 10.1016/j.jiph.2018.12.005. Epub 2018 Dec 29. PMID: 30600158.
17. Manikandan P, Abdel-Hadi A, Randhir Babu Singh Y, Revathi R, Anita R, Banawas S, Bin Dukhyil AA, Alshehri B, Shobana CS, Panneer Selvam K, Narendran V. Fungal Keratitis: Epidemiology, Rapid Detection, and Antifungal Susceptibilities of Fusarium and Aspergillus Isolates from Corneal Scrapings. Biomed Res Int. 2019;2019:6395840.