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FATAL DISSEMINATED CRYPTOCOCCOSIS WITH DIVERSE RISK FACTORS: A CASE SERIES FROM A TERTIARY CARE HOSPITAL IN EASTERN INDIA

Clinical Microbiology

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ABSTRACT

Background: *Cryptococcos noeformans* is a ubiquitous fungal infection that can cause life threatening meningitis and fungemia in both immunocompromised and immunocompetent host. Disseminated cryptococcosis is often a retrospective diagnosis when blood cultures are found positive for *Cryptococcus* species. **Case presentation:** We report two cases of Disseminated cryptococcosis with different risk factors emphasizing on early clinical suspicion, diagnosis and treatment. First case was of non- alcoholic steatohepatitis patient who presented with complain of abdominal swelling and hemiparesis. Other case was of retro positive male presenting with complains of headache and fever. These patients underwent empirical antibacterial and anti-tubercular therapy which was ineffective. Finally, diagnosis was made on the basis of India ink stain, Gram stain and fungal culture which were confirmed by polymerase chain reaction and gene sequencing of both the isolates. But the patients could not be saved due to delay in diagnosis. **Conclusion:** Disseminated cryptococcosis is an advanced potentially fatal disease. Cryptococcosis should be suspected not only in cases of HIV or transplant patients but also in cases of chronic liver diseases. An early clinical suspicion would help recognize these cases sooner. The microbiologist should always have a keen eye and an equally high index of suspicion especially in the presence of capsulated round yeast cells in any sample of sick patients. Early diagnosis and treatment is imperative to prevent mortality.

KEYWORDS

Disseminated cryptococcosis, non-alcoholic steatohepatitis, Human immunodeficiency virus

INTRODUCTION

Cryptococcosis is an invasive fungal disease acquired by inhalation of spores. This fatal infection affects immunocompromised & immunocompetent hosts both. Infection can range from asymptomatic colonization of the respiratory tract to widespread dissemination depending upon the host immune factors. Meningoencephalitis is the most common presentation but it can haematogenously disseminate to any part of the body like skin, kidney, liver, spleen, lymph nodes, peritoneum and adrenal glands. The diagnostic criteria of disseminated cryptococcosis have been defined as 2 or more non-adjacent organs being simultaneously affected with cryptococcosis.[1] Most cases show no specific clinical manifestations and can be misdiagnosed as tuberculosis and malignancy. Such cases have a poor prognosis. [2-4] Here we present a series of two cases of disseminated cryptococcosis diagnosed incidentally from samples other than cerebrospinal fluid (CSF).

CASE 1:

A 65-year-old male with diabetes, hypertension and hypothyroidism, was admitted in a tertiary care hospital in Bihar, India with complain of abdominal swelling for one month, left hemiparesis with incoherent speech for one week and fever for 3 days.

Multiple matted supraclavicular lymph nodes, bilateral pitting edema of lower limbs and decreased power in left upper and lower limbs were observed. There was decreased air entry in bilateral lower lobes. The abdomen was distended, non-tender with shifting dullness. A provisional diagnosis of ischemic stroke causing left hemiparesis with ascites was made.

Laboratory investigations revealed normal total leukocyte count and raised prothrombin time/ International Standardized Ratio, glycosylated hemoglobin, TSH and serum urea. Liver function tests were deranged. A routine urine examination revealed pyuria, hematuria and severe proteinuria. Therapeutic paracentesis was done which showed predominantly lymphocytic ascitic fluid with an LDH=359.60 U/L, ADA=48.14 U/L, Albumin=0.70 gm/dL, and glucose=75 mg/dL. HIV, hepatitis B, and hepatitis C tests were non-reactive.

Contrast-enhanced computed tomography (CECT) of the thorax showed multiple centrilobular nodules in linear and branching patterns in the left upper and lower lobes and bilateral effusion. The CECT of abdomen revealed gross ascites. Tuberculosis was radiologically suspected. Subsequently, a fine needle aspiration cytology from the supraclavicular lymph nodes confirmed tubercular lymphadenitis. In view of the clinical, radiological, and cytological findings, the patient was started on anti-tubercular therapy.

