



EMERGING MOULD -RHYTIDHYSTERON RUFULUM AN ETIOLOGICAL AGENT OF SUBCUTANEOUS PHAEOHYPHOMYCOSIS – A CASE REPORT

Medical Microbiology

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ABSTRACT

Subcutaneous phaeohyphomycosis is a fungal infection caused by dematiaceous fungi which can cause cutaneous, subcutaneous & disseminated infections. Here, we report a rare case of subcutaneous phaeohyphomycosis due to a rare dematiaceous fungus *Rhytidhysterion rufulum*, in an older woman, who presented with swelling over the dorsum of the left foot. Direct examination of the aspirate's 10% potassium hydroxide wet mount revealed septate phaeoid fungal hyphae. On culture, grey velvety colonies with black pigments on the reverse were observed. The fungal growth was identified as *Rhytidhysterion rufulum* by Internal transcribed spacer (ITS) sequencing. She was treated by surgical excision, amphotericin B, terbinafine & itraconazole. Laboratory diagnosis of *Rhytidhysterion rufulum* by conventional methods is challenging, as it does not show typical sporulation. *Rhytidhysterion rufulum* species should be considered as one of the etiological agents for subcutaneous phaeohyphomycosis. Molecular methods should be used for the identification of unusual pathogens where conventional methods fail to identify the organisms.

KEYWORDS

Subcutaneous phaeohyphomycosis, *Rhytidhysterion rufulum*, dematiaceous fungi

INTRODUCTION

Subcutaneous phaeohyphomycosis is a fungal infection caused by melanized environmental fungi. Phaeohyphomycosis mainly involves the skin and subcutaneous tissue and rarely causes disseminated infections.¹

Common aetiological agents under phaeohyphomycosis include *Sporothrix*, *Acremonium*, *Exophiala*, *Phialophora*, *Cladosporium*, *Curvularia*, *Fonsecaea*, *Alternaria*, *Chaetomium*, *Exherhilum* and *Phaeoacremonium*. Rarely it can also be caused by *Ochroconis* and *Rhytidhysterion* species.² *Rhytidhysterion rufulum* occupies diverse habitats, predominantly found in tropical & subtropical environments. The first case of *Rhytidhysterion rufulum* from India was reported in 2008. Thereafter, few cases from India as well as from other regions have been reported.³⁻⁹ Cases reported from India are mostly from Chandigarh, Delhi and Puducherry.³⁻⁸

Here, in an apparently healthy woman, we report a case of subcutaneous phaeohyphomycosis caused by a rare saprophytic fungus, *Rhytidhysterion rufulum*. To the best of our knowledge, this might be the first case reported from western India.

CASE STUDY

A 56-year-old woman, a farmer by occupation, presented with complaints of swelling on the dorsum of the left foot for one month. There was no other associated complaint & no history of diabetes, hypertension, tuberculosis, immunosuppression or other comorbidities. The patient could neither recollect any history of trauma to the site of the lesion nor a history of similar swellings elsewhere in the body. On examination, gradually increasing subcutaneous swelling was present without any discharge or draining sinuses over the left dorsal foot (5x3cm) (Fig.1). The swelling was soft, non-tender, non-motile, and cystic.



Fig. 1 Swelling On The Dorsum Of The Foot

Routine hematological and biochemical parameters were within normal limits, HIV antibody was negative. Sputum examination was negative for acid-fast bacilli. Fine needle aspiration of the swelling gave thick purulent material, without granules. Gram's stain, acid-fast stain, Periodic acid–Schiff stain (PAS) and 10 % KOH mount were performed on aspirate. Gram's stain and acid-fast stain did not reveal any organisms. Degenerated inflammatory cells with necrotic background, and a few thick, long septate hyphae were observed on the PAS stain. 10% KOH mount showed long, thick, brown, septate hyphae (Fig.2). Gene Xpert was negative for mycobacterium tuberculosis. The aspirate was inoculated onto Sabouraud's dextrose agar (SDA) & incubated at 25°C & 37°C. After 8-10 days of incubation, it showed greyish black velvety colonies with dark black pigmentation on reverse (Fig.3). All attempts to induce sporulation proved ineffective on potato dextrose agar & corn meal agar. Hence isolate was sent to PGIMER, Chandigarh, for molecular identification. The fungal growth was identified as *Rhytidhysterion rufulum* by Internal Transcribed Spacer (ITS) sequencing.

