Abstract

Risk Factors and Treatment Outcomes of Fungal Keratitis: A Tertiary Eye Care Centre Experience

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Purpose: To study the epidemiological characteristics, microbiological diagnosis and treatment outcome of fungal keratitis in a tertiary eye care centre of Western India

Materials and Methods: A retrospective non-comparative observational review of the medical and microbiological records was done for all the patients with laboratory proven fungal keratitis on 10% KOH wet mount. Data was compiled in Microsoft Office Excel 2010 spreadsheet and analysis was done using Med-Calc software. Chi square test was used for categorical data.

Results: Between July 2016 to August 2018, 104/354 (29.3 %) corneal scrapings of microbial keratitis showed presence of fungal mycelia on 10% KOH wet mount. 72/104 patients were included in the study. Vegetative trauma (31/72 eyes, 43.06%) was the commonest risk factor. Aspergillus and Fusarium species were the major fungi isolated. 52/72 (72.2%) eyes healed with a scar, of which 21 (40.38%) healed with medical management alone, 29 (55.77%) needed additional tissue adhesive with bandage contact lens (BCL) and 2 (3.85%) required intracameral Voriconazole. 334/524 eyes had a mean duration of presentation<=10 days (p<0.001) the need of keratoplasty (15/72) was seen in significantly larger number of patients presenting late (>10 days) (p<0.05).

Conclusion: History of late presentation, large extent of keratitis and presence of thinning/perforation at presentation were associated with the need for keratoplasty. Considering the recalcitrant course of fungal keratitis, the severity and time of presentation is highly predictive of the disease treatment and outcome.

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Keywords: Fungal, Koh, Trauma, Keratoplasty, Treatment Outcome

Introduction

Fungal infections of cornea present as suppurative, usually ulcerative lesions which may be potentially vision threatening. They can pose a major challenge to the ophthalmologist because of their tendency to mimic other types of stromal infections and because its management is restricted by the availability of effective antifungal agents and the extent to which they can penetrate into the corneal tissue. The microbiological and epidemiological pattern of fungal keratitis shows significant geographic variation. Though there is enough literature regarding the risk factors and microbial etiology of suppurative keratitis from various parts of India, only a few preceding studies from Western India describe the microbiological and epidemiological profile of fungal keratitis in particular. Besides, to the best of our knowledge, none of these studies report the outcome of different modalities of treatment of fungal keratitis in Western India.

The aim of our study was to elucidate the various predisposing factors, etiological agents and outcomes of different modalities of treatment in the management of patients with keratomycosis presenting to a tertiary care centre of Western India.

Methods

A retrospective analysis of the records of the patients presenting to the tertiary eye care centre in Western India with laboratory proven KOH wet mount positive fungal keratitis, over a period of two years between July 2016 to August 2018, was done. The study was conducted in accordance with the ethical standards of the institutional committee and the Declaration of Helsinki 1975, as revised in 2013. Documentation of the patients included.

1. socio-demographic characteristics,
2. duration of symptoms,
3. predisposing factors,
4. treatment received prior to presentation to the centre
5. slit lamp bio microscopy findings,
6. associated ocular and systemic diseases,
7. treatment given at the study centre
8. response to the treatment and final clinical outcome during the follow-up period.

Inclusion Criteria

Clinically suspicious fungal keratitis with unilateral central and para-central corneal ulcers, with stromal infiltration with or without thinning or impending perforation, with or without hypopyon.

Exclusion Criteria

The patients with pre-existing ocular diseases (lid, conjunctival and corneal affections, dacryocystitis, glaucoma), patients on prior anti-fungal treatment from outside, history of ocular surgery (within six months), scleritis, endophthalmitis on presentation and clinically suspicious as well as microbiologically mixed infections. All the patients had received a thorough slit lamp examination by a single experienced ophthalmologist. The size of the epithelial defect after staining with a fluorescein strip was measured with a variable slit on the bio- microscope in the greatest dimension and along the axis
perpendicular to it and recorded in millimetres. The size and depth of the stromal infiltrate was also recorded clinically on the slit lamp biomicroscope. Serial slit lamp photographs with documentation were used to record the findings on each follow-up. The presence or absence of hypopyon was recorded and its height measured in millimetres. Posterior segment evaluation was done by ultrasonography in cases where the fundus could not be evaluated on ophthalmoscopy. A systemic work-up of all the patients including the complete blood count, blood sugar, liver and renal function tests was done.