Despite treatment, patient's clinical condition worsened progressively. On day five of admission, patient was shifted to the intensive care unit, injection ceftriaxone and inotropes was started empirically. On day 8 & day 10, the culture of ascitic fluid & paired blood samples gave growth of *Cryptococcus spp*. respectively. The microscopy and culture findings were immediately conveyed to the treating unit. However, the patient had succumbed the same day, before receiving any antifungal agents. A final post-mortem diagnosis of disseminated cryptococcosis following cryptococcal peritonitis with non-alcoholic steatohepatitis was made. Details of further microbiological findings are shown in table 1.

Table 1: Microbiological Investigations

	Day of admission	Investigations	Result
Case 1	Day 5	Ascitic fluid Culture	Cream colored, mucoid colony
	Day 7	Paired Blood Culture	

		India ink stain	Narrow based budding yeast cell with capsule suggestive of *Cryptococcus spp.* (Fig.1)
Case 2	Day 1	Urine & sputum microscopy	Round, narrow based budding yeast cell with capsule
	Day 2	CSF microscopy (Gram stain & India ink stain) CSF (Acid Fast staining & Cartridge Based Nucleic Acid Amplification Test	Narrow based budding yeast cell with capsule suggestive of *Cryptococcus spp.* (Fig.1 & 2) Negative for acid-fast bacilli *Mycobacterium tuberculosis* not detected
	Day 4	Fungal culture of Urine & sputum India ink stain	Cream colored, mucoid colony Narrow based budding yeast cell with capsule suggestive of *Cryptococcus spp.*
	Day 6	CSF & paired blood culture	Confirmed growth of *Cryptococcus spp.*
		Skin scrapping Gram stain & KOH mount	Also showed narrow based budding yeast cells with capsule
Molecular identification of both culture isolates (ITS based molecular method)	PCR and genome sequencing	Genomic DNA was isolated from both the culture isolates. The ~500bp, DNA fragment was amplified using high–fidelity PCR polymerase. The PCR product was sequenced Bi-directionally. The sequence data was aligned and analyzed to identify the Fungus and its closest neighbor. The isolates were found to be most *Cryptococcus neoformans*. Phylogenetic tree was constructed. (Fig.3 & 4)	

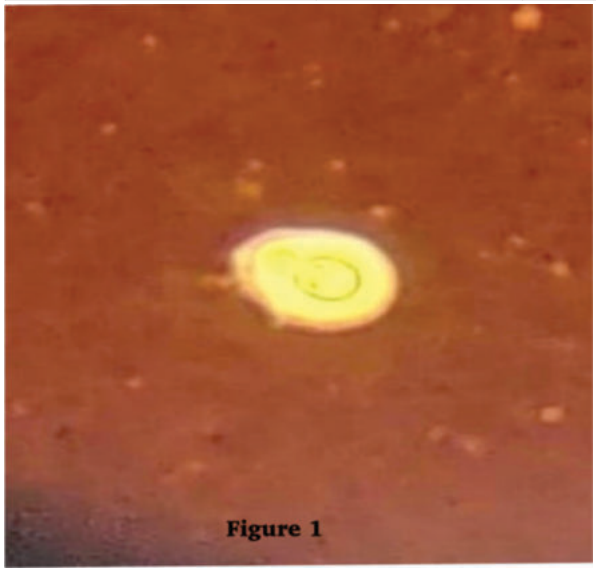

Figure-1: India ink microscopic finding of Cryptococcus spp. (400x)

