Original Research Paper

Volume - 11 | Issue - 09 | September - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

ISOLATION OF RHIZOPUS HOMOTHALLICUS FROM A PATIENT OF RHINO-ORBITAL CEREBRAL MUCORMYCOSIS : A RARE CASE REPORT.

Kanwar Bhanu	Postgraduate student, Department of Microbiology, DRPGMC, Tanda, Kangra (H.P.)
Sood Anuradha*	Associate professor, Department of Microbiology, DRPGMC, Tanda, Kangra (H.P.) *Corresponding Author
Gupta Veetheeanveshna	Postgraduate student, Department of Microbiology, DRPGMC, Tanda, Kangra (H.P.)
Rana Aditya	Postgraduate student, Department of Microbiology, DRPGMC, Tanda, Kangra (H.P.)
Thakur Daaman	Postgraduate student, Department of Microbiology, DRPGMC, Tanda, Kangra (H.P.)
Jaryal S.C	H.O.D. and professor, Department of Microbiology, DRPGMC, Tanda, Kangra (H.P.)

ABSTRACT We report a rare case of Rhino-orbital cerebral mucormycosis (invasive mucormycosis) caused by *Rhizopus homothallicus* in patient of uncontrolled diabetes mellitus. The organism was isolated from nasal scrapping and tissue obtained after surgery and was identified after fungal culture.

KEYWORDS : Rhino-orbital cerebral mucormycosis, invasive mucormycosis, Mucorales, *Rhizopus homothallicus*

Introduction :

Fungi belonging to the class Zygomycetes and order Mucorales are ubiquitous fungi often causes opportunistic infections. Mucormycosis are opportunistic fungal infection caused by the fungi belonging to order Mucorales, which causes devastating angioinvasive and life threatening infections in patients with underlying risk factors[1] with very high mortality rates even after active management[2-5]. It is common in immunocompromised host due to malignancy, immuno-suppressive therapy, steroid use, uncontrolled diabetes mellitus & diabetic ketoacidosis, neutropenia, lymphoma, autoimmune disorders,, illicit use of intravenous drugs, organ transplant recipients, desferoxamine therapy , prolonged antifungal treatment and breach of mucosal or cutaneous membrane barrier due to trauma, burns and surgical wounds[1,6].

These molds enter humans via respiratory tract or skin and rarely through gastrointestinal tract , eliciting an acute inflammatory response, invade blood vessels , cause extensive vessel thrombosis leading to ischemic tissue necrosis[2,6]. Infections due to *Rhizopus homothallicus* appear to be rare compared with those caused by other species of Zygomycetes[7]. We present a rare case report of rhino-orbital cerebral mucormycosis caused by *Rhizopus homothallicus.*

Case Report :

A 45 year old female patient, presented with pain and swelling over left eye for last 12 days along with discharge from left eye. She had accompanying severe headache. She was a newly diagnosed case of uncontrolled diabetes mellitus type II. The swelling was cystic, progressive with sudden onset along with history of decreased vision and inability to open eyes completely.

On local examination a clinical suspicion of orbital cellulitis with pansinusitis was made with probable cause may be mucormycosis .On laboratory investigations the blood sugar level(random) varied from 283-418 mg/dl and ketones bodies were detected in urine.HbA1C level varied between 13.1%-15.1% and serum ferritin level was 273 (range 4.63 – 204).

On radiological investigation, Magnetic resonance imaging(MRI), paranasal sinus and Orbit showed opacification and mucosal thickening in almost all paranasal sinuses with breech in the left lamina pypareacea and in left cribriform plate. There was erosion of anterior and posterior wall of the frontal sinus with soft tissue extension into anterior cranial fossa of left side. Surrounding frontal white matter was edematous. Proptosis of left eye with lateral displacement of the globe with features of left orbital cellulitis . There is enhancement of left optic nerve and optic nerve sheath.Overall imaging features were suggestive of invasive rhinosinusitis with intraorbital extension with left frontal infarct and a radiological diagnosis of Rhino-orbital cerebral mucormycosis was made.

Nasal scrapping were collected in normal saline and sent to Department of Microbiology for microscopic examination and fungal culture.

10% KOH mount on direct microscopic examination revealed plenty of broad aseptate ribbon like hyphae with width of 8-10µm and wide angled branching.

Surgery (left external ethmoidectomy and Functional endoscopic sinus surgery) was performed. The mucosal scrapping and tissue biopsy specimen was obtained, sent to department of Microbiology and Pathology. Histopathological examination of the tissue obtained post surgery showed necrotic areas revealling aseptate broad fungal hyphae and few yeast forms, which were supportive of oppurtunistic fungal infection ,possibly Zygomycetes.

