**INTRODUCTION**

Mycelial fungi (molds) are ubiquitous organisms found in soil, water, and decaying vegetation. Respiratory tract is the most common route of entry, with subsequent haematogenous dissemination to the central nervous system (CNS). Direct inoculation of CNS or paraspinal tissue as a result of surgery, trauma, intravenous drug use, or contaminated medical supplies may also occur in immunocompetent persons. Phaeohyphomycosis is an uncommon but frequent fungal infection mainly due to neurotropic black fungi belonging to the ascomycete order Chaetothyriales: Cladophialophora species, *Rhinocladiella* species and *Exophiala dermatitidis*. The infection may occur in immunocompetent patients following inhalation of conidia. A high proportion of primary cerebral and intracranial infections is reported in apparently immunocompetent individuals without any obvious predisposing factors. Mortality may be as high as 100% within weeks, months, or years if left untreated. For treatment of cerebral phaeohyphomycosis, in vivo and in vitro studies and single cases suggest that itraconazole, voriconazole and posaconazole may provide better outcome. *Rhinocladiella* species are found to be resistant to Amphotericin B.

**ABSTRACT**

Phaeohyphomycosis is a rare but frequently fatal fungal infection caused by neurotropic black fungi belonging to the ascomycete order Chaetothyriales: Cladophialophora species, *Rhinocladiella* species and *Exophiala dermatitidis*. An 8 year boy old presented with recurrent headache and fever, lumbar puncture done thrice over the course of 3 months. CSF cytology was consistent with lymphocytic pleocytosis, raised protein and hypoglycorrhachia. CSF was subjected to panfungal DNA detection by PCR followed by fungal identification by DNA sequencing. The assay targets multicopy genes, the ribosomal DNA (rDNA) genes (18S, 28S, and 5.8S) and the intervening internal transcribed spacer (ITS) regions (ITS1 and ITS2). Initial CSF sample submitted identified the etiologic agent as *Rhinocladiella similis* since it presented sequence identity at 99% and coverage at 100% with total score of 1000. As *Rhinocladiella* species are rare cause of chronic recurrent lymphocytic meningitis we had requested to submit repeat CSF specimens. Subsequent CSF sample submitted, identified the same etiologic agent on DNA sequencing. Here we present an unusual case of *Rhinocladiella similis*, a rare cause of chronic recurrent lymphocytic meningitis.

**CASE REPORT**

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**ARTICLE INFO**

Article History:
Received 5th January, 2018
Received in revised form 19th February, 2018
Accepted 24th March, 2018
Published online 28th April, 2018

Key words:

