**Nigrospora sphaerica** causing corneal ulcer in an immunocompetent woman: A case report

Ananya T.S., Anupma Jyoti Kindo, Anandhalakshmi Subramanian, Kalpana Suresh

**ABSTRACT**

**Introduction:** Corneal ulcers secondary to trauma can often pose a clinical challenge. The causative pathogen is at times an unusual and resistant microorganism which may not be identified by conventional laboratory techniques.

**Case Report:** A 45-year-old immunocompetent woman with a history of injury of right eye with a cow’s tail was diagnosed to have fungal corneal ulcer. Sporulation of the isolate occurred after prolonged incubation and the pathogen was found to be Nigrospora sphaerica. The number of cases of true infection caused by this fungus amount to only five in available literature. The patient did not improve with medical management using natamycin and ketoconazole and underwent voriconazole therapy.

**Conclusion:** Delayed sporulation of pathogenic fungal isolates may necessitate prolonged incubation and use of multiple sporulation techniques for the purpose of speciation. Uncommon fungi also need to be kept in mind when dealing with an unresponsive/worsening clinical situation.
**Nigrospora sphaerica** causing corneal ulcer in an immunocompetent woman: A case report

Ananya T.S., Anupma Jyoti Kindo, Anandhalakshmi Subramanian, Kalpana Suresh

**ABSTRACT**

Introduction: Corneal ulcers secondary to trauma can often pose a clinical challenge. The causative pathogen is at times an unusual and resistant microorganism which may not be identified by conventional laboratory techniques. Case Report: A 45-year-old immunocompetent woman with a history of injury of right eye with a cow's tail was diagnosed to have fungal corneal ulcer. Sporulation of the isolate occurred after prolonged incubation and the pathogen was found to be *Nigrospora sphaerica*. The number of cases of true infection caused by this fungus amount to only five in available literature. The patient did not improve with medical management using natamycin and ketoconazole and underwent voriconazole therapy. Conclusion: Delayed sporulation of pathogenic fungal isolates may necessitate prolonged incubation and use of multiple sporulation techniques for the purpose of speciation. Uncommon fungi also need to be kept in mind when dealing with an unresponsive/worsening clinical situation.

Keywords: Fungal keratitis, *Nigrospora sphaerica*, Black fungi, Voriconazole

**INTRODUCTION**

*Nigrospora* species are a filamentous melanized (dematiaceous) group of fungi that belong to the phylum Ascomycota [1]. Widespread in the environment and every so often found in the lab as a contaminant, it is only rarely encountered as a true pathogen. Thus far, literature includes only five cases of human infections caused by this violently spore discharging fungus [2], which includes infections of the skin, nail and eye in both immunocompetent and immunocompromised patients [3–7]. Herein, we report another incidence of this fungus exhibiting its infrequent pathogenic role.

**CASE REPORT**

A 45-year-old previously healthy immunocompetent woman hailing from a rural area in south India presented to the ophthalmology outpatient department of our institute on September 2013 with a history of injury of the right eye following a hit from a cow's tail one month
She had complaints of redness, pain and watering of the right eye since last one month, associated with decrease in vision which was gradual in onset, beginning after two days of the injury and progressive in nature. She was not a diabetic and was not on any medication.

Examination of the affected eye revealed the visual acuity to be limited to counting fingers at one meter, with a congested anterior chamber and corneal infiltration with extensive surrounding stromal edema in the left lower quadrant (Figure 1).

Gram stain and 10% KOH mount of the corneal scrapings were sent to the microbiology lab along with the inoculated culture plates of Sheep Blood Agar, Chocolate Agar and Sabouraud’s Dextrose Agar without cycloheximide (HiMedia Laboratories, Mumbai, India). The Gram stain showed the presence of occasional inflammatory cells but no microorganisms while the 10% KOH mount revealed the presence of septate hyaline hyphal elements.

The culture plates were incubated at 25°C. In seven days, the plates showed moldy growth along the sickle shaped stab lines of inoculation. The colonies were woolly in texture, with the color turning from white to grey to black (Figure 2), producing a reverse pigmentation which darkened from pale to dark brown and finally to black (Figure 3). A slide culture using Oatmeal Agar (HiMedia Laboratories, Mumbai, India) was put up for the purpose of speciation and incubated at room temperature. An LPCB (Lactophenol Cotton Blue) mount of the slide culture ten days later demonstrated only sterile hyphae and no spores.

In an attempt to induce sporulation, a banana peel culture was put up, which has been indigenously developed in our lab (Figure 4). Banana peel was cut up into smaller pieces of one square inch and autoclaved in a glass Petri dish. No agar needs to be added since the banana peel itself acts as the source of nutrition. The fungal isolate was inoculated onto the sterile pieces, moistened with a few drops of sterile distilled water and incubated at room temperature. The banana peel simulates the saprophytic environment. Examination with an LPCB mount three weeks later revealed branching septate hyphae, with the conidiophores exhibiting a single conidium at their inflated apex (Figure 5). The conidia were unicellular, very black, ovoid in shape, with the older spores showing a horizontal flattening (Figure 6). The description was found to fit the characteristic “black spores” of Nigrospora sphaerica.

