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Cryptococcal meningitis patients associated with HIV co-infection admitted in tertiary care hospital–A Case Series

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ABSTRACT

Meningitis is a significant infection of the central nervous system that is followed by inflammation of the meninges, resulting in catastrophic neurologic consequences. The inflammation may be caused by infection with viruses, bacteria, fungi, or other microorganisms and less commonly by certain drugs. Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore, the condition is classified as a medical emergency. In the present case series, we encompass patient demographics, initial clinical symptoms, physical examinations, laboratory results, cerebrospinal fluid examination findings, treatment side effects, the occurrence of complications, and hospital outcomes. Furthermore, documented were any instances of recurrent cryptococcal meningitis (CM) during follow-up, along with the potential causes of recurrence, the treatment modalities administered, any complications that arose, and the ultimate outcomes. This series reveals CM can manifest alongside HIV co-infection in male patients. Therefore, it is essential to consider the possibility of CM when an immune-compromised patient presents with symptoms such as headaches and other indications of central nervous system involvement. These cases reveal cryptococcosis with HIV-infected patients for rapid and early diagnosis and appropriate treatment of opportunistic infections.

Key words: Meningitis; Cryptococcal meningitis; Human immunodeficiency virus; Bacterial infection; Neurological complications; Inflammatory response

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INTRODUCTION

Cryptococcal meningitis (CM) is a significant suprainfection that is usually seen in immune-compromised patients, particularly in children.[1] It is a significant fungal infection, that is, estimated to cause approximately one million infections and 625,000 mortality annually as part of the central and peripheral nervous system disorders affecting HIV–infected patients globally.[2] The global annual incidence of CM in the years 2008–2009 was estimated at 957,900 (range 371700–1544000 cases).[2] Variations in geographical distribution, epidemiology, and clinical presentation have been observed among all HIV patients. The incidence of CM is rare in HIV infection[2] and is the major cause of death in HIV patients. CM-related mortality remains high (20–30%) despite the presence of antiviral treatment (ART).[3] The transmission incidence in AIDS patients varies between 23–48.6%, and around 70–90% of subjects develop indications and signs of sub-acute meningitis or meningoencephalitis, both of which have fatal outcomes.[4] The death rate could be as high as 30%, especially if identification and definitive treatment are delayed.[5] The mortality rate of CM varies regionally.[6-8]

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METHODOLOGY

The current research was carried out at the Microbiology Department at the Dr. Ram Manohar Lohia Medical Sciences Institute in Lucknow, India. The Institutional Ethics Committee (IEC 6/14) accepted the research's ethics, and the research was conducted between July 2016 and July 2019. In this study period, five cases of CM which were HIV co-infection. According to the inclusion and exclusion criteria, the subjects who were considered for the research were based on clinical, laboratory, and/or microbiological and pathological criteria.

Case 1

A 53-year-old man presented at the hospital, reporting a persistent headache that began one month before admission and had worsened over the past week. The headache, characterized by severe throbbing sensations throughout the entire head, typically developed gradually and did not respond to pain relievers. The headache remains constant and is not exacerbated by physical activity or coughing. There is no identifiable trigger or influencing factor for the ongoing headache. The patient has been experiencing a cough for the last month and has also lost approximately 10 kg of weight in the last three months. The patient has engaged in unprotected sexual activity. Notably, the patient was diagnosed with HIV one day before hospital admission and has not yet received antiretroviral treatment. On neurological examination, the patient displayed alert consciousness with stiffness in the neck. Laboratory tests on blood revealed positive results for HIV using three different testing methods. In addition, there was a decline in the CD4 count by 4%, along with a 2% decrease in the absolute CD4 count by 10 cells/uL. The examination of cerebrospinal fluid (CSF) revealed 71.4% MN cells and 28.6% PMN cells, with the total protein level within the normal range at 12.00. In the Indian ink staining, yeast-like cells were detected (Figure 1). A head CT scan with contrast showed no signs of visible bleeding, intracerebral space-occupying lesions, or other intracranial abnormalities (Figure 2). The patient's treatment regimen included dexamethasone injections at 5 mg every 6 h, with a gradual tapering off every 3 days. In addition, the patient was prescribed paracetamol at three doses of 1000 mg each, cotrimoxazole at 960 mg once daily, fluconazole injections at 400 mg per day for 2 weeks, and folic acid at one tablet per day. The patient also had a consultation with the infectious tropical diseases (PTI) section and was prescribed fixed-dose combination ARV therapy, including tenofovir, lamivudine, and efavirenz, to be taken once a day. During therapy, the patient experienced a reduction in headache symptoms and subsequently transitioned to outpatient care, returning home.