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course of 3 months was consistent with tubercular aetiology in the form of lymphocytic pleocytosis, raised protein and hypoglycorrhachia. Serial CSF examination did not show any improvement in the laboratory parameters. On clinical examination, heart rate - 90/min, respiratory rate - 18/min, weight- 39.4 Kg. Systemic examination, S1 and S2 +, no murmur, chest was clear. On neurological examination, GCS score 15/15, conscious, interactive and playful, speech normal, cranial nerve examination was normal. Fundus examination showed no papilledema, no ptosis and nystagmus, pupils were bilaterally equal and reacting to light, no facial asymmetry found. Motor system examination showed power 5/5 in all four limbs. No cerebellar signs. Serological markers like HBsAg, HCV and HIV were non-reactive. Haemoglobin 13.9 Gms/dl, Total count 9100 cells/mm³ with lymphocytes 62%, eosinophils 2%, and neutrophils 36%. Prothrombin time 14.9 seconds with INR 0.90. Random glucose 75 mg%, serum uric acid nitrogen 9 mg%, serum potassium 3.3 mEq/L, serum sodium 135 mEq/L, Serum creatinine 0.44 mg/dL, SGOT 14 IU/L. SGPT 30 IU/L, total bilirubin 0.3 mg%, total proteins 7.2 gm%, serum LDH 195 IU/L, creatinine phosphokinase 34 IU/L, calcium 9.3 mg/dL. TSH 3.64 mIU/ml, FT3 5.81 pmol/L, FT4 15.33 pmol/L. SGPT 30 IU/L, total bilirubin 0.3 mg%, total proteins 7.2 gm%, serum LDH 195 IU/L, creatinine phosphokinase 34 IU/L, calcium 9.3 mg/dL. TSH 3.64 mIU/ml, FT3 5.81 pmol/L, FT4 15.33 pmol/L. CSF VDRL was Non-reactive, India ink preparation did not show any capsulated yeast. Serum Lyme Borrelia burgdorferi IgM and IgG antibody were negative. ANA profile negative, Anti ds DNA, APLA, ANCA negative, serum Angiotensin converting enzyme (ACE) level 13.9 U/L, tumour markers CA 19.9, Serum AFP and serum LDH levels were 198 IU/L. MRI of Brain was essentially normal except for small evidence of old blood in the form of blooming in the cerebellar folia. MRI spine screening was normal. Digital subtraction angiography study showed no significant abnormality in intracranial vessels. No evidence of steno occlusive lesion or aneurysm seen. On neuropsychology evaluation on the Wechsler Intelligence Scale for Children (WISC) he obtained Full Scale IQ (FSIQ) of 112. CSF cytological evaluation showed total count of 2 cells/mm³, no polymorphs, no RBCs with 100% lymphocytes (lymphocytic pleocytosis). CSF glucose- 41 mg%, protein- 39 mg%, chloride- 720 mg%. Routine aerobic and tuberculosis liquid culture were sterile on 42 days of incubation. CSF adenosine deaminase (ADA) levels were 0.1 U/L. Nucleic acid amplification tests like Gene x pert and TB PCR, CMV, HIV, enterovirus and herpesvirus PCR were negative. CSF was subjected to pan fungal DNA sequencing following DNA extraction.

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<td>30 cycles</td>
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The assay targets multicity genes, the ribosomal DNA (rDNA) genes (18S, 28S, and 5.8S) and the intervening internal transcribed spacer (ITS) regions (ITS1 and ITS2). Initial CSF sample submitted identified the etiologic agent as Rhinocladiella similis since it presented sequence identity at 99% and coverage at 100% with total score of 1000. As Rhinocladiella species are rare cause of chronic recurrent meningitis we had requested to submit repeat CSF specimens. Subsequent CSF samples submitted identified the same etiologic agent on pan fungal DNA sequencing following DNA extraction.

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deeply invasive infections (phaeohyphomycosis), including infections of the central nervous system. Most of the agents causing phaeohyphomycosis grow very slowly on routinely used fungal culture media. Conventional mycologic identification can take around 3 weeks or longer. In comparison with aspergillus, mucorales, and fusarium, dematiaceous molds commonly cause infections of the CNS in immunocompetent hosts. Some dematiaceous molds within a narrow geographic range cause cerebral phaeohyphomycosis. Clade Exophialia spinifera contains several morphologically and genetically identical species, being Rhinocladiella similis and one of them as an agent of cerebral phaeo-hymycomycosis and chromoblastomycosis. With recent advances in the molecular biology techniques, it has been possible to identify and characterise these pathogenic molds based on DNA-DNA hybridization7,8. Conventionally, fungal identification is based on observation of microscopic characteristics, morphological identification on slide culture, pigment production and temperature variation, This has led to incorrect and prolonged fungal identifications8. It is not always possible to identify the fungus at a species level based on morphological characteristics. Some of the fungi are common laboratory contaminants leading to under or overestimation of the number of infective cases as there is lack of confidence in reporting such fungi in routine clinical practice. Panfungal DNA detection is a PCR based test for detection of fungi by panfungal PCR followed by fungal identification by DNA sequencing. The assays targets multiplicity genes, the ribosomal DNA (rDNA) genes (18S, 28S, and 5.8S) and the intervening internal transcribed spacer (ITS) regions (ITS1 and ITS2).

**Conventional PCR:** Polymerase Chain Reaction (PCR) is a molecular biology technique that allows for quick replication of DNA. With PCR, minute quantities of genetic material can be amplified millions of times within a few hours allowing for the rapid and reliable detection of infectious agents. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase (after which the method is named) are key components to enable selective and repeated amplification. To check whether the PCR has generated the anticipated DNA fragment, agarose gel electrophoresis is employed for size separation of the PCR products. The size(s) of PCR products is determined by comparison with a DNA ladder (a molecular weight marker), which contains DNA fragments of known size, run on the gel alongside the PCR products.