The patient was initially managed medically with oral ketoconazole and natamycin eye drops after the diagnosis of fungal keratitis was made but showed no improvement. Further to this, bandage contact lens was used for the descemetocele. A month after presentation to the outpatient department, she was started on oral voriconazole 400 mg per day for 10 days and 1% voriconazole eye drops two hourly (eight times a day) for 28 days. The patient showed full recovery on subsequent follow-up visits with reduction in stromal oedema, redness and clearing of corneal infiltration leading to full recovery of vision (6/9 with refraction) (Figure 1).
DISCUSSION

Spores of *Nigrospora* species are more abundant in regions with a tropical or subtropical climate, more commonly occurring outdoors than indoors and readily isolated from soil and decaying plants [8]. It is a frequent plant pathogen affecting grain and fruit [7, 8]. Our patient suffered a traumatic inoculation of the spores on being hit with a cow’s tail most likely contaminated with soil and vegetative matter which are constant habitats of this widely distributed fungus.

Of the seven species of *Nigrospora* that are known, *N. oryzae* and *N. sphaerica* are the more frequently encountered, with the former being a sizeable agricultural problem. The usual human response to this fungus is hay fever or asthma [8] but singular cases of infections have been reported, with *N. sphaerica* being the pathogen and trauma being associated with the infections in the immunocompetent [3–7]. *Nigrospora* has also been isolated as part of the conjunctival flora in a healthy conjunctival sac [9].

A study of the frequency of occurrence of the spores of *Nigrospora* species at every month of the year in the United States [8], revealed that *Nigrospora* spores are typically present outdoors throughout the whole year at a consistent but low density but tend to be higher from August to October. Although there is no data available on the spore density of *Nigrospora* in India, it is of interest to note that our patient suffered her injury during the month of August.

In culture, the color of the colony darkens in proportion with the increasing amount of sporulation during incubation [10]. A full grown culture was black on both the obverse and reverse sides.

The asexual spores (conidia) were found to be typical of the textbook description with conidia ranging from young ovoid to older flattened [8].

The teleomorphic stage (sexual phase) of *Nigrospora* are included in *Khuskia*, another genus of the phylum Ascomycota.

The fungal isolate has been deposited at The Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, The Netherlands, and can be accessed with the accession number CBS 137557.
CONCLUSION

The necessity of considering rarer, more resistant pathogens when managing a case of fungal keratitis is emphasized in this case, since our patient showed clinical improvement only after changeover to voriconazole therapy.

Although *Nigrospora sphaerica* grew very rapidly in culture of the clinical specimen, sporulation took nearly five weeks which would warrant prolonged incubation and usage of multiple techniques like slide culture and banana peel culture for the purpose of accurate speciation of those pathogenic fungi showing no or delayed sporulation.

Acknowledgements

We acknowledge The Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, The Netherlands, for depositing our clinical isolate in the CBS Collection of Fungi.

Author Contributions

Ananya T.S. – Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Anupma Jyoti Kindo – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Anandhalakshmi Subramanian – Acquisition of data, Revising it critically for important intellectual content, Final approval of the version to be published

Kalpana Suresh – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

Copyright

© 2014 Ananya T.S. et al. This article is distributed under the terms of Creative Commons Attribution License which permits unrestricted use, distribution and reproduction in any medium provided the original author(s) and original publisher are properly credited. Please see the copyright policy on the journal website for more information.

REFERENCES

1. De Hoog GS, Guarro J, Gene J, Figueras MJ. *Atlas of Clinical Fungi*, 2ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands 2000. p. 777.
2. Webster J. Spore projection in the hyphomycete *Nigrospora sphaerica*. *The New Phytologist* 1952;51(2):229–35.
3. Talwar P, Sehgal SC. Mycotic infections of the eye in Chandigarh and neighbouring areas. *Ind J Med Res* 1978;67:929–33.
4. Pritchard RC, Muir DB. Black fungi: A survey of dematiaceous hyphomycetes from clinical specimens identified over a five year period in a reference laboratory. *Pathology* 1987;19(3):281–4.
5. Fan YM, Huang WM, Li W, Zhang GX. Onychomycosis caused by *Nigrospora sphaerica* in an immunocompetent man. *Arch Dermatol* 2009;145(5):611–2.
6. Boralkar AN, Dindore PR, Fule RP, Bangde BN, Albel MV, Saoji AM. Microbiological studies in Conjunctivitis. *Indian J Ophthalmol* 1989;37(2):94–5.
7. Muralidhar S, Sulthana M. *Nigrospora sphaerica* causing corneal ulcer—a case report. *Indian J Pathol Microbiol* 1997;40(4):549–1.
8. Gregorio Delgado, Fungus of the Month: *Nigrospora* species, The Environmental Reporter 2006;4(4). Available at: http://www.emlab.com/s/sampling/env-report-04-2006.html. Accessed 14th May 2014.
9. Ando N, Takatori K. Fungal flora of the conjunctival sac. *Am J Ophthalmol* 1982;94(1):67–4.
10. Larone DH. Medically Important Fungi - A Guide to Identification, 3ed. Washington, D.C.: ASM Press 1995.
ABOUT THE AUTHORS

Article citation: Ananya TS, Kindo AJ, Subramanian A, Suresh K. *Nigrospora sphaerica* causing corneal ulcer in an immunocompetent woman: A case report. Int J Case Rep Images 2014;5(10):675–679.

