Rare Case of Meningoencephalitis Due to *Aspergillus* and *Acanthamoeba* Coinfection

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ABSTRACT: *Aspergillus* and *Acanthamoeba* cause central nervous system (CNS) infection mostly in immunocompromised hosts, disseminating via the hematogenous route from a primary focus in the respiratory tract. We are presenting a case of an immunocompetent patient diagnosed with *Aspergillus* and *Acanthamoeba* coinfection of the CNS, a rare finding. A positive cerebrospinal fluid (CSF) galactomannan antigen test confirmed the presence of *Aspergillus*, and direct visualization in CSF sample and culture confirmed the presence of *Acanthamoeba*.

KEYWORDS: *Aspergillus*, *Acanthamoeba*, CNS

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Introduction

Aspergillus is an omnipresent saprophyte organism in soil, water, and decomposing vegetation. It is second only to *Candida* in the incidence of opportunistic mycosis among immunocompromised hosts. This fungus gains entry into the body through the respiratory tract and paranasal sinuses.[1] The central nervous system (CNS) invasion follows direct inoculation into a region anatomically near to the brain or by hematogenous dissemination. Meningitis is infrequent in aspergillosis. Brain abscesses are common in disseminated aspergillosis.[2] Extended neutropenia and the use of high dose of corticosteroids are the key predisposing factors, particularly in patients with solid organ transplant and malignancies.[2,3] However, infection can occur in an immunocompetent human.[4]

Similar to CNS *Aspergillus* infection, cases of granulomatous amoebic encephalitis (GAE) due to *Acanthamoeba* species (*A. castellanii*, *A. polyphaga*, *A. culbertsoni*, and probably other amoebas) have also been documented in chronically ill and debilitated or naturally or iatrogenically immunologically suppressed individuals with severe derangements of the host defenses. In cases of GAE, the route of invasion of amoebas into the CNS is hematogenous, probably from a primary focus in the lower respiratory tract or the skin. The virulent *Acanthamoeba* organisms may enter the respiratory tract via inhaled air, aerosols, and dust containing cysts or trophozoites.[5]

We report a unique case of *Aspergillus* and *Acanthamoeba* coinfection of CNS in an immunocompetent patient.

Case Report

A male nonsmoker in his 30s was admitted to Kasturba Hospital, Manipal, with chief complaints of headache, blurring of vision, projectile vomiting, and double vision since 15 days.

On query, the patient gave history of left-sided nose bleed four years back for which he underwent Functional Endoscopic Sinus Surgery (FESS). Histopathology showed aspergillus sinusitis for which the patient was started on tablets. Itraconazole 200 mg was given 6 tablets/day. Computed tomography (CT) brain done three years back was normal. One year later, the patient returned with complaints of recurrent sinusitis, nose bleed, swelling of left cheek, loosening left upper teeth, and bleeding from the left upper gums. The patient was referred for dental checkup where radiograph revealed fungal mass in the left maxillary sinus. On investigation, hemoglobin was 19.3 gm% and hematocrit was 57.8%. Systemic examination revealed blood pressure of 160/100 mm Hg and inferior turbinate hypertrophy. Patient was evaluated for polycythemia, which showed normal arterial blood gas, no splenomegaly, and JAK 2 mutation was negative. Polycythemia was considered in view of raised hemoglobin and hematocrit, and subsequently, blood venesection was done twice. CT paranasal sinus showed growth in the left maxilla. Nasal swab was sent to the microbiology department for culture, which grew *Aspergillus flavus*. Biopsy was done and histopathological examination showed noncaseating granuloma composed of epitheloid histiocytes and langhans

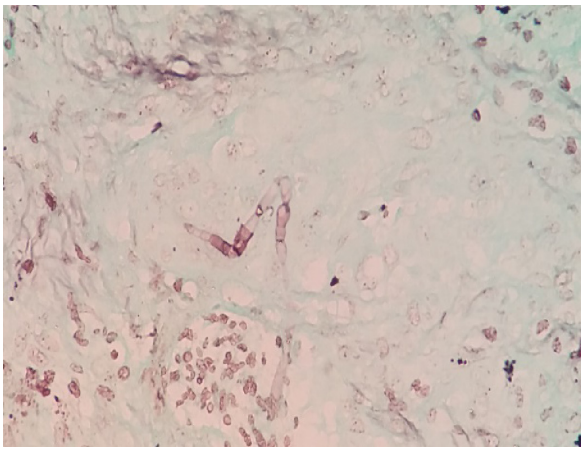

Figure 1. Gomori methenamine silver staining of tissue section showing retractile, septate, branching hyphae with histiocytes and foreign body giant cells.

