Evaluation of Agent and Host Factors in Progression of Mycotic Keratitis

A Histologic and Microbiologic Study of 167 Corneal Buttons

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Purpose: To evaluate the host and agent factors in the progression of mycotic keratitis through the microbiologic evaluation and histologic study of human corneal buttons obtained at the time of therapeutic keratoplasty.

Design: Retrospective noncomparative consecutive case series.


Methods: Therapeutic penetrating keratoplasty, review of microbiologic results, histopathologic and microbiologic evaluation of the corneal buttons of mycotic keratitis

Main Outcome Measures: Histologic evaluation of the buttons for morphologic changes, degree and distribution of inflammatory cells, presence or absence of fungal filaments, and their degree and distribution within the corneal buttons.

Results: The diagnosis of fungal infection was made on corneal scrapings in 36 cases; whereas in 131 (78%), the fungus was grown in cultures and identified as Aspergillus in 55 (42%), Fusarium in 42 (32%), unidentified hyaline fungi in 22 (17%), dematiaceous (unidentified) in 4 (3%), and others in 8 (6%). The mean interval between diagnosis and keratoplasty was 19 (±40) days. From the keratoplastic specimen, the fungus was identified at histologic examination in 127 of 167 (76%) buttons and grown by culture techniques in 76 of 115 (66%) buttons. The fungal species identified in the corneal button were Fusarium in 30 (39%); Aspergillus in 25 (33%); unidentified hyaline in 19 (25%), and others in 2 (3%). Fungus-positive corneal buttons had early surgery (mean, 15 days) compared with fungus-negative (39 days) corneal buttons (P = 0.0005), with 93% fungus positivity in the buttons removed within 2 weeks and 42% after 2 months. In the fungus-positive buttons, there was an inverse correlation between the degree, distribution of inflammatory cells, and fungal filaments (r = −0.255, P = 0.024; r = −0.199, P = 0.027), respectively. The factors necessitating an early keratoplasty were heavy fungal load, deeper penetration of fungus, and possibly insufficient inflammation to combat infection. A granulomatous reaction was noted in the posterior stroma and around the fragmented Descemet’s membrane in 23 buttons (13.8%), independent of fungal species. Inflammation was unaffected by elimination of fungus and increasing interval between diagnosis and treatment.

Conclusions: Rapid progression of mycotic keratitis in the early phases is by agent factors such as heavy load and deeper penetration of the fungus, insufficient inflammatory response, and possibly relative ineffectiveness of antifungal agents. Progression in the later phase of mycotic keratitis need not necessarily be agent mediated; it could be either host-modulated, species-related, or drug resistance, thereby suggesting that ideal treatment regimens should include sensitivity-based antifungal therapy aided by in vivo monitoring of fungal filaments. Ophthalmology 2002;109:1538–1546 © 2002 by the American Academy of Ophthalmology.

Keratomycosis is a suppurative, ulcerative sight-threatening infection of the cornea that sometimes leads to loss of the eye. Worldwide, the reported incidence of fungal keratitis is 17% to 36%, whereas in India, it is 44% to 47%. Despite advances in diagnosis and medical treatment of


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mycotic keratitis, 15% to 27% of patients require surgical intervention such as keratoplasty, enucleation, or evisceration because of either failed medical treatment or advanced disease at presentation.1–11 There is no existing method, except confocal microscopy,12 to assess whether the progression of the disease during treatment of mycotic keratitis is due to the persistence of the agent, an ongoing inflammatory response, or both. The outcome of mycotic keratitis depends ultimately on the interplay of the agent (virulence, resistance to drugs, and toxicity) and host factors (predisposing factors, inflammatory response, and hypersensitivity reactions) in addition to timely diagnosis and appropriate medical treatment. Insight into this complex pathogenic mechanism in keratomycosis could be obtained from animal experiments, impression debridement of corneal ulcers, diagnostic corneal biopsy, or from corneal buttons removed during penetrating keratoplasty for medically uncontrolled mycotic keratitis.13–17 We have used a histologic evaluation of corneal buttons of mycotic keratitis to assess the role of agent factors, such as persistence of fungus, degree and distribution of filaments, and host factors, such as inflammatory response, tissue damage, and vascularization, to determine the progression of mycotic keratitis. Microbiologic evaluation of the mycotic ulcers at the time of diagnosis and at the time of therapeutic keratoplasty was also undertaken to assess the response to medical treatment.