The patient underwent surgical excision of the swelling with intralesional and intravenous amphotericin B for five days. After five days, she was shifted to oral terbinafine & itraconazole, which was continued for the next three months. She did not report any relapse or recurrence on follow-up.

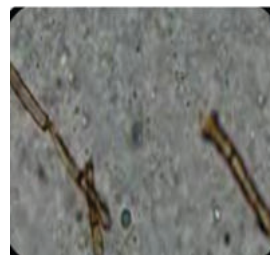


Fig. 2: KOH Mount Showing Septate Brown Pigmented Hyphae

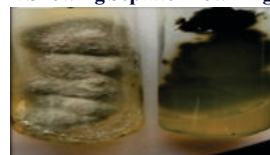


Fig.3 SDA Showing Growth On The Obverse And Pigment On The

Reverse Side

DISCUSSION

Subcutaneous phaeohyphomycosis is a chronic infectious disease characterised by hyperkeratotic nodular abscesses, nonhealing ulcers or sinuses.⁹ According to Revankar et al., the disease is classified into allergic superficial, deep local, pulmonary, central nervous system, and disseminated infections.¹⁰ It is more prevalent in immunocompromised patients but it can infect immunocompetent individuals also.¹¹

Rhytidhysterion species was first described by Spegazzini (1881) and belongs to the family Hysteriaceae. The genus has four species, *R. rufulum*, *R. hysterinum*, *R. opuntiae* and *R. dissimile*.¹² In India, Chowdhary et. al. reported the first human rare case of chromoblastomycosis in a 50-year-old male caused by *Rhytidhysterion rufulum* in renal transplant patients. In spite of itraconazole and supportive treatment, the patient died due to multiorgan failure. The predisposing factors for chromoblastomycosis could be impaired cell-mediated immunity because of immunosuppressive agents.³

Human infections with *Rhytidhysterion rufulum* are rare. These infections are acquired by traumatic implantation.⁹ It usually affects exposed parts. The severity of the disease also depends on the immune status and underlying predisposing factors. Certain genes of *Rhytidhysterion rufulum* express a mycotoxin, dothistromin, which lyses human red blood cells in vitro, which may explain its pathogenic potential on human cells.¹²

Cases have been reported from different parts of India. This might be the first case that is being reported from western India, in a 56-year-old immunocompetent woman. This infection is common in agricultural workers and gardeners because of the possibility of minor injuries that go unnoticed. All reported cases, from India were immunocompetent except one who had a history of renal transplant. Diabetes was observed in three cases of which one had hypertension as well as chronic renal disease.

Infection is more common in males. In the present case, painless, small nodule was observed at the site and became ulcerative and progressively increased in size. These lesions are often misdiagnosed as cutaneous tuberculosis. Diagnosis relies on histopathology and fungal culture. Histopathological diagnosis is by special stains such as periodic acid Schiff (PAS), Mayer's mucicarmine or Gomori methanamine silver (GMS) stain.⁹ It grows on Sabourauds dextrose agar but does not sporulate on sporulating media.⁹ Thus, it becomes challenging to identify this non-sporulating fungus by conventional methods. Therefore, molecular methods are of great help in the diagnosis of non-sporulating fungi. i.e. amplification of the conserved gene with polymerase chain reaction (PCR) followed by internal transcribed spacer (ITS) sequencing & BLAST searching in GenBank or other databases for sequence homology.¹³ Attempts at identification were made by other authors using ITS1 and ITS4 primer which were successful with approximately 98-100% homology with GenBank *Rhytidhysterion rufulum* sequences. MALDI-ToF mass spectrometry can be of help when database is augmented with in-house generated spectra.

There are no treatment guidelines available for *Rhytidhysterion rufulum*. Surgical debridement with antifungals for 4-6 weeks forms the mainstay of treatment. Amphotericin B, flucytosine, ketoconazole, and itraconazole are commonly used antifungals. Drainage, cryotherapy and laser therapy are other treatment options. Majority of the reported cases were treated with surgical excision and itraconazole. The present case was successfully managed by surgical intervention and antifungal treatment.