The samples were cultured on two sets of Sabouraud's dextrose agar(SDA) tubes ,each of which included SDA plain and SDA with gentamycin but without cycloheximide (actidione).One set was incubated at 25^0C and the other at 37 ^{0}C . After 3 days of incubation, fungal growth was obtained in both tubes incubated at 25^0Cand 37^0C. The isolates was thermotolerant and were able to grow when incubated up to 48^0C.The growth was cottony white with no pigmentation on reverse side of tubes. The colonies turned grey after prolong incubation.The colonies were teased and stained with lactophenol cotton blue (LCB).

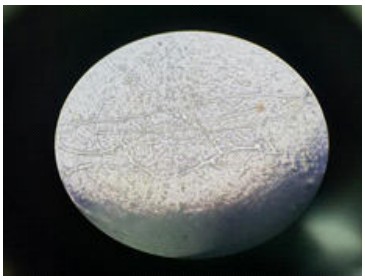
Microscopic examinations showed broad (8-10µm in size) hyaline, aseptate, wide branching hyphae with rhizoids tufts from which arose only few unbranched sporangiophores (100-115 µm) with columella of various shapes.

A slide culture was put up for species identification. Simultaneously , water agar method was done to induce sporulation in which SDA blocks (1cm×1cm) with growth were cut and transferred,taking all aseptic precautions to a plate containing 20ml of sterile distil water with few drops of 10% filter sterilized yeast extract.

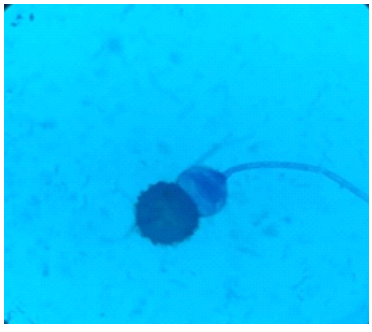
LCB stained preparation showed large number of dark brown zygospores measuring 60-100µm in diameter with stellate spines on their walls, developed after slide culture. Detached zygospores which were tuberculate was also seen. The zygospores showed unequal suspensor cells. After 3 days of incubation at 37^0C in water agar, plenty of zygospores were seen on LCB stain preparation.

Based on the these characteristic features, the isolates was identified as *Rhizopus homothallicus.* Patient was put on intravenous liposomal amphotericn B , 50mg intravenous once daily for ten days and showed improvement.

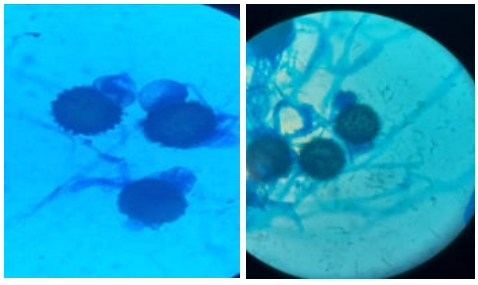
10% KOH MOUNT (40X):



LCB (40X)



LCB (40X): Zygospores **LCB (40X) : Zygospores**



Discussion :

The Mucorales belong to a distinct phylum called Glomeromycota[8] and are characterised by the formation of broad, ribbon like ,hyaline, aseptate hypahe and sexual reproduction with formation of zygospores[9].The pathogenic species cause acute angioinvasive infections which are fatal especially in the immunocompromised patients. Other predisposing factors include poorly controlled diabetes mellitus, haematological malignancies, trauma, neutropenia, iron chelation by desferoxime, corticosteroid use, protein calorie malnutrition and neonatal prematurity[10]. Mucorales after entry to susceptible host lead to angioinvasion, vessel thrombosis and tissue necrosis. Elevated levels of serum glucose, , iron and ketone bodies increase fungal growth resulting in increased ability of Rhizopus to invade host tissues and explaining the susceptibility of diabetic patients to mucormycosis[4]Rhino-orbital cerebral mucormycosis which represents 39% of infections by Mucorales, is common in patients of diabetes mellitus[4]. Rhizopus spp. are the most common agents causing human infection followed by Lichthemia and Mucor. Among Rhizopus , *Rhizopus arrhizus* is the predominant agent of invasive mucormycosis[7]. Colonies of Rhizopus are rapid growing and have coarse and floccose aerial mycelia with pigmented rhizoids, brown unbranched sporangiospores and terminal globose sporangia bearing numerous sporangiospores[9,12]. Zygospores are usually formed between oppositely oriented stains or heterothalic strains.They are large of size 40-104µm , bright brown to dark yellow brown in color and have stellate , spiny projections on the walls[9]. *R. homothallicus* an environmental isolate has been increasingly reported to cause invasive infections which includes pulmonary mucormycosis, rhino-orbital cerebral mucormycosis and cutaneous mucormycosis in six patients in India[13-17]and one case of pulmonary mucormycosis in France[18].The diagnosis of *Rhizopus homothallicus* was made due to presence of zygospores which is its sexual spore form with a single suspenser cell.