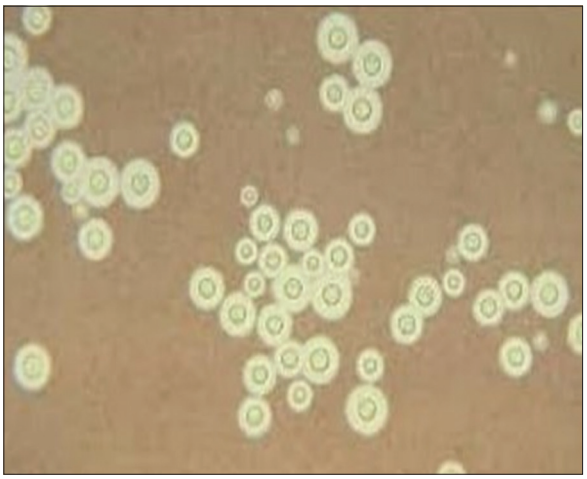

Figure 1: The Indian ink preparation showing encapsulated budding yeast cells characteristic of *Cryptococcus neoformans*. These yeast cells exhibited narrow-based budding and variable sizes ranging from 5 to 25 μ.

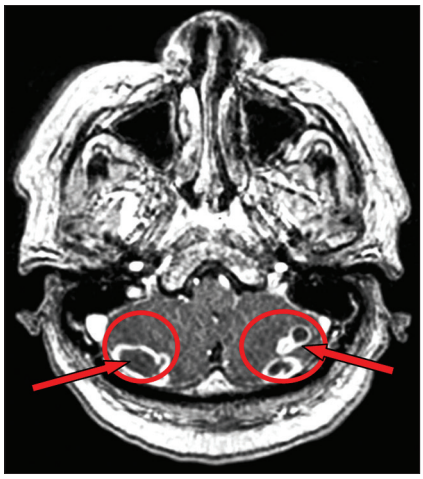

Figure 2: Several ring-enhancing lesions are present in the lower part of the cerebellum, consisting of four distinct lesions

Case 2

A 43-year-old male patient sought medical attention in the emergency department, reporting a persistent shortness of breath that had been bothering him for the past 2 weeks. Alongside the shortness of breath, he also experienced a cough. In addition, he had been dealing with a fever and a reduced appetite for the past 4 months, attributed to an oral ulcer. His recent diagnosis of HIV had been made at another hospital just 2 weeks before he was admitted to King George Medical University (Dr. Harpreet Malhotra). During the physical examination, the patient displayed drowsiness, registering a Glasgow Coma Scale score of 13. Rhonchi were detected in both hemithoraces on auscultation. A chest X-ray revealed a reticulogranular pattern in both hemithoraces. The trachea appeared centrally located, and there were no abnormalities observed in the soft-tissue or visualized bone within the left and right