After a detailed ocular examination and informed consent, the corneal scrapings were taken in all cases by a trained ophthalmologist under aseptic conditions using a sterile 15 number blade. The scraped material was obtained from the leading edge and base of each ulcer and was initially spread onto labelled slides in a thin even manner for 10% KOH wet mount and Gram’s stain. Direct inoculation on Sabouraud Dextrose agar in C shaped streaks and on blood agar and chocolate agar was done with meticulous care. Sabouraud’s dextrose agar was incubated at 27°C, examined daily and discarded in four weeks if no growth was seen. Blood agar and chocolate agar were incubated at 37°C examined daily and discarded at three weeks if there was no growth.

All laboratory methods followed a standard protocol. A definitive diagnosis of fungal keratitis was made if the corneal scrapings revealed fungal elements on 10% KOH wet mount with/ without,

1) confluent growth of fungus in any solid media or
2) fungal growth in more than one medium.

Treatment at the centre was started after the microbiological sample was taken depending on clinical suspicion and in most cases after getting an immediate positive smear result. Topical Natamycin (5%) eye drops were started as per the need and judgement of the clinician and tapered according to the clinical response in cases of resolving keratitis. Associated supportive treatment included topical cycloplegic Atropine (1%) eye drops with lubricating eye drops and intraocular pressure lowering agents as per need. Systemic Fluconazole (150 mg twice a day for initial three days followed by once a day for 18 days) was prescribed to patients with ulcer depth more than 50% of cornea, maximum linear diameter > 5mm, deep stromal infiltration and presence of hypopyon. Topical Voriconazole (1%) was added in cases which showed no improvement within seven to ten days of the start of initial anti-fungal therapy. Periodic therapeutic debridement of the ulcer bed was done unless corneal thinning prevented it. Additional procedures were performed at the discretion of the attending ophthalmologist for patients with extreme thinning or impending perforation at presentation or cases not responding to medical treatment and they included N butyl 2-cyanoacrylate glue with bandage contact lens (BCL) application, anterior chamber wash with intracameral injection of Voriconazole (50 mcg / 0.1 ml) and therapeutic/ tectonic penetrating keratoplasty.

Treatment was considered as a success if the keratitis healed with a visible scar on slit lamp bio-microscope. The final outcome measures included percentage of healed cases in each group of the different modalities of treatment and treatment failures like the requirement of keratoplasty or the eyes going into phthisis.

Statistical analysis
Data was compiled and entered in Microsoft Office Excel 2010 spreadsheet and analysis was done using MedCalc software. Proportions, mean and standard deviation were used for quantitative data. Chi square test was used for categorical data. A p value of <0.05 was considered as statistically significant.

Results
During the study period, corneal scrapings were done in 354 patients clinically diagnosed as microbial keratitis. Of these, no organisms were isolated on smears in 105 clinical samples (29.7%), while 145 (41%) showed presence of bacteria with or without presence of fungal mycelia on Gram staining and KOH wet mount and 104 (29.3%) showed presence of fungal mycelia on KOH wet mount. Of the 104 KOH positive scrapings, 63 (60.58%) showed fungal growth on cultures. Aspergillus was the commonest fungus isolated (40/63) (63.5%).

