Comparative study on the incidence and outcomes of pigmented versus nonpigmented keratomycosis

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Purpose: To determine the incidence, outcomes and establish factors determining visual prognosis of keratomycosis due to pigmented fungi in comparison with nonpigmented fungi. Materials and Methods: All culture-proven cases of fungal keratitis from January 2006 to August 2008 were drawn from a computerized database and cases with adequate documentation were analyzed for predisposing factors, clinical characteristics, microbiology and treatment methods. Outcomes of keratitis due to pigmented and nonpigmented fungi were compared using t-test and \( \chi^2 \) test. Results: Of 373 cases of keratomycosis during the study period, pigmented fungi were etiological agents in 117 eyes (31.3%) and nonpigmented fungi in 256 eyes (68.7%). Eyes with nonpigmented keratitis had significantly larger ulcers (14.96 mm\(^2\)) and poorer vision (1.42 logMAR) at presentation compared to those with keratomycosis due to pigmented fungi (\( P=0.01 \)). The characteristic macroscopic pigmentation was seen in only 14.5% in the pigmented keratitis group. Both groups responded favorably to medical therapy (78.1% vs. 69.1%) with scar formation (\( P=0.32 \)) and showed a significant improvement in mean visual acuity compared with that at presentation (\( P<0.01 \)). Visual improvement in terms of line gainers and losers in the subgroup of eyes that experienced healing was also similar. Location of the ulcer was the only factor that had significant predictive value for visual outcome (\( P=0.021 \)). Conclusion: Incidence of keratomycosis due to pigmented fungi may be increasing as compared to previous data. These eyes have similar response to medical therapy and similar visual outcome compared to nonpigmented keratitis. Central ulcers have a poor visual outcome.

Key words: Keratitis, keratomycosis, nonpigmented fungi, outcomes, pigmented fungi

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes. Corneal infection of fungal etiology (keratomycosis) is very common and represents 30-40% of all cases of culture-positive infectious keratitis. Moreover, fungi have replaced bacteria as the predominant cause of infectious keratitis in developing countries. Keratomycosis is most commonly caused by filamentous fungi which can be further classified into two types: pigmented (dematiaceous) fungi which produce characteristic black/brown pigment appreciable clinically and/or on culture media and nonpigmented (moniliaceous) fungi which do not produce such pigments. Most of the existing literature on keratomycosis is focussed mainly on nonpigmented filamentous fungi or Candida spp. as etiological agents. As a group, the pigmented fungi have increasingly gained importance as agents causing corneal ulcers, second only to Fusarium and Aspergillus species. Curvularia species are saprophytic dark pigmented fungi found in soil and are considered the commonest cause of pigmented fungal corneal ulcer. Other dematiaceous fungi such as Alternaria, Exserohilum, Cladosporium Botryodiplodia and Biopolaris are also known to cause human keratomycosis. Except for one large series by Garg et al., and another by Wilhelmus et al., rest of the literature on pigmented fungal keratitis have been published case reports, with emphasis on the appearance of the pigmented lesion in some. However, little is known about the outcomes of dematiaceous fungal keratitis especially in comparison with the more common nonpigmented keratitis.

The purpose of this study was to determine the clinical characteristics of dematiaceous fungal keratitis and compare the demographics and outcomes of this cohort with that of nonpigmented fungal keratitis. Factors predictive of visual outcome were also analyzed for keratitis due to pigmented fungi.