**Material and Methods**

The medical, microbiologic, and histopathologic records of all culture-proven cases of fungal keratitis, which were managed surgically between January 1995 and May 1998 at the L. V. Prasad Eye Institute, a tertiary referral eye care center, were reviewed retrospectively. Patients with a clinical diagnosis of corneal ulcer were directed to the ulcer clinic and worked up extensively for the etiologic diagnosis. Once diagnosed microbiologically as having a fungal ulcer, the patients were preferably admitted as inpatients. The treatment was initiated with 5% natamycin instilled every half hour, fungal ulcer, the patients were preferably admitted as inpatients. The treatment was initiated with 5% natamycin instilled every half hour, was added in “severe ulcers.” Surgical intervention with therapeutic keratoplasty was considered for failed medical treatment as assessed by an increase in the size of the epithelial defect, stromal infiltrates despite 5 to 7 days of treatment, limbal involvement, impending perforation, or advanced disease at presentation. Patients who underwent therapeutic penetrating keratoplasty were noted from the corneal database of our institution. We assessed the agent and host factors that are influenced by the duration of treatment (i.e., the interval between diagnosis and keratoplasty and also the influence of one on the other). The time interval was segregated into four phases for convenience: less than 2 weeks, 2 weeks to 1 month, 1 to 2 months, and more than 2 months. In 14 cases the data on time interval between diagnosis and keratoplasty was not available, hence these cases were excluded in the analysis when time frame was compared with other parameters. An attempt was made to evaluate the prognostic markers for perforation and regrafting. The statistical methods used in this study were chi-square test, Student’s t-test (risk factors for perforation and graft failure), Fisher’s exact coefficient, Spearman’s coefficient (inflammation vs. fungus degree and distribution), and analysis of variance (relationship of agent and host factors with duration of treatment).
Results

Patients

The number of patients with infectious keratitis attending our ulcer clinic between January 1995 and May 1998 was 1926, of which 644 (33.4%) were microbiologically diagnosed as having fungal keratitis; 148 patients (23%) required therapeutic penetrating keratoplasty. In addition, 19 patients required regrafting within the same period, resulting in 167 corneal buttons for this study.

The age/gender distribution is given in Figure 1. The age of these patients ranged from 12 to 80 years, with a mean of 41 ± 14 years. There was a peak in the third decade, constituting 28.4% of all cases; 53.4% of cases were 40 years of age or younger. There were 104 (70%) males and 44 females (30%), with a male/female ratio of 2.3:1. The mean duration between the initial scraping and the time of penetrating keratoplasty was 19 days (40 days with a median of 8 days (1–331). One hundred five (62%) cases had undergone penetrating keratoplasty within 2 weeks, 28 (17%) between 2 weeks and 1 month, 13 (8%) between 1 and 2 months, and 7 (4%) more than 2 months after diagnosis was established.

Microbiology

The microbiologic diagnosis of mycotic keratitis was based on the identification of fungal filaments in the potassium hydroxide and calcofluor preparations in 36 cases, whereas growth on culture media was noted in 131 (78.4%) cases. The species identified at the time of corneal scraping and from the corneal button obtained at the time of therapeutic keratoplasty are given in Table 1. Complete microbiologic study of the bisected corneal button obtained at the time of keratoplasty was available in 115 of 167 (68.9%) cases, of which 76 of 115 (66%) buttons revealed growth on cultures, as shown in Table 1.

Histologic Data

The morphologic changes in the epithelium, Bowman’s layer, and stroma are shown in Table 2. The absence of epithelium in two cases and intact Bowman’s layer in 13 cases possibly represent a sampling error during bisection of the button, embedding, or cutting of the sections. Vascularization was noted in 39 cases (23%): localized to superficial stroma in 35 cases and deep stroma in 4 cases. Type, degree, and distribution of stromal inflammation is shown in Table 3. Inflammation was seen mostly involving all the layers (94, 60%), whereas in some cases it was restricted to either the anterior two thirds (23, 16%) or posterior one third (38, 24%) (Fig 2). Involvement beyond Descemet’s membrane was noted in 55 (22%) cases, with or without demonstrable continuity thorough the posterior stroma. Perforation was evident on histologic examination in 40 (24%) buttons (Fig 3). The mean interval between diagnosis and keratoplasty in these cases was 21 days ± 54 days (median, 7 days). Giant cell reaction with or without granulomatous inflammation (Fig 4) was noted in 23 cases (13.8%). Granulomas were seen mostly in the deep stroma and along the fragmented Descemet’s membrane, except in two cases, where they were seen in the superficial and midstroma. They were associated with identifiable perforation in 10 (43%) cases.