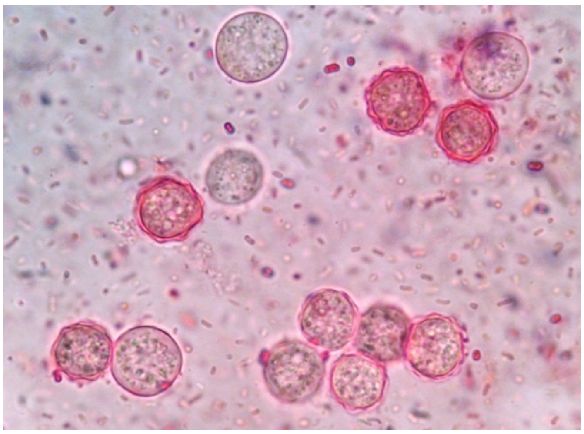

Figure 2. Showing star shaped cysts of *Acanthamoeba* species in culture.

and foreign body giant cells with intractoplasmic refrective (PAS and GMS stain) positive septate, occasionally branching hyphae suggestive of chronic invasive *Aspergillus* sinusitis (Fig. 1). The patient was advised left radical maxillectomy and was started on voriconazole. He took voriconazole for three months and later went for ayurvedic therapy because of financial constraints. One year later, left radical maxillectomy was done.

After surgery, the patient presented with symptoms of bilateral proptosis, more so in the left eye with restriction of left eye movement inferiorly. On CNS examination, no signs of meningeal irritation were present. No abnormality was detected on examination of cardiovascular system, respiratory system, and abdomen. The following laboratory investigations were done as shown in Table 1.

Ophthalmology consultation was sought and opined as bilateral papilledema with right eye showing enlarged blind spot and left eye showing constriction of visual field suggestive of optic nerve involvement. Provisional diagnosis of hypertensive encephalopathy was made, and patient was started on intravenous central nervous system antiedema measures (intravenous mannitol + intravenous Lasix). Oral beta blockers, calcium channel blockers, and angiotensin receptor blocker (ARB) were started for hypertension. Neurology consultation was taken, where it was advised to rule out meningitis with CSF analysis and magnetic resonance imaging (MRI) of the brain. Initial analysis showed that the CSF was clear and sterile, cultures and test for

Table 1. Laboratory parameters.

	AT THE TIME OF INITIAL HOSPITAL ADMISSION	TWO WEEKS AFTER INITIAL ADMISSION
Haemoglobin (gm%)	17.6	15.7
Haematocrit (%)	53.4	47
Total leucocyte count (/µl)	12,000	18,300
Neutrophils (%)	77.4	80
Lymphocytes (%)	13.4	12
Monocytes (%)	6.1	5
Platelet count (/µl)	295,000	418,000
ESR (mm)	5	2
Random blood sugar (mg/dl)	155	
Serum sodium (mmol/L)	136	124
Serum potassium (mmol/L)	5	4.4
Serum urea (mg/dl)	23	25
Serum creatinine (mg/dl)	0.9	0.8
Urine routine	Normal	
HIV status	Negative	

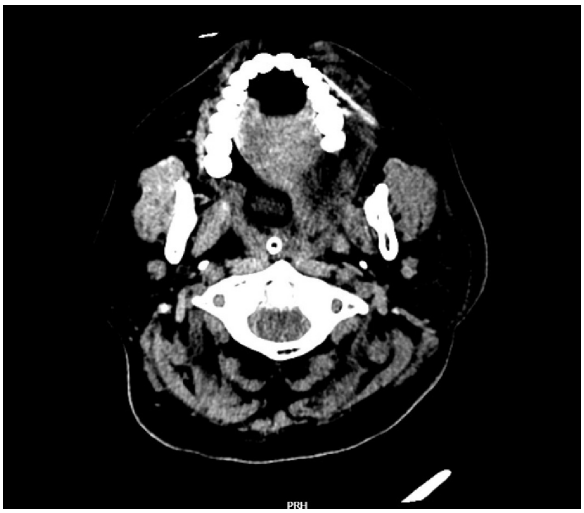

Figure 3. CT scan showing diffuse involvement of bilateral cerebellar hemispheres and tonsillar herniation; hydrocephalus.

cryptococcal antigen were negative, and MRI of the brain was normal (Fig. 4).