Socio-demographic variables of the study population-
Of the 104 KOH positive cases, 72 eyes of 72 patients with unilateral corneal ulcer, with above mentioned inclusion and exclusion criteria were enrolled in the study. The socio-demographic characteristics of the patients is described in (Table 1). Mean age of the patients was 47.44 ± 16.63 years (range: 13 to 70 years). Vegetative trauma was the most

Table 1: Socio-demographic characteristics of the patients with mycotic keratitis

| Characteristics | Patient Details | Number of patients (%) |
|-----------------|-----------------|------------------------|
| Age             |                 |                        |
| 11-30           |                 | 19 (26.39%)            |
| 31-50           |                 | 17 (23.61%)            |
| 51-70           |                 | 20 (28.57%)            |
| 36 (50%)        |                 |                        |
| Gender          |                 |                        |
| Males           |                 | 45 (62.5%)             |
| Females         |                 | 27 (37.5%)             |
| Residence       |                 |                        |
| Urban           |                 | 21 (29.17%)            |
| Rural           |                 | 41 (57.83%)            |
| 51 (70.83%)     |                 |                        |
| Visit           |                 |                        |
| Primary         |                 | 28 (38.89%)            |
| Referral        |                 | 44 (61.11%)            |
| Occupation      |                 |                        |
| Farmers         |                 | 47 (65.28%)            |
| Labourers       |                 | 9 (12.5%)              |
| House-wives     |                 | 9 (12.5%)              |
| Office job      |                 | 2 (2.78%)              |
| Unemployed      |                 | 6 (8.33%)              |
A common predisposing factor (Table 2). Mean duration of presentation was 12.3 ± 5.87 days (range: 3 days to 30 days) after the onset of symptoms and the mean follow-up of the patients was 6.19 ± 3.21 months.

**Slit lamp and microbiological examination**

Thirty seven eyes presented with central keratitis while the rest 35 had para-central keratitis. The area of ulcers and stromal infiltration ranged from 3x3 sq mm to 11x9 sq mm. Most common findings on clinical examination were as per (Table 3). Out of the 72 eyes, cultures were positive in 36 (50%) cases. Of these, Aspergillus was the most common organism isolated followed by Fusarium (Table 4).

**Treatment modalities and outcomes**

All the patients were under medical management. 44 eyes had significant thinning within 7 to 10 days of presentation, which necessitated the application of N butyl 2 cyanoacrylate glue and Bandage Contact Lens (BCL). The mean duration of presentation in these eyes was 12.21 ± 3.61 days. 22 eyes needed tissue adhesive and BCL application at presentation itself due to thinning or impending perforation. 10 eyes required application of the tissue adhesive twice due to premature dislodgement of the glue. The need for application of glue and BCL was seen in larger number of patients (24 out of 34 eyes) with late presentation (>10 days), though this was not found to be statistically significant (p =.11).
The final clinical outcome of healed scar was achieved in 52 (72.2%) patients with the mean healing time being 6.9 weeks. The healing was achieved within 4 weeks in 5 patients and within 6 weeks in 29 patients. The mean duration of topical antifungal therapy was 9.8±2.13 weeks and the topical therapy was continued 3 weeks after the healed scar was observed clinically. There was no statistically significant association between the age (p=.06) and gender and the number of healed eyes (p=.41) (Table 5). A majority of healed eyes (34 eyes) had a mean duration of presentation ≤10 days, which was found to be statistically significant. (p=.001)(Table-6). All the cases with a lesser extent of keratitis (maximum linear diameter<5 mm) healed. The final outcome of different modalities of treatment is described in (Table 5).