Though the diagnosis of mucormycosis depend on clinical, radiological findings and confirmed on histopathological and culture. It requires intensive efforts from the Microbiology laboratory for diagnosis. Management of invasive mucormycosis include antifungal therapy, surgery and control of underlying disease like diabetes mellitus & reduction in steroid/immunosuppressive therapy.

This case report highlights the need of Mycology expertise required for diagnosis and subsequent management of cases. As far as our knowledge goes this case is unique as *Rhizopus homothallicus* is very rarely reported worldwide as causative agent of rhino-orbital cerebral mucromycosis which being an emergency situation and antifungal therapy has to be initiated at the earliest to save patient's life. In addition, this is the first time we have isolated *Rhizopus homothallicus* from any clinical sample.

Conclusion :

Mucormycosis is a life threatening fungal infection causing angioinvasion and tissue necrosis and is associated with an increasing incidence and mortality. *Rhizopus homothallicus* , a rare fungi previously reported to be cause of pulmonary mucormycosis, is now a emerging cause of rhino-orbital cerebral mucormycosis.

Declaration of Conflicting Interests:

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding :

The author(s) received no financial support for the research, authorship, and/or publication of this article.

REFERENCES :

1. Chakrabarti A, Singh R. The emerging epidemiology of mould infections in developing countries. Curr opin infect Dis 2011 ; 24: 521-6.
2. Chakrabati A, M.D. epidemiology of mucormycosis in India. Curr Fungal Infect Rep 2013; 7: 287-92.
3. Chakrabati A, Chatterjee SS, Das A e*t al* .Invasive zygomycosis in India; experience in a tertiary care hospital. Postgrad Med J 2009;85 : 573-81.
4. Chakrabati A,Das A, Mandal J *et al.* The rising trend of invasive zygomycosis in patients with uncontrolled diabetes mellitus. Med Mycol 2006; 44: 335-42.
5. Chakrabati A,Das A, Sharma A *et al* . Ten years experience in zygomycosis at a tertiary care centre in India. J Infect 2001;42: 261-6.
6. Mantadakis E, Samonis G. Clinical presentaion of Zygomycosis. Clin Microbiol Infect 2009 ; 15(suppl.5): 15-20.
7. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, *et al*.(2005) Epidemiology and outcome of Zygomycosis: a review of 929 reported cases. Clin Infect Dis 41: 634-653.
8. Kwon-Chung KJ. Taxonomy of fungi causing mucormycosis and entomophthoramycosis (mucormycosis) and nomenclature of the disease : molecular mycologic perspective. Clin Infect Dis. 2012;54(Suppl 1): S8-15.
9. Ribes JA. Vanover-Sams CL. Baker DJ. Mucorales in human disease. Clin Microbiol Rev. 2000; 13:236-301.
10. Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis. 2012;54 (Suppl 1): S23-34.
11. Baldin C, Ibrahim AS (2017) Molecular mechanisms of mucormycosis- The bitter and the sweet. PLoSPathog 13: e1006408.
12. Schipper MAA. A revision of the genus Rhizopus.I. The Rhizopus stolonifer group and R. oryzae. Stud Mycol.1984;25:1-19.
13. Chakrabati A, Marak RS, Shivaprakash MR, Sunita G, Rajiv G, *et al*.(2010) Cavitary pulmonary zygomycosis caused by *Rhizopus homothallicus*. J Clin Microbiol 48: 1965-1969.
14. Kokkayil P, Pandey M, Agarwal R, Kale P, Singh G, *et al* .(2017) *Rhizopus homothallicus* Causing Invasive Infections : Series of Three Cases from a Single Centre in North India . Mycopathologia 182 : 921-926.
15. Chander J, Kaur M, Singla N, Punia RS, Singhal SK, *et al* .(2018) Mucormycosis: Battle with Deadly Enemy over a Five-Year Period in India. J Fungi (Basel) 4:46.
16. Compain F, Ait-Ammar N, Botterel F, Gibault L, Le Pimpec Barthes F, *et al* .(2017) Fatal Pulmonary Mucormycosis due to *Rhizopus homothallicus.* Mycopathoolgia 182: 907-913.