Original Article

Lasiodiplodia keratitis: A case series of 27 patients

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Purpose: To review 27 cases of microbial keratitis caused by *Lasiodiplodia theobromae* presenting to a tertiary eye care center in eastern India over seven-year period and analyze the risk factors, clinical profile, microbiological characteristics, treatment, and outcome of patients. **Method:** This is a retrospective review of 27 cases of microbial keratitis diagnosed with *Lasiodiplodia theobromae* between November 2015 and October 2022. Data were retrieved using the electronic medical record system. Additional outcomes such as average time of resolution, frequency, type, and outcome of surgical interventions, wherever performed, were also analyzed. Descriptive statistics were used to interpret the demographic data. **Results:** The mean age of patients was 56.8 ± 18.7 years (range: 23–86), the male: female ratio was 2.4:1, and the average follow-up was 90.2 days. Eleven (40.7%) patients had a history of trauma, and 16 (59.3%) presented with a large infiltrate (>6 mm). Samples from all the patients were culture-positive for *Lasiodiplodia theobromae*, of which 26 (96.3%) were corneal scrapings and one (4%) was a half-corneal button. Eight (29.6%) patients had coexisting bacterial infections. Successful management was achieved in 12 eyes (66.7%) with medical treatment alone (topical natamycin, n = 7; topical natamycin + oral ketoconazole, n = 3; and topical natamycin + topical gatifloxacin, n = 2). However, surgical intervention was required for nine (33.3%) eyes. **Conclusion:** Medical management with natamycin was effective in treating patients with *Lasiodiplodia* keratitis. However, advanced cases necessitated surgical intervention for complete resolution.

Key words: Fungus, keratitis, microbiology, risk factors

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Fungal keratitis (FK) is a corneal infection that causes significant ocular morbidity. It is common in developing countries, where agriculture is the primary occupation.[1] It has been estimated that ocular injuries resulting in corneal ulceration contribute to approximately two million new cases of blindness globally.[2]

In tropical countries, antecedent ocular trauma caused by vegetative matter is a major predisposing factor for FK. FK contributes to approximately one-third (34.5%) of all infectious keratitis and requires differentiating from other causal organisms.[3] In humans, FK is predominantly caused by *Aspergillus* spp., *Fusarium* spp., *Curvularia* spp., *Scedosporium apiospermum*, and *Paecilomyces*.[4] *Lasiodiplodia theobromae* is a relatively uncommon cause of keratomycosis, with only a few anecdotal cases reported globally.

Lasiodiplodia theobromae belongs to the *Coelomycetes* family. It is the anamorph of *Botryosphaeria rhodina*, a member of the subphylum *Pezizomycotina* of *Ascomycota*.[5] It is a common plant pathogen seen in countries with humid climates, causing rotting and dieback in infected plants. Although infections in humans are uncommon, they can affect the eye, primarily the cornea, causing microbial keratitis. Keratitis can progress rapidly with increased propensity and causes complications, such as corneal perforation, endophthalmitis, and panophthalmitis. Its variable response to antifungals makes the management challenging. Therefore, in this report, we present 27 cases of *Lasiodiplodia* keratitis and provide an analysis of its risk factors, clinical profile, microbiological characteristics, treatment, and patient outcomes.

Methods

Data mining

We conducted a retrospective review of the medical records of patients with microbiologically proven *Lasiodiplodia* keratitis, between November 2015 and October 2022. The project was initiated after approval by the Institutional Ethics Committee (2022-151-BHR-49) of L V Prasad Eye Institute, Bhubaneswar, India. Cases were identified from the electronic medical records (EMRs) and laboratory records of the Institute. The collected data included patient profile (age and gender), risk factors, previous topical medications, size of infiltrate, smear and culture reports, and details about medical and surgical management and their outcomes.

Sample processing for microbiological evaluation (smear and culture)

All patients presenting with microbial keratitis underwent a detailed slit lamp examination. In the clinics, patients without significant corneal thinning underwent corneal scrapings as an outpatient department procedure. The samples were put on

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sterile slides and different culture media for smear examination and culture, respectively.

Smear examination

Two slides were taken for smear examination, one for the potassium hydroxide with calcofluor white (KOH + CFW) mount and the other for gram staining. A drop of KOH was added to the sample, followed by one drop of CFW. A cover slip was put over the sample, and slides were visualized under a fluorescence microscope (Olympus, Japan) at 400x magnification. Gram-stained slides were visualized with 1000x magnification with bright field mode of the same microscope.