Two weeks after initial admission, the patient was readmitted with symptoms of headache, giddiness, and vomiting. The patient was started on IV ceftriaxone, IV fluids, antihypertensives, antiedema (Lasix + mannitol), vertin (meclizine), and stugeron (cinnarizine). Diagnostic and therapeutic CSF tap was done to relieve symptoms of papilledema; the results are shown in Table 2.

Based on high CSF eosinophil counts, two possibilities of eosinophilic meningitis and parasitic infestation of the brain were considered. The initial total leukocyte count of 400 cells/mm^3 with RBC count of 27,200 cells/mm^3 was not consistent with meningitis. This may be because of correction

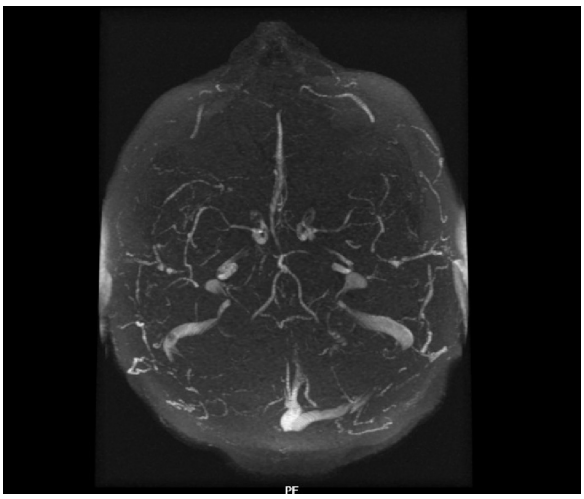

Figure 4. Noncontrast MR venography of brain: All the dural venous sinuses and major cerebral veins show normal flow-related hyperintense signals. No dural venous sinus/major cortical or cerebral venous thrombosis is seen.

of WBC count based on high RBC count. Thus, a repeat CSF analysis was done as shown in Table 2. CSF parasitic culture was requested in view of high eosinophil count. On CSF microscopy, suspected structures resembling ameba were present. Subsequently, sample was cultured on non-nutrient agar coated with *Enterobacter* spp. After an incubation for three to four days at room temperature, trophozoites and cysts of *Acanthamoeba* were seen (Fig. 2). This confirmed the presence of *Acanthamoeba* in CSF for which the patient was started on intravenous liposomal formulation of amphotericin B (5 mg/kg). Intravenous ceftriaxone, antihypertensives, and antiedema measures were continued. Three days after starting amphotericin B, kidney injury set in (creatinine 2.1 mg/dL), and so the drug was withheld. Neurology opined to add rifampicin in view of *Acanthamoeba* and advised to repeat HIV and CD4 count, with no other tests performed to ascertain the immune status of the patient.

If the CSF galactomannan test was positive then voriconazole 200 mg IV, 12 hourly, was started. After three days, renal failure improved and amphotericin B was added. Repeat CSF results are shown in Table 2. Repeat CSF culture showed persisting *Acanthamoeba* with further clinical deterioration of the patient with symptoms of drowsiness, altered sensorium, and right-sided hemiparesis. Patient was started on intravenous cotrimoxazole and tablet flucytosine. Lumbar drain was inserted. Ventriculoperitoneal (VP) shunt was performed. CT brain reported obstructive hydrocephalus (Fig. 3). Hypotension was refractory to IV fluids, and IV inotropes were added. External ventricular drain (EVD) placement was done in right Kocher's point and CSF drained under high pressure. After two weeks of continued management, patient finally succumbs.