costophrenic angles. A head computed tomography (CT) scan was conducted and indicated the absence of hypo/hyperdense lesions within the brain parenchyma, with no contrast enhancement detected. The sulcus and gyrus appeared to be within normal parameters. The ventricular system and cisterns were also found to be normal. Both the pons and cerebellum exhibited no abnormalities, including the absence of any evident abnormal calcifications. There was no midline deviation detected. Gram-positive cocci of *Staphylococcus aureus* and yeast cells of *Cryptococcus neoformans* were identified from sputum and blood cultures. However, the blood culture for yeast cells in the form of *C. neoformans* yielded negative results. Based on the patient's clinical signs, the outcomes of the head CT scan, and the blood culture findings, a diagnosis of *C. neoformans* in the context of AIDS was recognized. The patient commenced fluconazole treatment at a dosage of 750 mg/day and was prescribed ARV therapy, including duviral and neviral, to be taken twice daily. After 2 weeks of treatment, there was a noticeable improvement in the patient's clinical condition, leading to their discharge from the hospital.

Case 3

A 35-year-old farmer sought medical attention at our outpatient department due to a month-long history of progressive weakness in all four of his limbs. He has also been experiencing a low-grade fever over the same duration. For the past month, he has been experiencing neck pain, intermittent visual disturbances, and difficulty in feeding himself for the past 5 days. After examination, the patient appeared disoriented and only partially responsive to commands. He displayed clinical manifestations indicative of meningitis, such as a convergent squint and the bilateral paralysis of rectal muscles. The patient displayed a muscle power rating of 3/5 in all four of his limbs, with no abnormalities noted in the facial nerve. Sensory functions were intact, and there were no issues with bladder or bowel control. Routine blood tests, including blood sugar levels, total leukocyte count, differential leukocyte count, smear study, serum electrolytes, urea, and creatinine, all returned within normal ranges. The patient had an elevated erythrocyte sedimentation rate level. A CT scan without contrast and a chest X-ray revealed no abnormalities. A lumbar puncture was conducted, and the CSF, found to be under increased pressure, was sent to a central laboratory for analysis. A preliminary diagnosis of tuberculous meningitis was recorded. The biochemical analysis of the fluid revealed heightened protein levels at 130 mg/dL and diminished sugar levels at 18 mg/dL. Microbiological and pathological examinations were carried out, which included the standard wet mount preparation. This preparation revealed the existence of round to oval cells, several of which were in the budding stage. In addition, an Indian ink procedure was executed by applying a small drop of centrifuged CSF deposit onto a glass slide, combining it with a drop of Indian ink, covering it with a coverslip, and subsequently examining it under both ×10 and ×100 magnifications. Spherical to oval yeast-like cells with thick walls and a distinct clear halo were observed, and some of these cells exhibited budding. A preliminary diagnosis of CM was established. The patient's serum underwent HIV testing, initially with a rapid test, which was subsequently affirmed as positive through routine ELISA HIV antigen detection.

Furthermore, the CSF deposit was cultured on sabouraud dextrose agar and incubated at 25°C for 72 h. After 48 h, it displayed smooth, white, and flat irregular colonies, which became mucoid in appearance by the 3rd day (Figure 3). Based on standard methods including Gram's stain, cryptococcal capsular polysaccharide stain, and urease production test, the organisms were identified as *C. neoformans*. In addition, a latex agglutination test (LAT) was performed on the CSF deposit, confirming the diagnosis. The LAT for detecting the cryptococcal capsular polysaccharide antigen is a dependable and swift diagnostic method for *C. neoformans*. Further assessments, including sputum culture to identify bacterial pathogens, Gram's stain smear of sputum, acid-fast bacilli smear, urine wet mount, and stool assessment, were conducted to exclude the possibility of other opportunistic infections, given the patient's HIV-positive status. No indications of any other co-infections were found. The patient commenced treatment with intravenous Amphotericin B at a dose of 1 g twice daily, along with intravenous flucytosine at a dose of 1 g twice daily. Due to the patient's HIV-positive status, he was transferred to a private ward at Dr. Ram Manohar Lohia Institute of Medical Sciences in Lucknow, India. Unfortunately, the patient passed away on the 3rd day despite receiving appropriate treatment.