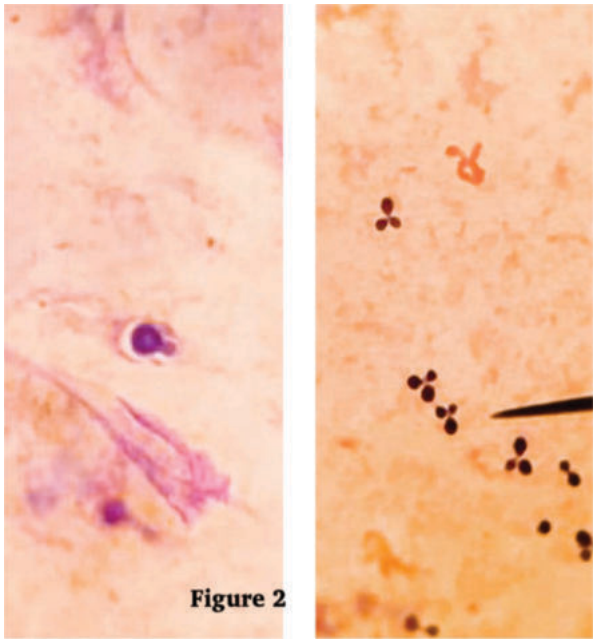

Figure-2: Gram Stained Preparation Showing Of Cryptococcus Spp. (1000x)

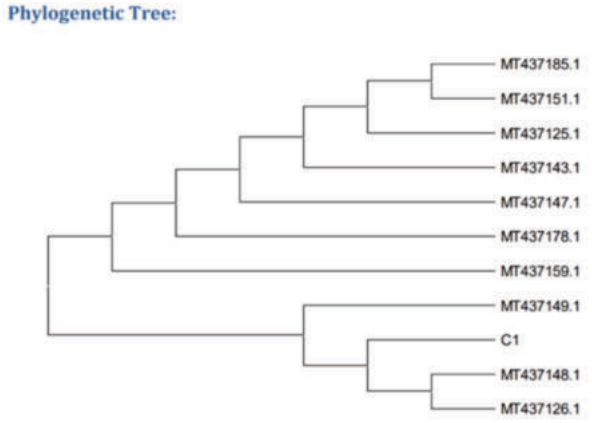

Figure-3: Phylogenetic tree of clinical isolate 1 (C1) (Accession number: Oq608654)

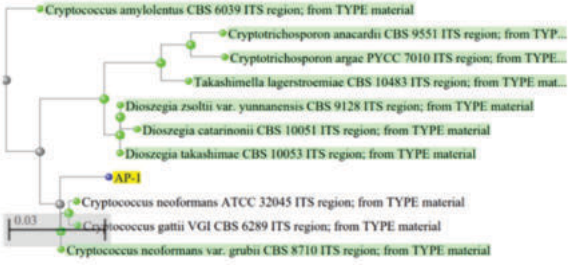

Figure-4: Phylogenetic Tree Of Clinical Isolate 2 (AP1)

CASE 2

A 43 years old male, from Bihar presented to emergency with complaints of low-grade fever, severe occipital headache, blurred vision and productive cough for 20 days. Temperature spiked in the evenings but settled down with antipyretics. History of exposure to pigeon guano was present for last 20 years.

On examination, the patient was confused with fever and raised blood pressure. Few supraclavicular lymph nodes measuring about 2.5 cm and oral thrush was observed. Systemic Examination showed neck stiffness and mild hepatosplenomegaly. Few hyperpigmented maculopapular rashes over the neck, trunk, and limbs were observed. A differential diagnosis of meningitis was made.

Laboratory investigations namely blood culture, urine for routine

microscopy and culture, and sputum for bacterial culture were sent before initiation of antibiotics on Day 1. He was reactive for Human Immunodeficiency Virus (HIV-1) and non-reactive for hepatitis B, hepatitis C, and syphilis. The patient was empirically started on inj. ceftriaxone 2 gm BD, vancomycin 0.5 gm TDS, tablet cotrimoxazole 960 mg BD and tablet fluconazole 400 mg BD. All tests performed for acute febrile illnesses (Widal test, Giemsa stain for malaria, RK-39, and scrub typhus) and reverse-transcriptase PCR for COVID-19 were negative. The patient was anemic with predominantly neutrophilic total leukocyte count.