Discussion

CNS *Aspergillus* infection can be a consequence of hematogenous dissemination from primary focus in the lungs. It can also be initiated by direct inoculation into the bloodstream via the middle ear, paranasal sinuses, eye, or mastoid, or as a result of illicit drug use, or following open heart surgery.[6] *Aspergillus fumigatus* is recognized as the most frequent species to cause invasive infection. *A. flavus* is considered as the agent of a great part of infections in paranasal sinus.[7] Although *A. terreus* is an unusual cause of CNS invasion, it is amphotericin B resistance, and consequently related with high mortality rate.[8]

CNS infection by *Aspergillus* is more frequent in patients with predisposing factors like leukemia, lymphoreticular neoplasms, those on long-term steroid therapy, large spectrum-antibiotics, cancer chemotherapy, and immunosuppressant drugs following renal or cardiac transplantation. More recently, CNS *Aspergillus* infection is a well-documented complication in patients with acquired immunodeficiency syndrome. Surprisingly, our patient was immunocompetent with none of the documented predisposing factors.

Aspergillus infections can be grouped in two types: localized or disseminated. Disseminated infections usually occur in

Table 2. CSF analysis.

	INITIAL CSF BIOCHEMICAL AND CULTURE RESULTS AFTER READMISSION	REPEAT CSF BIOCHEMICAL AND CULTURE RESULTS	CSF BIOCHEMICAL AND CULTURE RESULTS AFTER TREATMENT WITH AMPHOTERICIN B	CSF BIOCHEMICAL AND CULTURE RESULTS AFTER TREATMENT WITH AMPHOTERICIN B AND METRONIDAZOLE
Macroscopic appearance	Reddish (traumatic tap)	Clear		
WBC (cells/cumm)	400	200		300
RBC (cells/cumm)	27,200	2		
Neutrophils	51%	4%		51%
Lymphocytes	32%	19%		49%
Monocytes		1%		
Eosinophils	17%	73%		
Basophils		3%		
Adenosine deaminase	10 U/L (normal range: 0–5 U/L)			
Glucose, protein, chloride and lactate	Within normal limits			
Fungal culture	Negative	Negative		
Cryptococcal antigen test	Negative			
Galactomannan test		Positive		
Culture for parasite			Positive for *Acanthamoeba*	Positive for *Acanthamoeba*

immunocompromised hosts and involve the CNS in 40%–60% of cases. Invasion of the CNS occurs either by direct extension from an area adjacent to the brain or by hematogenous route. In some patients, the mechanism of CNS penetration is unclear. The direct extension to the brain may occur from the adjacent structure, ie, ear, nose, paranasal sinuses, orbit, and sphenoid sinus or following head trauma. The present case had a history of *Aspergillus* infection of maxillary sinus, which could be a probable mechanism of CNS penetration of the fungus. Iatrogenic introduction of *Aspergillus* into the CNS have also been reported following spinal tap, craniotomies, and after implantation of radioactive yttrium for pituitary ablation.[9,10]

CNS aspergillosis can present as subcortical hemorrhagic infarcts in cerebral hemispheres, abscesses, granulomas, mycotic aneurysms, and meningitis. The subcortical infarctions, hemorrhage, and mycotic aneurysms indicate vascular invasion of small and large cerebral vessels, resulting in thrombosis or destruction of the internal elastic lamina of cerebral arteries.[9] Brain abscesses present as multiple lesions with hyphae, a focal mixed polymorphonuclear and mononuclear reaction, and usually occur in the frontal and temporal lobes. Granuloma formation is seen in patients who have had longstanding aspergillosis, predominantly if the lesions are limited to the CNS and/or adjacent paranasal sinuses or orbit.[11] Spinal cord compression has also been recorded. Meningitis as the predominant clinicopathological process has been reported in only a few cases.[11]

As seen in our case, there are no pathognomonic features in CSF examination in patients with CNS aspergillosis. CSF is usually clear, with normal or increased opening pressure; the glucose level may be normal or depressed, the protein is elevated (>100 mg/dL), and pleocytosis is usually less than 600 cells/mm^3 with a variable distribution of polymorphonuclear and mononuclear cells. Positive CSF cultures to *Aspergillus* are rare.[12] *Aspergillus* rarely invades the meninges, and the absence of diffuse meningeal involvement might be the reason for relative paucity of CSF findings.[13]