Figure 3: White, flat, and mucoid colonies on sabouraud dextrose agar

Case 4

A 49-year-old patient with a one-month chronicle of watery diarrhea and a recent 2 kg weight loss was admitted to the hospital. He lived alone and had a daily drinking habit. Despite being HIV-positive with a CD4 count of 9, he did not exhibit typical symptoms of cryptococcal disease such as fever, headache, confusion, neck stiffness, or light sensitivity. The patient also showed mild dehydration and had oral candidiasis. Routine blood tests and stool examinations were normal, but the Cryptococcal antigen test returned positive. The patient was counseled to participate in preparatory sessions and instructed to revisit the clinic in a fortnight to initiate anti-retroviral therapy. On his discharge, he received prescriptions for nystatin mouthwash, bactrim (2 tablets daily), Vitamin B complex (2 tablets daily), and thiamine (200 mg daily) as a result of his past alcohol consumption and potential issues with nutrient absorption. Three months later, the patient was readmitted to the hospital, reporting symptoms of headache, neck stiffness, and confusion. Unfortunately, during this time, he had not returned to the clinic to initiate antiretroviral treatment as previously advised. On lumbar puncture, it was discovered that the patient had very high intracranial pressure (45 cm), and his CSF tested positive for *Cryptococcus*. Following that, the patient commenced treatment with amphotericin B alongside pre-hydration. Despite receiving suitable medical attention, the patient passed away on the 5th day of his hospital stay.

Case 5

After three months, the patient was readmitted to the hospital, complaining of symptoms such as headache, neck stiffness, and confusion. Regrettably, during this interval, he had not followed through with the previous recommendation to initiate antiretroviral treatment at the clinic. On undergoing lumbar puncture, it was determined that the patient was experiencing significantly elevated intracranial pressure (measuring at 45 cm), and his CSF tested positive for *Cryptococcus*. Consequently, the patient's treatment was initiated with amphotericin B, accompanied by pre-hydration. Despite receiving appropriate medical attention, the patient unfortunately succumbed to his condition on the 5th day of hospitalization. On the 4th day of his hospitalization, the patient's creatinine levels spiked, indicating kidney dysfunction. In response, he received intravenous fluids, and the dosage of amphotericin B was reduced. By the 10th day, the patient developed thrombophlebitis characterized by vein swelling due to blood clots, as a result of amphotericin B treatment. In addition, he experienced anemia. Despite these challenges, he completed a 14-day course of amphotericin B and was discharged with a prescription for fluconazole.

During a follow-up clinic visit after his hospital discharge, the patient's condition had improved significantly, and he no longer reported headaches. His treatment regimen included continuing fluconazole at a dose of 800 mg/day and initiating ART (AZT, 3TC, EVZ) due to his kidney dysfunction (elevated creatinine levels) and peripheral neuropathy (nerve pain).

DISCUSSION

The prevalence of cryptococcosis varies depending on geographical location.[9] Kisenge et al.,[10] reported a 60% positivity rate for Indian ink preparation, while Kumar et al.,[11] indicated a range of 70–90% positivity among AIDS patients in India. The higher prevalence of *Cryptococcus* infection in males compared to females seems to result from differences in exposure rather than variations in host susceptibility. Both Indian ink preparation and culture methods are considered essential for an accurate diagnosis of cryptococcosis, as they complement each other. Multiple studies have noted differences in their results, highlighting the importance of using both methods for an efficient diagnosis. The cellular responses and biochemical alterations observed in CSF often bear similarities to those seen in tuberculous meningitis. Therefore, the diagnosis primarily relies on isolating the organism from clinical samples and confirming its presence through culture.

Meningitis is a common clinical presentation in cases of cryptococcosis, with CM being the second most frequently observed cause of neurological disease in AIDS patients. *Cryptococcus* is identified in 50% of cases through Indian ink preparation of CSF deposits. The detection of cryptococcal antigen in spinal fluid exhibits high sensitivity (90%) and specificity. This rate of HIV positivity aligns closely with a study conducted in India for 3 years from 2009 to 2012, which reported an HIV prevalence of 0.31% among adults. All cases underwent screening for various opportunistic infections.