### Table 5: Outcomes of various modalities of treatment of fungal keratitis in relation to different parameters

| Parameters                        | Healed with medical management | Healed with tissue adhesive BCL and medical therapy | Healed with medical therapy + Intracameral Voriconazole | Total healed | Number of eyes gone into Phthisis | Number of eyes needing keratoplasty | Total |
|-----------------------------------|-------------------------------|-----------------------------------------------|---------------------------------------------|--------------|----------------------------------|-----------------------------------|------|
| Gender                            |                               |                                               |                                            |              |                                  |                                   |      |
| males                             | 14                            | 18                                            | 02                                         | 34           | 03                               | 08                                | 45   |
| females                           | 07                            | 11                                            | -                                          | 18           | 02                               | 07                                | 27   |
| Total                             | 21                            | 29                                            | 02                                         | 52           | 05                               | 15                                | 72   |
| Age (years)                       |                               |                                               |                                            |              |                                  |                                   |      |
| 11-30                             | 06                            | 06                                            | 01                                         | 13           | -                                | 05                                | 18   |
| 31-50                             | 06                            | 04                                            | -                                          | 10           | 02                               | 07                                | 19   |
| 51-70                             | 09                            | 19                                            | 01                                         | 29           | 03                               | 03                                | 35   |
| Total                             | 21                            | 29                                            | 02                                         | 52           | 05                               | 15                                | 72   |
| %                                 | 29.17%                        | 40.28%                                        | 2.78%                                      |              | 6.94%                           | 20.83%                            | -    |
| Mean duration of presentation    | 7.47±2.94                     | 12.14±3.57                                    | 15                                         |              | 10.36±4.05                      | 23±7.58                           | 15.66±5.61 |
| in days                           |                               |                                               |                                            |              |                                  |                                   | -    |
| Fungi isolated in culture         | Aspergillus -niger (4 eyes)   | -fumigatus (2 eyes)                            | -flavus (4 eyes)                            |              | None isolated                    | -                                 |     |
|                                   | -flavus (1 eye)                | Fusarium (2eyes)                              | Aspergillus -fumigatus (4 eyes)            |              | None isolated                    | -                                 |     |
|                                   | P boydii (1 eye)               | Fusarium (4 eyes)                             | None isolated                              |              |                                  | Aspergillus -niger (4 eyes)       |     |
|                                   | None (11 eyes)                 | -fumigatus(2 eyes)                            | -flavus (2 eyes)                            |              |                                  | -fumigatus (2 eyes)               |     |
|                                   |                                | Fusarium (2eyes)                              | -Fusarium (2eyes)                          |              |                                  | Curvularia (1 eye)                |     |
|                                   |                                |                                                | -None (5 eyes)                              |              |                                  |                                   |     |

The mean duration of presentation was late for all the eyes (23 ± 7.58 days).

### Discussion

This study presents the data regarding epidemiological, laboratory and clinical features and treatment outcomes of patients with mycotic keratitis from Western India. A
Higher during the monsoon from July to September when the agricultural activity is greater, with higher chances of vegetative trauma and a greater propensity for fungal growth in a favourable humid environment, in contrast to the findings of another study done in Gujarat, which noted it to be common from September to December.

The clinical presentation of fungal keratitis is varied and largely depends on the type of fungus, severity of the invading pathogen, liberation of toxin, resistance of the host tissue and the age of the patient. The typical slit lamp biomicroscopic presentation of a dry looking ulcer was seen in 66.67% and the feathery infiltration in 36.11%, as against 75.43% and 71.78% respectively observed in the study by Bharathi et al.

In almost all the studies, the diagnosis of fungal keratitis is remarkably efficiently done using simple methods such as KOH wet mount and Gram stain. Our study reported 104 (29.3%) of the total samples from corneal scrapings to be fungal on KOH wet mount, of which 72 were included in the study. Since the clinical acumen in the diagnosis of fungal keratitis varies according to the level of training and experience, it is essential to have a minimum facility of KOH wet mount in the clinic as a screening tool to diagnose it. In our study, the commonest causative organism isolated was Aspergillus niger and the second most common fungus was Fusarium, which is comparative to another study reported from north India. However, Saha et al reported Candida (19%) to be the second most common fungus isolated.

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Table 7: Comparison of microbiological and clinical data on mycotic keratitis from studies from various parts of India