A CECT of the head, neck, and thorax showed diffuse ground-glass opacities with interstitial thickening and cavitary changes in the bilateral lung parenchyma, hepatosplenomegaly and few enlarged supraclavicular lymph nodes.

CSF microscopy showed a 100% mononuclear cell count of 30 cells/mm^3. The patient had a low CSF glucose (16mg/dl). CSF protein and Adenosine deaminase (ADA) were 81.72 mg/dl and 2.77 U/L respectively. However, Microscopic examination of the CSF and skin scrapings and urine, sputum, CSF & blood culture was suggestive of *Cryptococcus spp*. Details of further microbiological findings are shown in table 1. A final diagnosis of disseminated cryptococcosis with Cryptococcal meningitis was made.

The patient was started on Liposomal Amphotericin B (0.7 mg/kg body weight/day) and Flucytosine (100 mg/kg body weight/day) but deteriorated clinically. On day 7 of admission, he was shifted to the ICU, intubated, and needed inotropic support. Despite the administration of antifungal agents, the patient succumbed to his illness on Day 8 of the presentation.

DISCUSSION:

Cryptococcosis is caused by the encapsulated basidiomycete yeast, *Cryptococcus neoformans*. According to the Asian Surveillance study, Cryptococcus is the most common non-*Candida* yeast to cause community-acquired fungal disease in Asia. [5] Infection is primarily acquired by inhalation of spores but it may also be acquired by ingestion and inoculation. Primary pulmonary infections are usually subclinical and *Cryptococcus* can disseminate to other organs via blood and lymphatics from the lungs.

Can Chronic Liver Disease Be One Of The Risk Factors For Disseminated Cryptococcal Infections?

Historically, cryptococcal infections have been associated with immunosuppressive conditions such as HIV, hematopoietic malignancies, and transplants. HIV remains most common risk factor for acquiring the disseminated disease. Reports suggest that between 12 to 34% of patients with HIV present with cryptococcemia.[6,7] However, there is a rising appreciation of newer risk factors following the decline of the HIV epidemic. In a recent analysis of comorbidities associated with Cryptococcus in the West, it was found that up to 70% of patients with the disease have no history of HIV or transplant.[8] Notably, chronic liver disease has emerged as a strong risk factor for cryptococcosis. Bhatt et al. conclusively established that chronic liver disease has a significant impact on cryptococcal diagnostic results and eventual mortality.[8] Likewise, Lin et al. emphasized considering cryptococcosis as a differential diagnosis in cases of cirrhotic patients presenting with altered mentation and community-acquired sepsis in Asia.[5] In our report, case 1 was a patient with non-alcoholic steatohepatitis and diabetes. A study from Kentucky, USA reports only 5 cases of cryptococcosis with NASH as a predisposing disease in over a decade.[8] Because of the rarity of NASH as a risk factor, *Cryptococcus* was not suspected in our patient throughout his clinical course. Another comorbidity in our first case, diabetes mellitus, is being increasingly reported in patients with cryptococcal disease. Large retrospective cohorts have recorded a remarkably high rate of diabetes in patients with cryptococcosis. Studies from Asia have reported diabetes in 9 to 12% of patients with disseminated cryptococcosis. [6,7]. In contrast, a Western study reports diabetes as a predisposing morbidity in up to 28% of patients with cryptococcosis.[8]

Is Co-infection Of Tuberculosis And Cryptococcosis Common?

Our first case is also unique because of the comorbid conditions of tubercular lymphadenitis along with cryptococcosis. However, radiological features of cryptococcosis and tuberculosis are often confounding.[9] Unfortunately, no respiratory specimens were sent for microbiological or pathological confirmation of the infective etiology. Co-infections of tuberculosis and cryptococcosis have frequently been reported in HIV-infected patients, but their presence in HIV-uninfected patients is rare. [9-12] We offer a plausible explanation of this co-infection in our patient. Tuberculosis infection is known to impair host defenses. Tuberculosis also leads to immune response evasion and disrupts cross-talk between the innate and adaptive immunity.[13-15]

A unique characteristic of *Cryptococcus* species is the presence of a polysaccharide capsule, this is essential for virulence and also endows *Cryptococcus* with potent immunoregulatory properties. Studies suggests that polysaccharide Glucuronoxylomannan (GXM) of Cryptococcus, is highly immunosuppressive. Hence, the cryptococcosis may have resulted in the tuberculosis presentation. [16]

As tuberculosis and cryptococcosis both leads to impaired host defenses, it is quite likely that any one disease could act as risk factor for other in our patient.