CONCLUSION

Our study findings suggest that cryptococcosis is prevalent among AIDS patients. Given the indistinct symptoms, and to reduce mortality among individuals affected by AIDS, it is advisable to incorporate routine checkups into the overall management of AIDS. We strongly advocate for the routine laboratory diagnosis of cryptococcal infection in all HIV patients, along with an enhanced focus on intensifying antiretroviral treatment. This approach aims to minimize the risk of cryptococcosis in individuals living with HIV.

ETHICAL APPROVAL

The protocol underwent review and received approval, and the work was carried out in alignment with the directives set forth by the Institutional Ethical Committee of Dr. Ram Manohar Lohia Institute of Medical Sciences.

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REFERENCES

1. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of America. Clin Infect Dis. 2010;50(3):291-322.
   https://doi.org/10.1086/649858
2. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG and Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23(4):525-530.
   https://doi.org/10.1097/QAD.0b013e328322ffac
3. Chan M, Lye D, Win MK, Chow A and Barkham T. Clinical and microbiological characteristics of cryptococcosis in Singapore: Predominance of *Cryptococcus neoformans* compared with *Cryptococcus gattii*. Int J Infect Dis. 2014;26:110-115.
   https://doi.org/10.1016/j.ijid.2014.05.019
4. Jarvis JN, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: Implications for improving outcomes. Clin Infect Dis. 2014;58(5):736-745.
   https://doi.org/10.1093/cid/cit794
5. Pyrgos V, Seitz AE, Steiner CA, Prevots DR and Williamson PR. Epidemiology of cryptococcal meningitis in the US: 1997-2009. PLoS One. 2013;8(2):e56269.
   https://doi.org/10.1371/journal.pone.0056269
6. Musabende M, Mukabatsinda C, Riviello ED and Ogbuagu O. Concurrent cryptococcal meningitis and disseminated tuberculosis occurring in an immunocompetent male. BMJ Case Rep. 2016;2016:bcr2015213380.
   https://doi.org/10.1136/bcr-2015-213380
7. Swe Han KS, Bekker A, Greeff S and Perkins DR. Cryptococcus meningitis and skin lesions in an HIV negative child. J Clin Pathol. 2008;61(10):1138-1139.
   https://doi.org/10.1136/jcp.2008.056119
8. Ellis JP, Kalata N, Joekes EC, Kampondeni S, Benjamin LA, Harrison TS, et al. Ischemic stroke as a complication of cryptococcal meningitis and immune reconstitution inflammatory syndrome: A case report. BMC Infect Dis. 2018;18:520.
   https://doi.org/10.1186/s12879-018-3386-0
9. Perfect JR. Cryptococcosis. Infect Dis Clin North Am. 1989;3(1):77-102.
   https://doi.org/10.1016/S0891-5520(20)30248-8
10. Kisenge PR, Howkins AT, Marvo VP, Mchele JP, Swai NS, Muller A, et al. Low CD4 count plus coma predicts cryptococcal meningitis in Tanzania. BMC Infect Dis. 2007;7:39-43.
    https://doi.org/10.1186/1471-2334-7-39
11. Kumar S, Wanchu A, Chakrabarti A, Sharma A, Bambery P and Singh S. Cryptococcal meningitis in HIV infected: Experience from a North Indian tertiary center. Neurol India. 2008;56(4):444-449.

Authors Contribution:
PY: Conceptualization, formal analysis, resources, methodology; **RM:** Validation, formal analysis, writing–review; **AKS:** Writing original draft, data curation, methodology, resources; **SY:** Concept and design, manuscript preparation, revision of manuscript and treating physician; **GTS:** Writing–review, methodology; **MS:** Validation, conceptualization.

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