Materials and Methods

All patients, with culture proven diagnosis of keratomycosis, who presented to the cornea services, from January 2006 to August 2008, were identified from a computerized database. Medical and microbiological records of these patients were reviewed for demographic features, predisposing factors, prior therapy, clinical features, microbiological findings, medical and surgical treatment, and outcome of therapy. Only those with thorough slit-lamp examination and good documentation in the case records were included in the analysis. The location, size, depth, nature of the infiltrate; presence of clinically detectable pigmentation; endothelial exudates; and anterior chamber reaction or hypopyon were recorded, at the time of presentation. Best corrected visual acuity (BCVA) was measured using Snellen’s distance visual acuity chart and was converted to logarithm of the minimum angle of resolution (logMAR) units for analysis.
As a routine, corneal scrapings were obtained from all patients with corneal ulcers using a sterile Kimura spatula under slit-lamp magnification. Material obtained from the base and edges was examined microscopically using freshly prepared potassium hydroxide (10%) and Gram staining methods, and was directly inoculated on various solid media in the form of multiple rows of C streaks; growth occurring only on the C streaks was considered to be significant. All inoculated media were incubated aerobically. Cultures were checked every day during the first week and twice a week for the next 3 weeks. The inoculated blood agar, chocolate agar, thioglycolate broth and brain-heart infusion broth were incubated at 37°C. The inoculated potato dextrose agar (PDA) were incubated at 27°C and discarded at 4 weeks if no growth was seen.

Microbial cultures were considered significant if, growth of the same organism was demonstrated on more than one solid-phase medium, and/or if there was a confluent growth at the site of inoculation on one solid medium, and/or if growth of one medium was consistent with direct microscopy findings and/or if the same organism was grown from repeated scrapings. Any growth present on the medium was identified by standard laboratory techniques viz., the rate of growth, colony morphology on PDA and microscopic appearance of fungal hyphae and conidia in lactophenol cotton blue mount and slide culture. Speciation was based on the characteristics of conidia and spore bearing structures wherever possible. An isolate was considered dematiaceous, if fungal colonies revealed black or brown pigmentation [Fig. 1] and Lactophenol cotton blue mount from the culture revealed black or brown pigmented hyphae, conidia or both [Fig. 2]. Medical treatment was based on the smear results and was modified depending on the clinical response.

Clinical cure was defined as healing of the corneal epithelium with scarring of the cornea. This was assessed by absence of epithelial defect, resolution of corneal infiltrate, absence of cellular reaction in the anterior chamber, presence of corneal vessels and scarring from the case records.

The primary outcome measures were mean BCVA at time of presentation and at last followup for the pigmented and nonpigmented fungal keratitis groups. The visual acuity at the end of treatment was classified as good recovery if there was >4 lines gain (Snellen's visual acuity) compared with visual acuity at presentation and poor recovery if there was >4 lines loss. Visual stabilization was noted if final visual acuity was within 4 lines of the vision at presentation. Patients with less than 1-month followup and incomplete resolution at the time of last followup were excluded from the analysis. All eyes that experienced healing and scarring were included for visual outcome analysis in terms of line gainers and losers; and those eyes subjected to surgical interventions were excluded.

For comparison of means, t tests or their nonparametric equivalents were used when appropriate, and for proportions chi-square tests were used. Point estimates of the “treatment” effect were calculated as differences between means or as proportion ratios (for binomial outcomes) together with their 95% confidence limits. An initial univariate analysis was followed by a multivariate analysis to identify and select important risk factors predicting visual outcome. P<0.05 was considered statistically significant.
Results

Out of total 413 cases of culture-proven keratomycosis at our institute during the study period, 373 with good documentation were included in the analysis. Nonpigmented fungal keratitis dominated the spectrum with 256 eyes (68.63%) while the pigmented group of fungi accounted for infection in 117 eyes (31.37%). Table 1 shows the fungal pathogens isolated from the two groups in our study. The different isolates of pigmented fungi identified in our series comprised predominantly of *Curvularia* species. Table 2 shows the comparative demographics of the patients in the two groups. The mean visual acuity at the time of presentation was 1.42 logMAR (+/-0.80) in the nonpigmented group and 1.21 logMAR (+/-0.78) in the pigmented group (*P* = 0.01). The mean size of the infiltrate (length x breadth) was 14.96 mm² (range of 8.16-31.92 mm²) in the nonpigmented group and 10.5 mm² (range of 4.23 mm²) in the pigmented group (*P* = 0.01). The characteristic macroscopic pigmentation was seen in only 17 eyes (14.5%) in the pigmented keratitis group [Fig. 3].