Culture

As per our Institute protocol, all corneal scrapings were inoculated on culture media, including chocolate agar (CA), 5% sheep blood agar (BA), Sabouraud dextrose agar (SDA), non-nutrient agar with *Escherichia coli*, brain heart infusion broth, Robertson's cooked meat medium, and thioglycollate broth. All media were incubated aerobically at 37°C except SDA, which was incubated at 27°C. The media were checked regularly for two weeks for the appearance of any significant growth. The fungus grown on either BA or CA plate was sub-cultured further on PDA for sporulation. The fungus grown on PDA was identified using the lactophenol cotton blue (LPCB) mount.

A culture was defined as "significant" if there was confluent growth at the site of inoculation on any solid medium, growth of the same organism on two or more culture media, growth in any one culture medium with consistent smear examination results, or growth of the same pathogen on repetitive corneal scrapings.[6] Those corneal ulcers that were presented with significant thinning underwent corneal scraping and application of cyanoacrylate glue at the thinned area. The procedure was performed in the operating room under sterile conditions. The remaining microbiological examinations followed the same protocol.

Results

Demographics

Between November 2015 and October 2022, 3036 cases of culture-positive microbial keratitis were reported, of which 1537 were of fungal etiology. Among the FK cases, *Lasiodiplodia theobromae* was detected in 27 patients. The average age of the patients was 56.8 ± 18.7 years (range: 23–86), and the gender ratio was 2.4:1 (male: female). The mean follow-up for the patients from the day of presentation was 90.2 days (median: 42 days, range: 7–538 days).

Visual acuity

The mean best corrected visual acuity (BCVA) at presentation was 2.06 ± 0.89 logMAR units, and the mean BCVA at the final visit was 1.75 ± 0.96 logMAR units.

Risk factors

Eleven (40.7%) out of the 27 patients reported a history of trauma before the development of keratitis, of which five (45.4%) had vegetative trauma, three (27.2%) had injuries with wooden material, two (18%) had injuries with metal objects, and one (9%) had an accidental injury with a cow's tail. Moreover, one patient underwent a prior corneal transplantation, and another patient had associated systemic co-morbidity of chronic kidney disease.

Prior topical medications

Five patients were treatment-naïve, and the remaining 22 were on topical medications for microbial keratitis before presentation. Among the 22 patients, 11 (50%) were on topical fluoroquinolones (gatifloxacin 0.3%: n = 3; ofloxacin 0.3%: n = 5; and moxifloxacin 0.5%: n = 3), three (13.6%) were on topical antibiotics and antifungals, two (9%) were on topical antifungals (natamycin 5%), three (13.6%) were on a combination of topical fluoroquinolones (gatifloxacin 0.3%: n = 1 and moxifloxacin 0.5%: n = 1) and steroids, two (9%) were on antivirals (ganciclovir 0.15%: n = 2) alone, and one (4.5%) was on a combination of topical antibiotic and antiviral medications (moxifloxacin 0.5% and ganciclovir 0.15%: n = 1).

Clinical features

Eleven (40.7%) patients had an infiltrate ≤6 mm, and the remaining 16 (59.3%) had a large infiltrate (>6 mm). At the time of presentation, five (18.5%) patients had infiltrates involving the limbus, and one (3.7%) had significant corneal thinning. Seven (25.9%) patients had endoexudate, and five (18.5%) had ring infiltrates [Fig. 1a]. At the time of presentation, 12 (44.4%) and four (14.8%) patients had hypopyon and anterior chamber exudates, respectively.

Microbiological analysis

Smear examination

Fungal hyphae were seen in 26 (96%) out of 27 patients with either gram stain, KOH + CFW mount, or both, while one patient revealed gram-positive cocci on gram stain [Table 1]. Of the 26 patients, 21 only showed fungal filaments with KOH + CFW mount [Fig. 1b] and gram stains, four showed fungal filaments with gram-positive cocci, and one showed fungal filaments with gram-positive bacilli on gram stain.