**DNA Sequencing:** DNA sequencing is the determination of the precise sequence of nucleotides in a sample of DNA or PCR product. DNA sequencing has been performed using the chain termination method developed by Frederick Sanger. It involves addition of deoxy nucleotides (ddNTPs- synthetic nucleotides that lack the -OH at the 3’ carbon atom), which gets incorporated along with dNTPs to a growing DNA strand and stops elongation because there is no 3’ -OH for the next nucleotide to be attached to. At the end of the cycling reaction, there are various products of different lengths tagged to fluorescence labeled ddNTPs. The fragments are separated by length from longest to shortest on a DNA sequencer. Presence of amplified product in the range of 350bp –880 bp indicates detection of fungal DNA. Fungal identification is done by sequencing of amplified product followed by BLAST analysis with the sequences in the GeneBank Database. Absence of amplimer indicates absence of Fungal DNA in the given specimen. Absence of an internal control as well as fungal DNA indicates an invalid result. The frequency of invasive fungal infections (IFIs) in critically ill and immunocompromised patients is continuing to increase and the spectrum of fungal pathogens has expanded well beyond Aspergillus fumigatus and Candida species. Early, rapid, and accurate identification of pathogenic fungi is important in order to guide the selection of appropriate antifungal therapy and thus improve patient outcomes, as well as for epidemiologic purposes. Culture-independent methods such as PCR can offer sensitive and specific diagnosis of viable and nonviable fungal pathogens in a variety of clinical specimens. The assay can detect clinically important fungi such as Candida, Cryptococcus, Aspergillus, Saccharomyces etc. Currently, in research, the identification based on sequencing of the Internal Transcribed Spacer (ITS) region of ribosomal DNA has been considered a reliable source of identification for this clade, but it has not been frequently used in clinical practices. Using the cutoff proposed by clinical laboratory standard institute (CLSI)10 the isolate was considered sensitive to posaconazole and itraconazole. Itraconazole was considered to be drug of choice in such cases of invasive fungal infections. Amphotecin B is considered to have higher MFCs, not used for rhinocladiella species. The studies which have isolated R. Similis and R. aquaspora as an agent of cutaneous chromoblastomycosis have reported that the response was intermediate to voriconazole and resistant to amphotericin B. For terbinafine and ketoconazole, there is no reference of cutoff to estimate minimum fungicidal concentrations (MFC). The MICs of posaconazole and voriconazole against R. similis were similar to those of both antifungals against R. Aquaspera11, 12. In the present case, itraconazole was chosen as the initial treatment of choice in a dose of 10 mg/kg/day in three divided doses along with caspofungin in the loading dose of 70 mg followed by 30 mg four times a day for 21 days of inpatient admission. With this treatment there was improvement in both clinical and lab parameters. On discharge patient has been prescribed oral itraconazole 200 mg twice day for 6 months with follow up every month till 6 months. The duration of treatment with itraconazole is variable, but a range from 8 to 10 months has been reported in many case studies13. This case report present R. similis as a agent an of chronic recurrent lymphocytic meningitis. This species could be more resistant to antifungals such as amphotericin B. The duration of treatment in such cases is prolonged and combination of antifungals should be considered in view of drug resistance following monotherapy. In addition, this report also stress the importance of species level identification by using pangungal DNA PCR and DNA sequencing as conventional methods lack sensitivity.

**Acknowledgements**

We are thankful to the Department of Microbiology, Dept of Molecular Biology, Metropolis Healthcare Limited. Mumbai, India-

**Conflict of Interest**

Author declares no conflicts of interest

**Funding**

No source of funding has been received
Rhinocladiella Similis A Rare Cause of Chronic Recurrent Lymphocytic Meningitis - A Case Report

Consent
Written informed consent has been taken

Guarantor
First (corresponding) and Second author

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How to cite this article:
DOI: http://dx.doi.org/10.24327/ijcar.2018.12064.2113

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