Diagnosis of CNS aspergillosis is challenging. Positive CSF culture gives a direct diagnosis; however, they are generally negative. Therefore, other tests may be helpful in the diagnosis of this life-threatening pathology. Morrow et al[14] described the presence of antibodies by double immunodiffusion in two out of three patients, who had negative cultures. These patients were cured after treatment with antifungal drugs. Western blot analysis can be used for detection of *Aspergillus* antigen in CSF. The presence of prominent 110 KD band can be a prognostic marker in fatal aspergillosis.[15] In the present case, CSF was positive for galactomannan antigen. There have been false-positive *Aspergillus* galactomannan tests in patients on intravenous fluids containing gluconate or citric acid, such as transfusion platelets, parenteral nutrition, or PlasmaLyte. The present patient did not receive any of the above therapies, thus excluding any possibility of false-positive test results.

The treatment of choice for CNS meningitis is amphotericin B or amphotericin plus flucytosine.[16] However, successful treatment is not the rule, perhaps reflecting a late diagnosis or may be related to poor response to antifungal drug.[16] This was one of the most probable reasons for treatment failure in our case. The use of intrathecal or intralesional antifungal chemotherapy has not been recommended for treatment of CNS aspergillosis. Intrathecal administration of AMB does

not allow penetration beyond the pia mater and may induce chemical arachnoiditis, seizures, severe headache, and altered mental status. Instead, high-dose systemic antifungal therapy is recommended to achieve higher parenchymal concentrations.[17] Other therapeutic alternatives include the use of parenteral or oral imidazole compounds, eg, fluconazole and itraconazole.[18] Mikolich et al[19] reported the use of itraconazole in an immunocompetent patient with *Aspergillus* meningitis with good outcome. More recently, Renard et al[20] reported another case of neuroaspergillosis associated with neurotuberculosis in an immunocompetent woman treated successfully with antituberculous agents and itraconazole. We, in the past, had successfully treated patients with GAE with a combination of rifampicin, amphotericin B, cotrimoxazole, and flucytosine. Therefore, the same treatment regimen was followed in this case too. Miltefosine was not available easily in our setup.

Acanthamoeba, free-living ameba, gains access to the human body through the skin or upper respiratory tract and reaches the CNS through hematogenous routes, where it causes severe edema and hemorrhagic necrosis.[21] Invasion of the olfactory mucosa and the olfactory bulbs, with hemorrhagic necrosis of both cerebral gray and white matters, and an acute inflammatory infiltrate are the histopathologic characteristics. Trophozoites and cysts may be found within the CNS lesions. Giant cells may not be present in immunosuppressed individuals.

However, some patients have no apparent underlying disease, definite predisposing factor, or immunodeficiency. It is imperative to assess the functional and morphologic features of the thymus, lymph nodes, spleen, and other reticuloendothelial system organs to evaluate the possibility of impairment of the cell-mediated immune response or other immunological deficiencies in such patients.[22] Associated disorders of GAE include skin ulcers, liver disease, pneumonitis, diabetes mellitus, renal failure; rhinitis, pharyngitis, and tuberculosis. Predisposing factors of GAE include steroid treatment, chemotherapy, alcoholism, radiation therapy, lymphoproliferative disorders, pregnancy, systemic lupus erythematosus, AIDS, and hematologic disorders (agammaglobulinemia, thrombocytopenia, splenectomy, and glucose 6-phosphate dehydrogenase deficiency).

As there are no lymphatic channels within the brain, the route of invasion must be through the bloodstream. Chronic ulceration of the skin containing amoebic trophozoites and cysts has been reported in patients who have died of GAE. These cases may represent a terminal hematogenous dissemination of amoebic trophozoites and cysts from primary foci in the lungs or from the CNS rather than a primary dermatologic lesion. Other cases, however, may represent true primary amoebic ulcerations.

Conclusion

This is the first report of *Aspergillus* and *Acanthamoeba* coinfection of CNS. The findings in this case emphasize the importance of establishing an early etiologic diagnosis in CNS infections in order to decide on the most appropriate therapy.

Author Contributions

Provided clinical care: MP. Analyzed the data: VK. Wrote the first draft of the manuscript: VK. Contributed to the writing of the manuscript: KT. Agree with manuscript results and conclusions: RK and PP. Jointly developed the structure and arguments for the paper: VK and KT. Made critical revisions: MP. All authors reviewed and approved of the final manuscript.

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