| Parameters | Eastern India | South India | West Bengal | North India | Western India | Present study |
|------------|--------------|-------------|-------------|-------------|---------------|---------------|
| Type of study | Retrospective | Retrospective | Retrospective | Prospective | Prospective | Prospective |
| Period of study with duration | July 2006-December 2009 (3.5 years) | January 1999-December 2000 (10 years) | January 2008-December 2008 (1 year) | December 2004 to December 2009 (5 years) | November 2008-December 2008 (11 months) | September 2003-June 2005 (20 months) |
| Sample size (total number of patients) | 997 | 3399 | 289 | 485 | 852 | 150 |
| Number of patients with proven fungal keratitis | 264 (26.4%) | 1352 (39.8%) | 310 (38.06%) | 191 (39%) | 311/537 (57.91%) | 22.5% |
| A. General | | | | | | |
| Type of study | Retrospective | Retrospective | Retrospective | Prospective | Prospective | Prospective |
| Period of study with duration | July 2006-December 2009 (3.5 years) | January 1999-December 2000 (10 years) | January 2008-December 2008 (1 year) | December 2004 to December 2009 (5 years) | November 2008-December 2008 (11 months) | September 2003-June 2005 (20 months) |
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| Number of patients with proven fungal keratitis | 264 (26.4%) | 1352 (39.8%) | 310 (38.06%) | 191 (39%) | 311/537 (57.91%) | 22.5% |
| B. Epidemiological factors | | | | | | |
| Commonly affected Age range | 50-60 years (63%) | 6-49 years (64.4%) | >50 years (60.81%) | 31-40 years (37%) | 21-30 years (52.6%) | 51-70 years (50%) |
| Commonly affected Gender | Males (70%) | Males (71.2%) | Males (65%) | Males (68%) | Males (68.31%) | Males (61%) |
| Residence | Urban | 21% | NA (Data not available) | NA | 29.7% | 20.83% |
| Rural | 79.5% | | | | | |
| Commonest risk factor | Ocular trauma (40%) | Ocular trauma (54.4%) | Vegetative trauma (13.9%) | Ocular trauma (42%) | Ocular trauma (47.29%) | Ocular trauma (46.11%) |
| Commonest months of presentation | October to January & June to September | NA | NA | NA | NA | July to September |
| C. Microbiology | | | | | | |
| Positive direct microscopy 10% KOH wet mount | 186 (70.5%) | 1277 | 4174 (55%) | 19 (62%) | NA | 2 | 68.4% | 72/104 included in the study |
| Gram stain positive | 199/252 (78.9%) | 118/121 (98.2%) | NA | 114 (60%) | NA | NA | NA | 60/100 (57.69%), 45 included in study |
| Culture positive for fungus | 215 | 1532 | 74 (62.27%) | 191 | 283 | 31 | 57 (49.5%) | 36 (50.7%) |
| Most common fungus isolated | Aspergillus (27.91%) | Fusarium (37.21%) | Aspergillus (55.43%) | Aspergillus niger (34%) | Aspergillus (35.04%) | Fusarium (29.82%) | Aspergillus (72.22%) | Aspergillus niger (42.35%) |
| D. Treatment given with outcome | | | | | | |
| Healed scar with medical management | 35.6% | - | 40.55% | NA | - | - | - | 21 (29.16%) |
| Tissue adhesive and BCL application | 18.9% | - | NA | NA | - | - | - | 29 (40.28%) |
| Tectonic/therapeutic keratoplasty | 19.7% | - | 59.45% | Aspergillus in 39.02% | 18.8% | - | - | 20.8% Aspergillus flavus in 4 eyes |
| Evisceration/ phthisis | 3.4% | - | - | - | 5 eyes | 6.94% phthisis | - | 5 eyes (6.94%) phthisis |
| Follow up | 43±115 days | NA | NA | 5.5 months | NA | NA | 6.19±3.21 months | NA (Data not available) |

**Conclusion**

The regional information of common etiological agents and predisposing factors from our study can be helpful in starting an empirical therapy based on high degree of clinical suspicion and formulating preventive measures in a population at risk of fungal keratitis.

Our study also emphasizes that direct microscopy with KOH wet mount preparation can be relied upon as an easily accessible screening test for rapid diagnosis of mycotic keratitis. Cyanoacrylate glue, previously thought to be a temporary measure is effective and can be a definitive treatment in the absence of surgical quality donor tissue for
therapeutic or tectonic keratoplasty, thereby decreasing the need of early surgical intervention in severe cases of fungal keratitis. History of late presentation, large extent of keratitis and presence of thinning/perforation at presentation were associated with the need for keratoplasty.

Future prospective studies can be done to include more number of patients with detailed analysis of visual outcomes of different modalities of treatment in the same as well as different zone of India.

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