All patients were initially treated with topical natamycin drops on an hourly basis for 48 hours followed by tapering, after the diagnosis was confirmed microbiologically by smear. Patients with deep mycosis (endothelial plaque) and hypopyon were treated on an in-patient basis. The rest were treated as out patients and closely followed up. After 1 week, an additional topical antifungal drug was added in cases of inadequate response (either worsening or slow response) and oral fluconazole was added to the regimen if deep mycosis occurred. The commonest topical agent used in combination with topical natamycin was itraconazole. Oral fluconazole was commonest systemic antifungal used. Superficial keratectomy was done for superficial plaque like pigmented infiltrates.

Outcome data were available for 206 cases in the nonpigmented group and 81 cases in the pigmented group, because of loss to followup. Table 3 shows the comparative outcomes between the two groups. One hundred and sixty-one (78.1%) eyes in the nonpigmented group and 56 eyes (69.1%) in the pigmented group responded to medical therapy with resolution of the infiltrate and scar formation (*P* = 0.32). Comparing visual outcomes in these subgroups of eyes (that experienced scarring) in terms of line gainers and losers; there was no significant difference in the two groups [Table 4]. However, in eyes with deep mycosis (endothelial plaque), 16 out of 34 eyes (47%) in pigmented group healed with scarring compared with 28 out of 61 (62%) eyes in nonpigmented group (*P* = 0.013). There was a statistically significant improvement in mean visual acuity after completion of treatment compared with that at presentation in both groups [Fig. 4] (*P* < 0.01). Fig. 5 shows a comparison of final visual acuity in the two groups.

Table 1: Fungal pathogens isolated from the two groups

| Fungal isolate     | Total number (n=373) |
|--------------------|----------------------|
| Pigmented fungi    | 117                  |
| *Curvularia*       | 84 (72%)             |
| *Exserohilum*      | 8 (6.8%)             |
| *Alternaria*       | 4 (3.4%)             |
| Unidentified       | 21 (17.9%)           |
| Nonpigmented fungi | 256                  |
| *Fusarium*         | 134 (52.34%)         |
| *Aspergillus*      | 97 (37.89%)          |
| Unidentified hyaline fungus | 25 (9.7%) |

Table 2: Comparison of baseline demographics in the two groups

| Variable                      | Nonpigmented (n=256) | Pigmented (n=117) | *P*-value |
|-------------------------------|----------------------|-------------------|-----------|
| Age (years)                   | 46.27 (+/- 15)       | 42.57 (+/-16.40)  | 0.03      |
| Gender (Male:Female)          | 1.46:1               | 1.92:1            | 0.33      |
| History of trauma             | 150 (58.59%)         | 62 (53%)          | 0.06      |
| Delay in Presentation         | 5 days (3-10days)    | 6 days (3-10days) | 0.91      |
| Visual acuity at presentation (Mean logMAR) | 1.42 (+/-0.80) | 1.21 (+/-0.78) | 0.01 |
| Ulcer location: Central       | 140 (54.68%)         | 51(48.58%)        | 0.07      |
| Paracentral                   | 67(26.17%)           | 46(39.3%)         |           |
| Peripheral                    | 29 (11.3%)           | 11(9.4%)          |           |
| Total                         | 20 (7.8%)            | 9 (7.6%)          |           |
| Size of the infiltrate: Mean (mm²) | 14.96              | 10.5              | 0.01      |
| Range (mm²)                   | 4.2 – 23.34          | 8.16 - 31.92      | ---       |
| Endothelial plaque            | 61 (23.82%)          | 34 (29%)          | 0.06      |
| Hypopyon at presentation      | 130 (50.7%)          | 55 (47%)          | 0.49      |
Nine eyes (11%) had perforation in the pigmented group and 28 eyes (13.6%) in the nonpigmented group, during the course of treatment \((P=0.84)\). Small perforations were typically managed with cyanoacrylate glue application and bandage contact lens. However, larger perforations especially with iris prolapse and shallow anterior chambers underwent therapeutic keratoplasty (TPK). TPK was also done for very large ulcers involving the visual axis. Evisceration was performed for large perforations with uveal prolapse, frank fungal endophthalmitis, panophthalmitis and extensive scleral spread in our study.