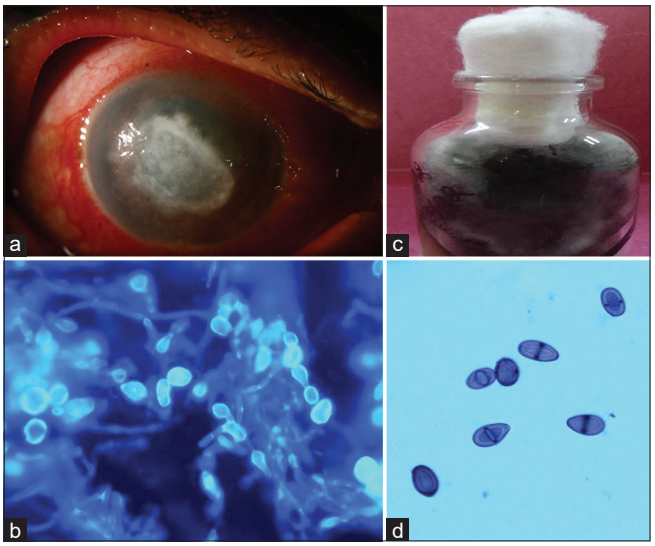

Figure 1: (a) Slit lamp photograph showing central corneal infiltrate in diffused Illumination, (b) Photomicrograph showing the hyphae of *Lasiodiplodia theobromae* in KOH + CFW mount (captured with 40x magnification), (c) Growth of *Lasiodiplodia theobromae* on Sabourd's dextrose agar (SDA), (d) Photomicrograph showing the two celled proconidia of *Lasiodiplodia theobromae* in lactophenol cotton blue (LPCB) mount (captured with 40x magnification)

Culture

All the patients included in this study were culture-positive for *Lasiodiplodia theobromae*, [Fig. 1c] of which 26 had positive corneal scrapings and one had corneal tissue retrieved during therapeutic penetrating keratoplasty (TPK). The fungus grown on culture was identified using the LPCB mount [Fig. 1d]. Of the total of 27 patients, 19 (70.3%) had *Lasiodiplodia* alone and eight (29.6%) had *Lasiodiplodia* and other bacteria, such as *Staphylococcus epidermidis* (*n* = 3), *Staphylococcus aureus* (*n* = 1), *Staphylococcus warneri* (*n* = 1), *Staphylococcus* spp. (*n* = 1), coagulase-negative *Staphylococcus aureus* (*n* = 1), and *Streptococcus pneumoniae* (*n* = 1) [Table 2].

Treatment

Topical antifungal medication was initiated in all 27 patients, of which topical natamycin (5%) alone and topical natamycin along with oral ketoconazole (200 mg, twice daily) were prescribed to 10 (37%) patients. Topical voriconazole (1%) along with topical natamycin (5%) was prescribed to two (7.4%) patients. Another two patients (7.4%) received adjuvant intracameral voriconazole (100 µg/0.1 mL). Topical antibiotics were prescribed to three (11.1%) patients due to the presence of mixed infections.

Surgical intervention

Surgical interventions were performed in nine patients (33.3%); TPK (*n* = 6, 22.2%), cyanoacrylate glue application (*n* = 2, 7.4%), anterior chamber wash with intracameral voriconazole (*n* = 2, 7.4%), and intraocular antibiotic (*n* = 1, 3.7%).

Outcome

In our series, nine patients were lost to follow-up (LTFU), and 12 patients (66.7%) were resolved with medical management. TPK was done in six eyes, of which one patient was LTFU. The remaining five eyes had complete resolution of infection with no recurrence. However, all six eyes developed graft failure. One patient who had a graft infiltrate with *Lasiodiplodia theobromae* resolved after applying cyanoacrylate glue; however, the eye went into phthisis. There was a significant difference in the outcome in relation to duration of symptoms, size of infiltrate, and trauma [Table 3].

Table 1: Smear from corneal scrapings

Group	*n*	(%)
Fungus	21	(77.8%)
Fungus + Gram-positive cocci	4	(14.8%)
Fungus + Gram-positive bacteria	1	(3.7%)
Gram-positive cocci	1	(3.7%)

Table 2: Culture from corneal scraping

Group	*n*	Organism
Fungus	19	*Lasiodiplodia theobromae*
Fungus + Gram-positive cocci	8	*L. theobromae* + *Staphylococcus epidermidis* (*n*=3) *L. theobromae* + *Staphylococcus aureus* (*n*=1) *L. theobromae* + *Streptococcus pneumoniae* (*n*=1) *L. theobromae* + *Staphylococcus spp.* (*n*=1) *L. theobromae* + *Staphylococcus warneri* (*n*=1) *L. theobromae* + Coagulase-negative *Staphylococci* (CoNS) (*n*=1)