The degree, orientation, and distribution of fungal filaments identified in 127 of 167 (76%) corneal buttons are shown in Table 2. Histopathologic Features of Corneal Buttons Diagnosed as Fungal Keratitis

<table>
<thead>
<tr>
<th>Features</th>
<th>No. of Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial ulceration</td>
<td>165 (98)</td>
</tr>
<tr>
<td>Stromal thinning</td>
<td>109 (65)</td>
</tr>
<tr>
<td>Stromal necrosis</td>
<td>138 (81)</td>
</tr>
<tr>
<td>Perforation</td>
<td>40 (24)</td>
</tr>
<tr>
<td>Vascularization</td>
<td>39 (23)</td>
</tr>
</tbody>
</table>

Figure 1.

The age and gender distribution of the patients who underwent penetrating keratoplasty for fungal keratitis.
4. It is to be noted that fungal filaments were either seen in all layers (62, 49%) or restricted to the anterior two thirds (50, 39%) or to the posterior one third of the stroma (14, 11%) (Fig 5). Fungal filaments were noted in 32 of 40 (80%) corneal buttons with perforation. The fungal load in this group was mild/moderate in 13 buttons each and heavy in 6 cases.

Regrafting was done in 19 cases during the study period. The mean interval between the initial graft and the regraft was 28 days ± 24.4 days (range, 5–98 days). Seventeen of these 19 cases (90%) had fungus in the primary (host) corneal button, with recurrence in 6 (32%) cases, proven by histology in 5 cases and by microbiologic techniques in 1 case. The fungus load in these cases was moderate (8) or heavy (7), accounting for 88% of fungus-positive host corneas. The distribution was diffuse, involving all layers in nine cases, the anterior two thirds in eight cases; moderate in eight cases; and mild in two cases. Posterior and/or lateral resected margins were seen in 14 of 17 (82%) cases; anterior chamber involvement was seen in 8 (47%) cases.

Figure 2. Light microscopic photographs of fungal keratitis showing epithelial ulceration with inflammatory infiltrates involving the full thickness of the corneal stroma. The inflammation was seen involving all layers (A) (stain, periodic acid–Schiff; original magnification, ×125), restricted to either the anterior two thirds (B) (stain, hematoxylin–eosin; original magnification, ×125), deeper one third of the stroma (C) (stain, hematoxylin–eosin; original magnification, ×125), and beyond the confines of the corneal stroma suggestive of extension into the anterior chamber (D) (stain, hematoxylin–eosin; original magnification, ×125).

Figure 3. Section of the cornea showing perforation with a small fragment of uveal tissue seen adherent to the area of perforation.

Figure 4. Granulomatous reaction (asterisks) in the posterior stroma and around the fragmented ends of Descemet’s membrane in a microbiologically and histologically proven case of fungal keratitis (stain, hematoxylin–eosin; original magnification, ×250).
After treatment, fungus was identified in the corneal buttons by histologic evaluation in 127 of 167 (76%) cases, whereas by microbiologic techniques it was grown from 76 of 115 (66%) buttons. The mean duration of medical treatment (i.e., interval between diagnosis and keratoplasty) in the fungus-positive cases was 15 days, whereas it was 39 days in the fungus-negative group, which was statistically significant ($P = 0.0005$). The presence of fungus when plotted against duration of treatment revealed that fungus was present in 92% of corneal buttons that had a penetrating keratoplasty within 2 weeks compared with 43% of buttons that underwent penetrating keratoplasty after 2 months (Fig 7).

The cases with heavy fungal load had a mean interval of 9 days between diagnosis and keratoplasty, whereas the moderate load had a mean interval of 12 days, and the mild load had a mean interval of 29 days. Corneal buttons with heavy fungal load were seen early compared with mild and moderate fungal load ($P = 0.048$). Diffuse involvement of fungus had an interval of 11 days, whereas that in the anterior two thirds had 21 days, and the posterior one third had 6 days. The various species of fungus as seen at different time points revealed a slight increase in the presence of *Aspergillus* species after 2 months, which, however, was not significant (Fig 8).

The presence or degree of inflammation did not vary with the interval between diagnosis and keratoplasty (Fig 7). The mean duration of treatment for buttons with mild inflammation was 21 days; for moderate inflammation, 20 days; and for severe inflammation, 16 days ($P = 0.536$). However, diffuse inflammation involving all layers had a mean interval of 25 days, whereas that in the anterior two thirds had 31 days, and the posterior one third had 6 days. The granulomatous reaction noted in 23 cases had a mean interval of 12 days between diagnosis and keratoplasty. It did not vary significantly with any particular species of fungus. There was no increase in the risk of perforation or graft failure with increasing time.