**Ananya T.S.** is an MD-PhD postgraduate student at Sri Ramachandra Medical College and Research Institute, Department of Microbiology, Sri Ramachandra University, Porur, Chennai, India. She earned her MBBS (Bachelor of Medicine and Bachelor of Surgery) undergraduate degree from Father Muller Medical College, Mangalore affiliated to Rajiv Gandhi University of Health Sciences, Karnataka, India. Her research interests include medical mycology, antifungal resistance and *Fusarium*. She intends to complete her PhD under the ICMR-TSS Fellowship by the year 2017. Email: ts.ananya87@gmail.com

**Anandhalakshmi Subramanian** is M.S. Ophthalmology postgraduate at Sri Ramachandra Medical College and Research Institute, Department of Ophthalmology, Sri Ramachandra University, Porur, Chennai, India. She earned her MBBS undergraduate degree from Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai, India. Her research interests include cornea, medical retina and oculoplasty. Email: ananthasub88@gmail.com

**Anupma Jyoti Kindo** MD is Professor in the Department of Microbiology and is In-charge of the Mycology section at Sri Ramachandra University, Porur, Chennai, India. She earned the undergraduate degree MBBS from JIPMER Pondicherry, India and postgraduate degree MD in Microbiology from AIIMS, New Delhi India. She has published 48 research papers in national and international academic journals in the field Mycology. Her research interests includes medical mycology. Email: anupmalakra@gmail.com

**Kalpana Suresh** is Professor & Head in the Department of Ophthalmology at Sri Ramachandra University, Chennai, India. She earned the undergraduate degree MBBS from Coimbatore Medical College, Tamilnadu, India and postgraduate degree from MS ophthalmology from Madras Medical College – Regional Institute of Ophthalmology, Chennai, India. She went on further to complete FICO (Fellow of International Council of Ophthalmology, Cambridge), FRCS (Glasgow) & MBA (Health service Management, Anna University, Chennai). She has published 32 research papers in national and international academic journals and presented 137 scientific papers, delivered 57 guest lectures, conducted around 30 workshops/Instruction courses/CME in various state, national and international conferences. Her research interests include amniotic membrane transplants, corneal endothelial cell isolation and expansion. She intends to pursue research activities involving corneal endothelial cell proliferation studies in future. Email: kalpanasrao@hotmail.com
About Edorium Journals
Edorium Journals is a publisher of high-quality, open access, international scholarly journals covering subjects in basic sciences and clinical specialties and subspecialties.

Invitation for article submission
We sincerely invite you to submit your valuable research for publication to Edorium Journals.

But why should you publish with Edorium Journals?
In less than 10 words - we give you what no one does.

Vision of being the best
We have the vision of making our journals the best and the most authoritative journals in their respective specialties. We are working towards this goal every day of every week of every month of every year.

Exceptional services
We care for you, your work and your time. Our efficient, personalized and courteous services are a testimony to this.

Editorial Review
All manuscripts submitted to Edorium Journals undergo pre-processing review, first editorial review, peer review, second editorial review and finally third editorial review.

Peer Review
All manuscripts submitted to Edorium Journals undergo anonymous, double-blind, external peer review.

Early View version
Early View version of your manuscript will be published in the journal within 72 hours of final acceptance.

Manuscript status
From submission to publication of your article you will get regular updates (minimum six times) about status of your manuscripts directly in your email.

Our Commitment

Six weeks
You will get first decision on your manuscript within six weeks (42 days) of submission. If we fail to honor this by even one day, we will publish your manuscript free of charge.

Four weeks
After we receive page proofs, your manuscript will be published in the journal within four weeks (31 days). If we fail to honor this by even one day, we will publish your manuscript free of charge and refund you the full article publication charges you paid for your manuscript.

Mentored Review Articles (MRA)
Our academic program “Mentored Review Article” (MRA) gives you a unique opportunity to publish papers under mentorship of international faculty. These articles are published free of charges.

Favored Author program
One email is all it takes to become our favored author. You will not only get fee waivers but also get information and insights about scholarly publishing.

Institutional Membership program
Join our Institutional Memberships program and help scholars from your institute make their research accessible to all and save thousands of dollars in fees make their research accessible to all.

Our presence
We have some of the best designed publication formats. Our websites are very user friendly and enable you to do your work very easily with no hassle.

Something more...
We request you to have a look at our website to know more about us and our services.

We welcome you to interact with us, share with us, join us and of course publish with us.

CONNECT WITH US

Edorium Journals: On Web
Browse Journals

This page is not a part of the published article. This page is an introduction to Edorium Journals and the publication services.