Table 3: Correlation between the duration of symptoms, size of infiltrate, and history of trauma with outcome

Risk factors	Outcome		*P*
	Complete success *n*=12 (%)	Partial success/ failure n=6 (%)	
Duration of symptoms			
• ≤15 days	9 (75)	5 (83)	0.70
• >15 days	3 (25)	1 (17)	
Size of infiltrate			
• Small	7 (58)	1 (17)	0.10
• Large	5 (42)	5 (83)	
Trauma			
• Yes	4 (33)	3 (50)	0.49
• No	8 (67)	3 (50)	

Discussion

Lasiodiplodia theobromae (*Botryodiplodia theobromae*) is an ascomycete fungus that belongs to the order *Sphaeropsidales* and family *Botryosphaeriaceae*.[5] It is responsible for 0.5% to 9% of all FK, especially in tropical countries.[7-9] It is responsible for causing fruit rot and dieback diseases in plants and is associated with more than 500 different plant hosts.[5] In humans, it can affect both immunocompetent and immunocompromised individuals, causing sinusitis, keratitis, pneumonia, and cutaneous lesions.[10] More than 30 species of *Lasiodiplodia* have been identified, of which *theobromae* and *pseudotheobromae* cause human infections.[11,12] Both species are also established plant pathogens and are responsible for postharvest fungal diseases across grape vineyards, mango orchards, and citrus orchards, resulting in huge financial losses. The first recorded case of keratitis caused by *Lasiodiplodia* was reported by Puttanna in 1967.[13]

L. theobromae is a saprophytic coelomycete that forms pycnidia. The hyphae grow into an aggregated thick layer of densely packed conidiophores.[14] These conidiophores further produce conidia that are initially unicellular, hyaline, granulose, subovoid to ellipsoid, thick-walled, and non-septate in morphology. Young hyphae are usually hyaline and non-septate, and the mature conidia become dark brown and septate with longitudinal striations and truncated bases. In our series, fungal filaments were isolated from the corneal scrapings of 26 (96.3%) patients. The only remaining patient was subjected to TPK, and *Lasiodiplodia* was isolated from the half-button retrieved during TPK. Out of 27 cases, 19 grew *Lasiodiplodia* alone and eight (29.6%) had a coexisting bacterial growth in the culture.

In our series, trauma with vegetative matter was documented in 40.7% of cases, which is similar to previous publications [Table 4].[15-20] In contrast, previously reported risk factors, such as contact lens wear, ocular surgery, chronic ocular surface disease, or diabetes, were not observed in our

cohort. There was a variation in clinical features and severity ranging from small (<6 mm, 40.7%) to large ulcers (>6 mm, 59.3%), endothelial plaque (25.9%), and anterior chamber involvement (59.25%). da Rosa *et al* reported a case of full-thickness corneal ulcer with anterior chamber invasion of the fungus.[18] Microscopy showed the presence of dematiaceous fungi, which were identified as *L. theobromae* and confirmed by polymerase chain reaction. The patient underwent TPK, followed by intravitreal injection of amphotericin B, topical amphotericin B (0.1 mg/mL), and natamycin (5%) resulting in a favorable outcome. da Rosa *et al*. noted that this organism has the propensity to invade deeper tissues of the eye. Borderie *et al*. have reported a case of FK and endophthalmitis, which was previously managed with topical steroids and gentamicin.[19] Despite all possible treatments, the eye had to be enucleated. *L theobromae* was identified from the histopathology report. Therefore, it was concluded that like any other fungus, *L theobromae* has a poor prognosis in cases with prior use of topical steroids.