### Discussion

Mycotic keratitis is an important ophthalmic problem in all parts of the world, because it leads to corneal blindness and sometimes loss of the eye. Despite advances in diagnostic methods and medical treatment, 20% to 25% of fungal keratitis cases required surgery in the form of therapeutic penetrating keratoplasty. Fungal keratitis is much more prevalent in tropical countries and South India, where fungi are present in the atmosphere and in the conjunctival sacs of asymptomatic individuals.

The most thought-provoking aspect of fungal keratitis is its pathogenesis, which involves the interplay of host and agent factors in the eye. A few histologic studies on fungal keratitis published earlier included cases with histologic evidence of fungus on sections, whereas in this study we included the cases that were clinically diagnosed and treated as fungal ulcer, irrespective of presence or absence of fungus in the histologic sections. These cases were microbiologically proven cases of fungal ulcers, adequately treated under supervision, and taken for penetrating keratoplasty only after ascertaining the disease progress at our institution. This study gives us the advantage of assessing the histologic and microbiologic factors involved in the progression of the disease (e.g., tissue inflammatory response, persistence of fungus after treatment), possible prognostic factors in evaluating the response to antifungal therapy, and thereby understanding the pathogenesis and progression of fungal keratitis.
The age group involved in this study ranged from 12 to 80 years, with a mean of 41 years. Although none of our cases were younger than 10 years old, as observed by Panda et al., more than half of the patients were younger than 40 years of age; that could have a great impact on the economical burden. There was a preponderance of males as noted in other series from India and other countries, which is possibly due to the relative frequency of outdoor activities of the males. Our hospital data revealed that 46.7% of patients with fungal keratitis are either agriculturists or manual laborers (unpublished data).

The corneal morphology noted in this study is mostly similar to that described in other studies, except for the granulomatous reaction in the corneal stroma. The granulomatous reaction was noted in 14% of cases, predominantly in the posterior stroma and around Descemet’s membrane, similar to the 17.3% prevalence observed by Panda et al. It did not show any specific association with the different species of fungus as identified from the corneal scraping or buttons. This type of inflammatory response in the cornea has been reported to be of nonspecific nature because of herpetic keratitis or Acanthamoeba keratitis.

Figure 5. The silver staining for fungus revealed fungal filaments involving the full thickness of the corneal stroma (A), restricted to the anterior two thirds (B), involving the posterior stroma and the anterior chamber (C), and extending into the anterior chamber (D) (stain, Gomori’s silver methenamine; original magnification, ×125).

Figure 6. A and B. Section of the corneal button from a patient who underwent keratoplasty within 10 days of treatment shows minimal inflammation in the anterior stroma and mild inflammation in the posterior stroma. The same corneal section on silver staining shows dense fungal filaments anteriorly and few filaments extending into the posterior stroma, highlighting the inverse relationship of inflammation to fungal filaments (A: stain, hematoxylin–eosin; original magnification ×125; B: stain, Gomori’s methenamine silver stain; original magnification, ×125).

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In herpetic keratitis, the granulomatous reaction is suggested to be due to a viral antigen, altered cellular and/or basement membrane structures from the host, or both. In light of existing knowledge, the granulomatous inflammation in our series of microbiologically proven fungal keratitis, with evidence of fungus in 78% cases, could be either nonspecific, immune-mediated, or due to primary preexisting undetected viral infection in the patient. It is beyond the scope of this study to confirm or eliminate these causes; however, it does point toward the role of immunity and hypersensitivity in the progression of fungal keratitis.

It is important to note that in the buttons positive for fungal filaments, the degree and distribution of inflammatory cells had a negative correlation with intensity and distribution of the fungal filaments. Although the correla-

**Figure 7.** Graph depicting the percentage of corneal buttons of various degrees of inflammation and fungus positivity in the corneal buttons removed at various intervals between diagnosis and treatment.

**Figure 8.** Graph representing the frequency of corneal buttons of varying fungal species as identified at the time of scraping compared with the time at which keratoplasty was undertaken for these cases. This is to assess the persistence of any particular species with increasing duration of medical treatment.
tion was weak ($r = 0.0255$), it could be of clinical significance. This was our observation over the past few years, which could be confirmed by statistical evaluation in this large series of cases. It could be speculated that in the initial phase of mycotic keratitis, the polymorphonuclear cell infiltrates are beneficial in reducing the load of fungus by phagocytosis of the fungal filaments and destruction through the free radicals generated from the polymorphonuclear cells. Another possible explanation for the inverse relationship of inflammatory infiltrates and fungus could be that the heavy fungal load and the toxins or enzymes inhibit the inflammatory response to perpetuate their growth.