Univariate analysis for factors predicting visual loss in the pigmented keratitis group revealed that male gender and central location of ulcers were associated with increased risk of visual loss [Table 5]. None of the other risk factors analyzed reached statistical significance. On performing multivariate analysis, central location of the ulcer \((P=0.021)\) was the only factor that had statistically significant predictive value for visual outcome.

**Discussion**

The 373 cases of culture proven fungal keratitis during the study period constituted 51% of all culture-proven cases of infectious keratitis in our institute, which is in accordance with previous reports on the incidence of such infections from tropical countries\([1-5]\). Among the fungal isolates, pigmented fungi (31.37%) were the second-most common etiological agents after the nonpigmented filamentous species i.e., *Fusarium* (35.92%) and *Aspergillus* (26%). Garg et al.,\([6]\) reported a much lower incidence of keratomycosis due to pigmented fungi (15%) in their series over a decade ago while Bharathi et al.,\([5]\) reported a higher incidence of 25% recently from South India and Chowdhary reported a 29% incidence of *Curvularia* keratitis from North India.\([19]\) Wilhelmus et al., in a retrospective series spanning 30 years (1970–1999), have reported only 43 patients from Houston, Texas. Our study possibly points toward an increasing trend in the incidence of keratitis due to pigmented fungi, especially in this part of the world.
Table 6: Comparison of previous studies on pigmented keratitis with the present study

| Variables                        | Garg et al[6] | Wilhelmus et al[7] | Forster et al[8] | Sengupta et al (Present study) |
|----------------------------------|---------------|--------------------|------------------|-------------------------------|
| Total number (N)                 | 88            | 43                 | 16               | 117                           |
| Study period (Years)             | Jan 1991 – Dec 1996 (6 years) | 1970 - 1999 (30 years) | NS               | Jan 2006 – Aug 2008 (2.6 years) |
| Prevalence of pigmented keratitis| 88/557 (15.7%) | NS                 | 7/53 (11.1%)     | 117/373 (31.3%)               |
| Mean size of the infiltrate      | 4.61 mm (max diameter) | 7 mm² +/- 5 mm²    | NS               | 10.5 mm²                      |
| Commonest fungus recovered       | Curvularia    | Curvularia         | Curvularia       | Curvularia                    |
| Macroscopical pigmentation       | 27%           | 6%                 | NS               | 14.5%                         |
| Hypopyon                         | 53.4%         | 12.5%              | NS               | 47%                           |
| VA at presentation               | 71% < 20/400  | NS                 | 31%<20/400       | 80% < 20/400                  |
| Final VA                         | NS            | >20/40 (78%)       | >20/40 (44%)     | > 20/40 (16%)                 |
| First-line drug                  | Natamycin     | Natamycin          | Natamycin        | Natamycin                     |
| Healed ulcers                    | 72%           | 81%                | NS *             | 48%                           |
| Penetrating keratoplasty         | 15.3%         | 9.3%               | 6%               | 8.6%                          |
| Superficial keratectomy          | NS            | 9.3%               | 6%               | 7%                            |
| Evisceration                     | 9%            | None               | None             | 11%                           |

*NS-Not specified and outcome data not uniform

Table 6 shows a comparison of studies on keratomycosis due to pigmented fungi thus far.

Eyes with nonpigmented keratitis had significantly worse vision at the time of presentation compared to the pigmented group. This can be explained by the fact that this group had significantly larger ulcers compared to the pigmented group. The characteristic macroscopical pigmentation was seen clinically in only 14.5% of eyes in the pigmented keratitis group. A similar incidence of clinically visible pigmentation was reported by Garg et al., (27%).[6] Hence the clinical picture is usually insufficient to establish a diagnosis of dematiaceous fungal keratitis or to distinguish it from nonpigmented fungal keratitis. A definitive diagnosis by microbiological investigations is a must in such cases.