Saha *et al*. reported a case of keratitis in an immunocompetent individual who was unresponsive to topical voriconazole therapy and ultimately underwent TPK. They reported satisfactory *in vitro* activity of amphotericin B (4 μg/mL) and voriconazole (1 μg/mL) and resistance to other antifungal agents.[17] Another study reported the role of antifungal susceptibility tests and showed their sensitivity to amphotericin B and voriconazole. In contrast, other antifungal agents, such as itraconazole and fluconazole, were resistant.[18] Even though oral itraconazole (400 mg daily dose) reaches the therapeutic blood level, the vitreous level of the drug is not enough to treat endophthalmitis. Vannam *et al*. reported the first successful case treated with 2% voriconazole without keratoplasty.[16]

Table 4: Comparative table of published case reports of Lasiodiplodia keratitis

Study	Cases	Risk factor	Clinical picture	Method of diagnosis	Follow-up duration	Medical management	Surgical management	Outcome
Lekhanont[15]	2	Case 1: Injury by insect	Multiple satellite lesions, stromal abscess	Smear and culture	3 months	Topical natamycin + Voriconazole	Intrastromal voriconazole	Resolved with cornea scar (20/200)
		Case 2: Injury by wood material	Full-thickness abscess, fungal ball in anterior chamber	Smear, culture, and DNA sequencing	–	–	Intrastromal voriconazole; Therapeutic keratoplasty	Failed graft (counting fingers close to face)
Vanam[16]	1	Injury with mango tree branch	2×3 mm superficial infiltrate with hyphate edges	Smear and fungal culture	8 weeks	Natamycin + Topical voriconazole	–	Resolved with macular scar (20/200)
Saha[17]	1	Injury with vegetative matter	9.8×8.2 mm infiltrate with hypopyon	Smear, fungal culture, and PCR	3 months	Amphotericin B + Voriconazole	Therapeutic keratoplasty	Failed graft
da Rosa[18]	1	Type 2 diabetes topical steroids usage	Large corneal ulcer with melt	Smear, culture, DNA sequencing	2<>months	–	Therapeutic keratoplasty	Graft edema with pupillary membrane (hand motion)
Borderie[19]	1	Myeloid leukemia, topical steroid usage	Corneal abscess; endophthalmitis	Smear, culture, and histopathology	9 weeks	Topical and systemic amphotericin B; Later replaced with systemic itraconazole	Vitrectomy + Intravitreal Amphotericin B; Enucleation	Poor
Rebell[20]	4	Case 1: Trauma	Central corneal ulcer	Smear and culture	–	Topical Amphotericin B + Natamycin	–	Good (20/30)
		Case 2: injury with dry tree branch	Infiltrate with hyphate edges	Smear and culture	–	–	–	Healed (20/25)
		Case 3: Injury with television antenna	Mid-stromal abscess with hypopyon	Smear and culture	5 weeks	Topical natamycin	Subconjunctival amphotericin B	Healed with mild scar (20/20)
		Case 4: Topical steroid usage	Gross corneal ulcer; later impending perforation	Smear, culture, and histopathology	–	Topical natamycin	Therapeutic keratoplasty	Good (20/50)

Moreover, the antifungal susceptibility test showed a low minimum inhibition concentration (MIC) to amphotericin B and voriconazole and a high MIC to itraconazole and posaconazole. Previous *in vitro* studies have shown that polyene antimycotic antibiotics have a clear-cut fungicidal effect on *L. theobromae*.[20] In our series, we successfully treated 12 eyes (66.7%) with medical management alone (topical natamycin, n = 7; topical natamycin + oral ketoconazole, n = 3; and topical natamycin + topical gatifloxacin, n = 2). Nine eyes (33.3%) required surgical intervention, and six eyes required TPK. A complete resolution of the infection was seen in five eyes, and one patient was LTFU. In cases where the infection was limited to cornea alone, the infection resolved well with medical management. However, involvement of deeper structures (endoexudates, hypopyon, anterior chamber exudates, or vitreous) necessitated surgical interventions in the form of anterior chamber wash with voriconazole injection, intraocular amphotericin B injections, or even TPK for resolution. We did not find significant difference in outcome in relation to duration of symptoms, size of infiltrate, and trauma. This might be due to the small sample sizes in each group. No molecular identification methods were used in the study, which can be considered as a limitation of the current study.

Conclusion

Lasiodiplodia theobromae is an uncommon cause of FK in humans, with no definite treatment reported in the literature. However, medical management can be initiated with topical natamycin (5%) eyedrops. Severity at presentation and response to the treatment can guide toward the need for any further surgical interventions or change in topical regimen. Multiple intravitreal amphotericin B injections can be considered in cases of intraocular spread into the vitreous cavity. A prospective randomized study with an adequate sample size can help in establishing the treatment protocol.

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Conflicts of interest: There are no conflicts of interest.

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