Interestingly, the corneal buttons obtained at the time of keratoplasty were negative for fungus in one fourth (24%) of the cases by histologic techniques and in one third (34%) by microbiologic techniques. Inflammation in this group was similar to that in the fungus-positive group, suggesting the persistence of inflammation after elimination of the agent (i.e., the fungus). It is evident that inflammation, once initiated, continues uninhibited even after the removal of the inciting agent. Further damage to the corneal tissues is possibly contributed to by the inflammatory mediators and not directly by agent factors. It is also evident from this study that the clinical parameters of the progression of the disease do not necessarily indicate the persistence of fungus, as has been generally assumed. It could be due to persisting inflammation, which may also be contributed to by the hypersensitivity reaction, as suggested by Thomas. The hypothesis gains support in our study with a longer interval (39 days) between diagnosis and keratoplasty in the fungus-negative corneal buttons compared with the fungus-positive (15 days) group. We therefore speculate that the addition of an immunomodulating drug (possibly an antisteroidal drug) in addition to antifungal therapy would help in containing infection. In vivo monitoring of fungal filaments by confocal microscopy could aid the clinician in assessing the persistence or absence of fungus in the corneal tissues.

The status of the fungus in the corneal buttons obtained at keratoplasty from patients who have been on continuous antifungal therapy could also reflect the in vivo effectiveness of the treatment protocols. Fungus was identified in 76% of our cases, similar to the 74% reported by Rosa et al., whereas it was grown in 66% of our cases compared with 71% in their series. The presence of fungus and inflammation in the buttons after treatment suggests either overwhelming infection at presentation, inadequate medical treatment, lack of penetration of drugs, or in vivo resistance to the drugs. Although the treatment regimen in our series was not based on culture and sensitivity studies, a few interpretations are feasible. Our results suggest that the load of fungus and its penetration are important determinants of progression of the disease, necessitating early surgical intervention. The location of fungus in all layers or in the anterior two thirds in 88% of cases compared with 12% restricted to the posterior third contradicts previous observation of absence of fungus on the corneal surface. This would allow us to speculate that progression of the disease in the early stages with persistence of fungus anteriorly is possibly due to ineffectiveness of drugs rather than penetration of drugs. The presence of the fungus after 2 months, however, could be due to poor penetration of drug, resistance of fungus to drugs, or both. There is a relative increase in the number of cases with Aspergillus species, suggesting that the drug resistance could be species-specific. An in vitro susceptibility test for the fungal isolates could shed light on these findings. Another noteworthy observation was the presence of fungal filaments in 28 of 39 (78%) of the corneal buttons with vascularization. This is in contrast to the belief that vascularization represents the healing phase of the disease process.

Other clinical factors such as history of trauma and systemic illness, which we did not include in this study, could shed light on the role of these factors in the progression of the disease. Although the need for surgical intervention by itself suggests a progressive disease, the addition of individual clinical parameters such as size of the infiltrate, stromal thinning at the time of diagnosis, and at the time of keratoplasty could have shed more light on the factors responsible for progression of this devastating disease. These factors that could not be addressed in this retrospective study should pave the way for another prospective study.

By comparing the histologic parameters in the host and the donor corneal tissues, a few prognostic factors in the graft can be assessed. Regrafting was done in 19 cases, of which 90% had fungus in the host button and 5 (26%) had recurrence in the donor tissue. There was no statistical difference in the degree of inflammation, fungal load, and the duration of treatment in failed grafts and the other corneal tissues. However, graft failure with Aspergillus species was significant. There was no increased risk of recurrence or regrafting with involvement of the margins by the fungal filaments ($P = 0.2477$). Earlier studies have shown that the results of surgical intervention are not adversely affected by the presence of fungus at the surgical margins. It could be speculated that factors such as stromal necrosis, cytokines, associated local diseases, or systemic diseases may be more important than the presence of fungal filaments. Although not addressed in this study, our hospital ulcer data revealed predisposing ocular factors in 11.5% of cases, whereas systemic factors were noted in 9% of cases. Further prospective studies or animal experiments could help in assessing the prognostic factors in patients with fungal keratitis.

In summary, both agent factors (heavy fungal load with deep penetration) and host factors (insufficient inflammatory response), possibly in addition to the relative ineffectiveness of antifungal agents, seem to influence the progression of mycotic keratitis in early stages, thereby justifying prompt surgical intervention. The late stages of mycotic keratitis could be attributed to the deleterious persistence of inflammation or possibly because of the resistant species of fungus. We therefore suggest that an ideal treatment protocol should include antifungal agents chosen by in vitro susceptibility of the fungus, the duration of which should be assessed by in vivo monitoring of fungal filaments, and, if required, the addition of antiinflammatory drugs should be considered in prolonged cases.