Response to medical treatment alone was favorable, with a significant improvement in logMAR visual acuity and comparable rates of healing and scar formation in both groups. In the study by Garg et al.,[6] 49 eyes with pigmented keratitis (72%) responded to medical therapy with resolution of infiltrate and scar formation. However, subgroup analysis revealed that healing was significantly better in the superficial infiltrate group (88%) compared with patients having deep infiltrate at initial examination (46.1%). Our study showed similar results in eyes with deep mycosis (endothelial plaque) and in this subgroup, nonpigmented group had a better outcome compared to the pigmented group.

As eyes with nonpigmented keratitis had a significantly worse mean visual acuity at presentation compared to eyes with pigmented keratitis, a direct comparison between the mean final visual outcomes of the two groups was not possible. To overcome this statistical barrier, we classified patients in each group into those with good visual outcome (> 4 line gainers), poor visual outcome (> 4 line losers) and visual stabilization. Classifying outcomes as line gainers and losers makes the study conclusions statistically robust. On analyzing these subgroups, we found no difference in visual outcome in the two groups. We analyzed visual outcomes only in those eyes that experienced healing and scar formation. Excluding eyes that underwent surgical intervention viz., TPK and evisceration in visual outcome analysis made the groups more comparable and eliminated intraoperative and postoperative factors responsible for visual outcome. This issue is an interesting one and may be analyzed separately. Similar study designs may be followed in other studies before and after treatment.

Topical natamycin suspension (5%) used as the first line medication in our study has been reported to have excellent efficacy (in vitro) against pigmented fungi by Wilhelmus et al., (MIC <4μg/L) about a decade ago and recently by Prajna et al.[7,10] However, increasing incidence and poor outcomes of pigmented keratitis as seen in our study may be pointing toward increasing drug resistance to natamycin. Alternative routes for drug delivery such as intrastromal and subconjunctival injections are available. However, in our study, neither alternative topical agents (other than natamycin) nor subconjunctival and intrastromal antifungals were used, thus the possible beneficial effect of these agents could not be assessed. Systemic agents available for fungal infections are oral ketoconazole, itraconazole and fluconazole. Studies testing efficacy and MIC<sub>50</sub> of these drugs conclude that all these are not very effective in filamentous fungi and that imidazoles viz., ketoconazole are superior to triazoles.[20,21] However, we prefer fluconazole for its low cost, superior safety profile and fewer drug interactions. For these reasons, topical/oral fluconazole is an acceptable alternative to ketoconazole.[22]

Both groups experienced equal number of perforations. TPK was performed more often in the nonpigmented group presumably because of the significantly larger ulcers in this group. The visual outcomes of TPK were poor in both the groups. Most common causes of failure of TPK were graft re-infection and endothelial rejection. Garg et al., also reported poor results with TPK.[6] Therapeutic superficial keratectomy followed by frequent topical antifungal instillation is a good option for treating plaque like infiltrates limited to the surface.
of the cornea. However, it was performed in less than 10% eyes in both the groups in our study. This can be explained by the fact that superficial pigmented plaque like lesions were seen in only about 14% eyes and most patients had deep mycosis as well. Prajna et al., in their work mainly on nonpigmented keratitis, reported slower healing rates and worse outcomes with larger ulcers (14 mm) and in those eyes where *Aspergillus* was recovered from clinical specimens. However, their outcome measures were purely clinical and did not report on visual status. The authors did not comment specifically on outcomes of pigmented keratitis. The depth of the infiltrate rather than the actual size may be a better predictor of treatment outcome.

On analyzing factors predictive for visual outcome, location of the ulcer was the only factor that reached statistical significance. Eyes with central corneal ulcers were found to have an increased risk of visual loss compared to peripheral and paracentral ulcers. Delay in presentation (>7 days) was also associated with visual loss, though this did not attain statistical significance (P=0.06).

In conclusion, this study highlights the relative importance of pigmented fungi in the etiopathogenesis of fungal keratitis. Response to medical management and visual outcomes are similar in both groups. More research needs to be done on pigmented fungal keratitis in isolation. It would be valuable to compare the efficacy of newer antifungal agents with broader spectrum like voriconazole with traditional polyene antifungals like natamycin for pigmented fungal keratitis alone.

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