This case highlights the importance of a rare etiological agent of phaeohyphomycosis in an immunocompetent patient.

CONCLUSIONS

Pheohyphomycotic infections are on the rise in India as well as globally. These infections mimic tuberculosis and other granulomatous infections which delays the diagnosis and treatment. Molecular diagnosis is indispensable in the identification of these non-sporulating fungi. Further studies are needed to understand the pathogenesis and treatment of this saprophytic fungus which is emerging as a human pathogen.

REFERENCES

- Chander, J., Singla, N., Kundu, R., Handa, U., & Chowdhary, A. (2017). Phaeohyphomycosis Caused by *Rhytidhysterion rufulum* and Review of Literature. *Mycopathologia*, 182(3-4), 403-407. <https://doi.org/10.1007/s11046-016-0064-x>
- Queiroz-Telles, F., McGinnis, M. R., Salkin, I., & Graybill, J. R. (2003). Subcutaneous mycoses. Infectious disease clinics of North America, 17(1), 59-viii. [https://doi.org/10.1016/s0891-5520\(02\)00066-1](https://doi.org/10.1016/s0891-5520(02)00066-1).
- Chowdhary, A., Guarro, J., Randhawa, H. S., Gené, J., Cano, J., Jain, R. K., Kumar, S., & Khanna, G. (2008). A rare case of chromoblastomycosis in a renal transplant recipient caused by a non-sporulating species of *Rhytidhysterion*. *Medical mycology*, 46(2), 163-166. <https://doi.org/10.1080/13693780701630420>.
- Visakha K, Krishna Karthik M. (1017) A rare case of subcutaneous phaeohyphomycosis caused by *Rhytidhysterion rufulum*. *Indian J. Microbiol Res*, 4(4), 468.
- Sarita, Y., Ruchi, A., Jagdish, E., & Anuradha, C. (2017). A Rare Case Report of Subcutaneous Mycoses by *Rhytidhysterion Rufulum*. *International Journal of Medical Research and Health Sciences*, 6, 12-15.
- Mishra, K., Das, S., Goyal, S., Gupta, C., Rai, G., Ansari, M. A., Saha, R., & Singal, A. (2014). Subcutaneous mycoses caused by *Rhytidhysterion* species in an immunocompetent patient. *Medical Mycology Case Reports*, 5, 32-34. <https://doi.org/10.1016/j.mmcr.2014.07.002>
- Mudhigeti, N., Patnayak, R., Kalawat, U., & Yeddula, S. R. C. (2018). Subcutaneous *Rhytidhysterion* Infection: A Case Report from South India with Literature Review. *Cureus*, 10(4), e2406. <https://doi.org/10.7759/cureus.2406>
- Dingle, T. C., Jansen, B., Walker, C., Sam, M., Verity, B., Purdy, D., Paul, P., & Schwartz, I. S. (2022). Implantation subcutaneous phaeohyphomycosis caused by *Rhytidhysterion rufulum*: A case report. *Medical mycology case reports*, 36, 16-18. <https://doi.org/10.1016/j.mmcr.2022.03.002>
- Chester R, Cooper, JR. Deep Phaeohyphomycosis, Topley and Wilson's Microbiology and Microbial Infections, 1998 9th edn London Hodder and Stoughton (pg.724-732) In *Medical Mycology* v. 4
- Revankar, S. G., & Sutton, D. A. (2010). Melanized fungi in human disease. *Clinical microbiology reviews*, 23(4), 884-928. <https://doi.org/10.1128/CMR.00019-10>
- Ge, Y. P., Lv, G. X., Shen, Y. N., Li, M., Deng, S. W., De Hoog, S., Sameripitak, K., & Liu, W. D. (2012). First report of subcutaneous phaeohyphomycosis caused by *Ochroconis tshawytschae* in an immunocompetent patient. *Medical mycology*, 50(6), 637-640. <https://doi.org/10.3109/13693786.2011.653834>
- Kabir MS, Ganley RJ, Bradshaw RE. (2015) Dothistromin toxin is a virulence factor in dothistroma needle blight of pines. *Plant Pathol*, 64, 225-234.
- White, T.J. (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocols, a Guide to Methods and Applications, 315-322.