Despite diverse risk factors and clinical presentations, both our patients had fatal outcomes. Studies report a 14-day mortality rate of over 50% in immunosuppressed patients with cryptococcemia.[6,17] Such high mortality rates can be attributed to late presentation, lack of clinical suspicion due to non-specific symptoms, and a delay in diagnosis and treatment. As seen in both our cases, different immunosuppressive risk factors can present with diverse clinical features and unusual routes of infection. While in the first case, cryptococcosis was not thought of as a differential diagnosis at all, a strong clinical suspicion of fungal meningitis early in the course of disease in the second case led to prompt diagnosis. In their study, Zhao et al, report a significantly shorter duration of diagnosis in patients with HIV compared to those in HIV-uninfected patients. [7] The same study also concluded that a considerably shorter time of diagnosis is needed when patients present with cryptococcal meningitis than with non-meningitis clinical features. In concordance with the above findings, diagnosis of our second case, presenting with HIV and features of fungal meningitis, was prompter than in the first case. A high index of clinical suspicion, early in the course of the disease, would help early initiation of antifungal therapy.

Can early and appropriate selection of specimens be helpful for diagnosis?

An important learning pearl for clinical microbiologists from both our cases is the early and appropriate selection of specimens for diagnosis. In case 1, specimens for microbiological examination were not sent until day five of presentation, and blood cultures were sent only a week after admission. The second case stresses upon rapid and meticulous microscopy and processing of non-invasive specimens like urine, sputum, and skin scrapings all of which yielded *Cryptococcus spp*. In both our cases, non-blood specimens gave early clues to the presence of cryptococcal infection. A search for *Cryptococcus* from initial, non-invasive specimens is of even more relevance in resource-limited centers. Such clinical settings are fraught with several clinical and diagnostic challenges. In a recent survey of mycology laboratory practices across Asia, rapid identification methods like MALDI-TOF and genotypic methods like PCR and sequencing were available in only 12 and 17% of laboratories respectively. [18] Both patients in our report belonged to an underdeveloped state, Bihar, in the North of India without access to rapid mycological diagnostic modalities. Careful microscopic examination of non-invasive specimens collected early in the course of the disease could be valuable in decreasing the time to diagnosis.

An environmental surveillance study of *Cryptococcus* by Haldar *et al* done in Bihar demonstrated a prevalence of 3% in the area. [19] This may represent only the tip of the iceberg as a large population in the state is based in rural and semi-urban areas presenting late or not at all. The state also has a very high prevalence of several risk factors like TB, diabetes and HIV. [20, 21] Given the indisputable prevalence of the disease, the late presentation, as well as the long time taken to diagnose and initiate anti-fungal treatment, the authors emphasize the need for rapid diagnostic methods with good sensitivity and specificity like latex agglutination, and lateral flow assays.

CONCLUSION

It's difficult to diagnose cryptococcosis in non-HIV infected, apparently immunocompetent individuals. Cirrhosis, alcoholic liver disease, end-stage liver diseases have emerged as a major cause of disseminated cryptococcosis. An early clinical suspicion would help recognize these cases sooner. So, the microbiologist should always

have a keen eye and an equally high index of suspicion especially in the presence of capsulated round yeast cells in any sample of sick patients. This will help in timely etiological diagnosis and guide the clinicians in starting appropriate treatment.

Conflict Of Interest

None of the authors has conflict of interests. Written consent has been obtained from the legal guardians of the patients whose cases are presented in this paper.

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